At a Glance...

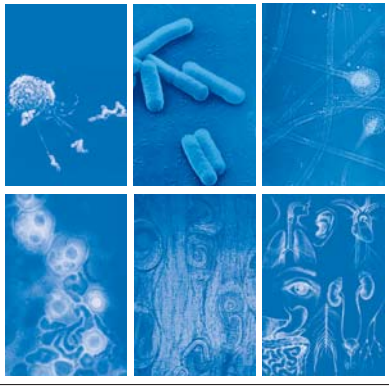
The book is divided into six main sections. The color-coded reference guide on the first page will help you find what you need.

The aspects of each pathogen are covered systematically, using the following order wherever practicable:

- Classification
- Localization
- Morphology and Culturing
- Developmental Cycle
- Pathogenesis and Clinical Picture
- Diagnosis
- Therapy
- Epidemiology and Prophylaxis

A **summary** at the beginning of a chapter or section provides a quick overview of what the main text covers. Students can use the summaries to obtain a quick recapitulation of the main points.

The Main Sections at a Glance



a The many **colored illustrations** serve to clarify complex topics or provide definitive impressions of pathogen morphology.
b The **header caption** above each illustration gives the reader the essence of what is shown.
c The **detailed legends** explain the illustrations independently of the main text.

Additional information

In-depth expositions and supplementary knowledge are framed in boxes interspersed throughout the main body of text. The headings outline the topic covered, enabling the reader to decide whether the specific material is needed at the present time.

Medical Microbiology

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Preface

Medical Microbiology comprises and integrates the fields of immunology, bacteriology, virology, mycology, and parasitology, each of which has seen considerable independent development in the past few decades. The common bond between them is the focus on the causes of infectious diseases and on the reactions of the host to the pathogens. Although the advent of antibiotics and vaccines has certainly taken the dread out of many infectious diseases, the threat of infection is still a fact of life: New pathogens are constantly being discovered; strains of „old" ones have developed resistance to antibiotics, making therapy more and more difficult; incurable infectious diseases (AIDS, rabies) are still with us.

The objective of this textbook of medical microbiology is to instill a broad-based knowledge of the etiologic organisms causing disease and the pathogenetic mechanisms leading to clinically manifest infections into its users. This knowledge is a necessary prerequisite for the diagnosis, therapy, and prevention of infectious diseases. This book addresses primarily students of medicine, dentistry, and pharmacy. Beyond this academic purpose, its usefulness extends to all medical professions and most particularly to physicians working in both clinical and private practice settings.

This book makes the vast and complex field of medical microbiology more accessible by the use of four-color graphics and numerous illustrations with detailed explanatory legends. The many tables present knowledge in a cogent and useful form. Most chapters begin with a concise summary, and in-depth and supplementary knowledge are provided in boxes separating them from the main body of text.

This textbook has doubtless benefited from the extensive academic teaching and the profound research experience of its authors, all of whom are recognized authorities in their fields.

The authors would like to thank all colleagues whose contributions and advice have been a great help and who were so generous with illustration material. The authors are also grateful to the specialists at Thieme Verlag and to the graphic design staff for their cooperation.

Zurich, fall of 2004

On behalf of the authors
Fritz H. Kayser

Abbreviations

- ABC: antigen-binding cell
- ABS: antigen-binding site
- ADA: adenosine deaminase
- ADCC: antibody-dependent cellular cytotoxicity
- ADE: antibody-dependent enhancement (of viral infection)
- AE: alveolar echinococcosis
- AFC: antibody-forming cell
- AFP: alpha-fetoprotein
- AIDS: acquired immune deficiency syndrome
- ANA: antinuclear antibodies
- APC: antigen-presenting cell
- APO: apoptosis antigen
- aPV: acellular pertussis vaccine
- ASL titer: antistreptolysin titer
- AZT: azidothymidine

- BAL: bronchoalveolar lavage
- BALT: bronchus-associated lymphoid tissue
- BCG: bacillus Calmette-Guerin
- BCGF: B-cell growth factor
- Bcl2: B-cell leukemia 2 antigen
- BSE: bovine spongiform encephalopathy

- C: complement
- CAH: chronic aggressive hepatitis
- CAM: cell adhesion molecules
- CAPD: continuous ambulant peritoneal dialysis
- CCC: covalently closed circular (DNA)
- CD: cluster of differentiation/ cluster determinant
- CDR: complementarity-determining regions
- CE: cystic echinococcosis
- CEA: carcinoembryonic antigen
- CFA: colonizing factor antigen
- CFT: complement fixation test
- CFU: colony forming units
- CJD: Creutzfeldt-Jakob disease
- CLIP: class II-inhibiting protein
- CMI: cell-mediated immunity
- CMV: cytomegaly virus (cytomegalovirus)
- CNS: central nervous system/ coagulase-negative staphylococci
- Con A: concanavalin A
- CPE: cytopathic effect
- CPH: chronic persistent hepatitis
- CR: cistron region
- CSF: colony-stimulating factor
- CTA: cholera toxin A
- CTB: cholera toxin B
- CTL: cytotoxic $CD8^+$ T cell
- CTX: cholera toxin (element)

- DAF: decay accelerating factor
- DAG: diacyl glycerol
- DARC: Duffy antigen receptor for chemokines
- DC: dendritic cells

DHF: dengue hemorrhagic fever
DHPG: dihydroxy propoxymethyl guanine
D vaccine: diphtheria toxoid vaccine
DNA: deoxyribonucleic acid
DNP: dinitrophenol
DR: direct repeats
ds: double-stranded nucleic acid
DSS: dengue shock syndrome
DTH: delayed type hypersensitivity
DtxR: diphtheria toxin repressor

EA: early antigen
EAE: experimental allergic encephalitis
EAF: EPEC adhesion factor
EaggEC: enteroaggregative *Escherichia coli*
EB: elementary body
EBNA: Epstein-Barr nuclear antigen
EBV: Epstein-Barr virus
EDTA: ethylene diamine tetraacetic acid
eEF2: eucaryotic elongation factor 2
EF: edema factor in spotted fevers
EHEC: enterohemorrhagic *E. coli*
EIA: enzyme immunoassay
EIEC: enteroinvasive *E. coli*
EITB: enzyme-linked immunoelectrotransfer blot
ELISA: enzyme-linked immunosorbent assay
EM: electron microscopy
EMB: ethambutol
EMCV: encephalomyocarditis virus
EPEC: enteropathogenic *E. coli*
EPS: extracellular polymer substance
ETEC: enterotoxic *E. coli*
EU: European Union

F factor: fertility factor
FA: Freund's adjuvant
FACS: fluorescence-activated cell sorter
Fas: F antigen
FcR: Fc receptor
FDC: follicular dendritic cell
FHA: filamentous hemagglutin
FITC: fluorescein isothiocyanate
FTA-ABS: fluorescent treponemal antibody absorption test

G6PDD: glucose-6-phosphate dehydrogenase deficiency
GAE: granulomatous amebic encephalitis
gag: group-specific antigen
GALT: gut-associated lymphoid tissue
GC: guanine-cytosine/gas chromatography
GM-CSF: granulocyte-macrophage colony-stimulating factor
GP: glycoprotein
GSS: Gerstmann-Sträussler-Scheinker (syndrome)
GVH: graft-versus-host (reaction)

H: heavy chain
HACEK: *Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, *Kingella*
HAT: hypoxanthine, aminopterin, thymidine
Hb: hemoglobin

HBs: hepatitis B surface antigen
HBV: hepatitis B virus
HB vaccine: hepatitis B vaccine
HCC: hepatocellular carcinoma
HCV: hepatitis C virus/ (human corona virus)
HDCV: human diploid cell vaccine
HDV: hepatitis D virus
HEV: hepatitis E virus/high endothelial venules
Hfr: high frequency of recombination
HGE: human granulocytic ehrlichiosis
HGV: hepatitis G virus
HHV: human herpes virus
HI: hemagglutination inhibition
Hib: *Haemophilus influenzae*, type b serovar
HIV: human immunodeficiency virus
HME: human monocytic ehrlichiosis
HPLC: high-pressure liquid chromatography
HPS: hantavirus pulmonary syndrome
HRF: homologous restriction factor (also histamine releasing factor)
HFRS: hemorrhagic fever with renal syndrome
hsp70: heat shock protein 70
HSV: herpes simplex virus
HTLV: human T cell leukemia virus
HuCV: human calicivirus
HUS: hemolytic-uremic syndrome
HVG: host-versus-graft (reaction)

IB: initial body
IEP: immunoelectrophoresis
IFAT: indirect immunofluorescent antibody test
IFN: interferon
Ig: immunoglobulin
IHA: indirect hemagglutination
(I)IF: (indirect) immunofluorescence
IL: interleukin
In: integron
INH: isoniazid (isonicotinic acid hydrazide)
IP_3: inositol trisphosphate
IPV: inactivated polio vaccine
IR: inverted repeats
Ir genes: immune response genes
IS: insertion sequence/intercistron space

K cells: killer cells

L: light chain
LA: latex agglutination
lac operon: lactose operon
LAK: lymphokine-activated killer cells
LB: leprosy bacterium
LCA: leukocyte common antigen
LCM(V): lymphocytic choriomeningitis (virus)
LE: lupus erythematosus
LFA: lymphocyte function antigen
LGL: large granular lymphocyte
LIF: leukemia inhibitory factor
LL: lepromatous leprosy
LM: light microscopy
LMC: larva migrans cutanea

LMV: larva migrans visceralis
LOS: lipo-oligosaccharide
LPS: lipopolysaccharide
LT: heat-labile *E. coli* enterotoxin
LTR: long terminal repeats

MAC: membrane attack complex
MAF: macrophage activating factor
MALT: mucosa-associated lymphoid tissue
MBC: minimal bactericidal concentration
MBP: major basic protein/myelin basic protein
MCP: membrane cofactor protein
M-CSF: macrophage colony-stimulating factor
MF: merthiolate-formalin
Mf: microfilaria
MHC: major histocompatibility complex
MIC: minimal inhibitory concentration
MIF: migration inhibitory factor/microimmune-fluorescence
MLC: mixed lymphocyte culture
MLR: mixed lymphocyte reaction
MMR: live, attenuated, trivalent measles, mumps, and rubella vaccine
MMTV: murine mammary tumor virus
MOMP: major outer membrane protein
MOTT: mycobacteria other than TB (see NTM)
MZM: marginal zone macrophages

NANB: nonA, nonB hepatitis
NCVP: noncapsidic viral protein
NE: *Nephropathica epidemica*
Nfa: nonfimbrial adhesin
NGU: nongonococcal urethritis
NIDEP: German study on assessment and prevention of nosocomial infections
NK cells: natural killer cells
NTM: nontuberculous (atypical) mycobacteria (see MOTT)
NTR: nontranslated region

OC: open circular (DNA)
OM: opportunistic mycosis
OMP, Omp: outer membrane protein
OPV: oral polio vaccine
OSP, Osp: outer surface protein

P: promoter
PAE: postantibiotic effect
PAIR: puncture, aspiration, injection, respiration
PAS: para-aminosalicylic acid/periodic acid-Schiff stain
PAM: primary amebic meningoencephalitis
PAP: pyelonephritis-associated pili
PBL: peripheral blood lymphocytes
PC: phosphoryl choline/primary (tuberculous) complex, Ghon's complex
PCA: passive cutaneous anaphylaxis
PCR: polymerase chain reaction

PEG: polyethylene glycol
PFC: plaque-forming cell
PHA: phytohemagglutinin
PI: pathogenicity island
p.i.: post infection
PIP_2: phosphatidylinositol bisphosphate
PKC: protein kinase C
PLC: phospholipase C
PMA: pokeweed mitogen
PML: progressive multifocal leukoencephalopathy
PMN: polymorphonuclear neutrophilic granulocytes
PNP: purine nucleoside phosphorylase
PPD: purified protein derivative
PRP: polyribosylribitol phosphate
PrP: prion protein
Ptx: pertussis toxin
PZA: pyrazinamide

QBC: quantitative buffy coat analysis

R: rubella vaccine
RAST: radioallergosorbent test
RES: reticuloendothelial system
RF: rheumatoid factor
RFFIT: rapid fluorescent focus inhibition test
Rh antigen: rhesus antigen
RIA: radioimmunoassay
RIBA: recombinant immunoblot assay
RIG: rabies immunoglobulin
RIST: radioimmunosorbent test
RMP: rifampicin
RMSF: Rocky Mountain spotted fever
RNA: ribonucleic acid
RNP: ribonucleoprotein
RS: respiratory syncytial virus
RT: reverse transcriptase
RT-PCR: reverse transcriptase-polymerase chain reaction
RTI: respiratory tract infection
RVF: Rift Valley fever

SAF: sodium acetate-acetic acid-formalin
SALT: skin-associated lymphoid tissue
SCF: stem cell factor
SCID: severe combined immunodeficiency disease
SDS: sodium (Na^+) dodecyl sulfate
SEA-E: staphylococcal enterotoxins A-E
SEM: scanning electron microscopy
SEP: sepsis
SEPEC: septic *E. coli* pathovar
SFT: Sabin-Feldman test
SLE: systemic lupus erythematosus
SPE: streptococcal pyrogenic exotoxin
SRBC: sheep red blood cells
SRSV: small round-structured virus
ss: single-stranded (nucleic acids)
SSME: spring-summer meningoencephalitis
SSPE: subacute sclerosing panencephalitis
ST: heat-stable *E. coli* enterotoxin

sp.: species
spp.: species (plural)
SV: simian virus
SWI: surgical wound infection

TATA: tumor-associated transplantation antigen
TB: tuberculosis bacterium
Tc: cytotoxic T cell
TCGF: T cell growth factor
TCP: toxin coregulated pili
TCR: T cell receptor
Td: tetanus/low-dose diphtheria toxoids
T-dep: thymus dependent antigens
T-DTH: delayed type hypersensitivity (T cells)
TEM: transmission electron microscopy
Th, TH: T helper cell
T-ind: thymus-independent antigens
TL: tuberculoid leprosy
TME: transmissible mink encephalopathy
Tn: transposon
TNF: tumor necrosis factor
TPHA: *Treponema pallidum* hemagglutination assay
TPI test: *Treponema pallidum* immobilization test
TPPA: *Treponema pallidum* particle agglutination assay
Tra: transfer
TSE: transmissible spongiform encephalopathy
TSS: toxic shock syndrome
TSST-1: toxic shock syndrome toxin-1
TU: tuberculin units

UPEC: uropathogenic *E. coli*
UTI: urinary tract infection

VacA: vacuolating cytotoxin
var.: variety
VCA: viral capsid antigen
VCAM: vascular cell adhesion molecule
VDRL: Venereal Disease Research Laboratory
VLA: very late antigen
vmp: variable major protein
VPv: viral protein
VPg: genome-linked viral protein
VSA: variant surface antigen
VSV: vesicular stomatitis virus
VTEC: verocytotoxin-producing *E. coli*
VZV: varicella zoster virus

WB: Western blot
WHO: World Health Organization

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I
Basic Principles of Medical Microbiologie and Immunology

Macrophage hunting bacteria

1 General Aspects of Medical Microbiology

F. H. Kayser

Infectious diseases are caused by subcellular infectious entities (prions, viruses), prokaryotic bacteria, eukaryotic fungi and protozoans, metazoan animals, such as parasitic worms (helminths), and some arthropods. Definitive proof that one of these factors is the cause of a given infection is demonstrated by fulfillment of the three Henle-Koch postulates. For technical reasons, a number of infections cannot fulfill the postulates in their strictest sense as formulated by R. Koch, in these cases a modified form of the postulates is applied.

The History of Infectious Diseases

The Past

Infectious diseases have been known for thousands of years, although accurate information on their etiology has only been available for about a century. In the medical teachings of Hippocrates, the cause of infections occurring frequently in a certain locality or during a certain period (epidemics) was sought in "changes" in the air according to the theory of miasmas. This concept, still reflected in terms such as "swamp fever" or "malaria," was the predominant academic opinion until the end of the 19th century, despite the fact that the Dutch cloth merchant A. van Leeuwenhoek had seen and described bacteria as early as the 17th century, using a microscope he built himself with a single convex lens and a very short focal length. At the time, general acceptance of the notion of "spontaneous generation"—creation of life from dead organic material—stood in the way of implicating the bacteria found in the corpses of infection victims as the cause of the deadly diseases. It was not until Pasteur disproved the doctrine of spontaneous generation in the second half of the 19th century that a new way of thinking became possible. By the end of that century, microorganisms had been identified as the causal agents in many familiar diseases by applying the Henle-Koch postulates formulated by R. Koch in 1890.

The Henle–Koch Postulates

The postulates can be freely formulated as follows:

- The microorganism must be found under conditions corresponding to the pathological changes and clinical course of the disease in question.
- It must be possible to cause an identical (human) or similar (animal) disease with pure cultures of the pathogen.
- The pathogen must not occur within the framework of other diseases as an "accidental parasite."

These postulates are still used today to confirm the cause of an infectious disease. However, the fact that these conditions are not met does not necessarily exclude a contribution to disease etiology by a pathogen found in context. In particular, many infections caused by subcellular entities do not fulfill the postulates in their classic form.

The Present

The frequency and deadliness of infectious diseases throughout thousands of years of human history have kept them at the focus of medical science. The development of effective preventive and therapeutic measures in recent decades has diminished, and sometimes eliminated entirely, the grim epidemics of smallpox, plague, spotted fever, diphtheria, and other such contagions. Today we have specific drug treatments for many infectious diseases. As a result of these developments, the attention of medical researchers was diverted to other fields: it seemed we had tamed the infectious diseases. Recent years have proved this assumption false. Previously unknown pathogens causing new diseases are being found and familiar organisms have demonstrated an ability to evolve new forms and reassert themselves. The origins of this reversal are many and complex: human behavior has changed, particularly in terms of mobility and nutrition. Further contributory factors were the introduction of invasive and aggressive medical therapies, neglect of established methods of infection control and, of course, the ability of pathogens to make full use of their specific genetic variability to adapt to changing conditions. The upshot is that physicians in particular, as well as other medical professionals and staff, urgently require a basic knowledge of the pathogens involved and the genesis of infectious diseases if they are to respond effectively to this dynamism in the field of infectiology. The aim of this textbook is to impart these essentials to them.

Table 1.**1** provides an overview of the causes of human infectious diseases.

Table 1.1 Human Pathogens

Subcellular biological entities	Prokaryotic microorganisms	Eukaryotic microorganisms	Animals
Prions (infection proteins)	Chlamydiae (0.3–1 µm)	Fungi (yeasts 5–10 µm, size of mold fungi indeterminable)	Helminths (parasitic worms)
Viruses (20–200 nm)	Rickettsiae (0.3–1 µm)	Protozoa (1–150 µm)	Arthropods
	Mycoplasmas		
	Classic bacteria (1–5 µm)		

Pathogens

Subcellular Infectious Entities

Prions (proteinaceous infectious particles). The evidence indicates that prions are protein molecules that cause degenerative central nervous system (CNS) diseases such as Creutzfeldt-Jakob disease, kuru, scrapie in sheep, and bovine spongiform encephalopathy (BSE) (general term: transmissible spongiform encephalopathies [TSE]).

Viruses. Ultramicroscopic, obligate intracellular parasites that:

- contain only one type of nucleic acid, either DNA or RNA,
- possess no enzymatic energy-producing system and no protein-synthesizing apparatus, and
- force infected host cells to synthesize virus particles.

Prokaryotic and Eukaryotic Microorganisms

According to a proposal by Woese that has been gaining general acceptance in recent years, the world of living things is classified in the three domains *bacteria*, *archaea*, and *eucarya*. In this system, each domain is subdivided into

kingdoms. Pathogenic microorganisms are found in the domains bacteria and eucarya.

Bacteria, Archaea, Eucarya

Bacteria. This domain includes the kingdom of the heterotrophic eubacteria and includes all human pathogen bacteria. The other kingdoms, for instance that of the photosynthetic cyanobacteria, are not pathogenic. It is estimated that bacterial species on Earth number in the hundreds of thousands, of which only about 5500 have been discovered and described in detail.

Archaea. This domain includes forms that live under extreme environmental conditions, including thermophilic, hyperthermophilic, halophilic, and methanogenic microorganisms. The earlier term for the archaea was archaebacteria (ancient bacteria), and they are indeed a kind of living fossil. Thermophilic archaea thrive mainly in warm, moist biotopes such as the hot springs at the top of geothermal vents. The hyperthermophilic archaea, a more recent discovery, live near deep-sea volcanic plumes at temperatures exceeding 100 °C.

Eucarya. This domain includes all life forms with cells possessing a genuine nucleus. The plant and animal kingdoms (animales and plantales) are all eukaryotic life forms. Pathogenic eukaryotic microorganisms include fungal and protozoan species.

Table 1.**2** lists the main differences between prokaryotic (bacteria and archaea) and eukaryotic pathogens.

Bacteria

- **Classic bacteria.** These organisms reproduce asexually by binary transverse fission. They do not possess the nucleus typical of eucarya. The cell walls of these organisms are rigid (with some exceptions, e.g., the mycoplasma).
- **Chlamydiae.** These organisms are obligate intracellular parasites that are able to reproduce in certain human cells only and are found in two stages: the infectious, nonreproductive particles called elementary bodies (0.3 μm) and the noninfectious, intracytoplasmic, reproductive forms known as initial (or reticulate) bodies (1 μm).
- **Rickettsiae.** These organisms are obligate intracellular parasites, rod-shaped to coccoid, that reproduce by binary transverse fission. The diameter of the individual cell is from 0.3–1 μm.

Table 1.2 Characteristics of Prokaryotic (Eubacteria) and Eukaryotic (Fungi, Protozoans) Microorganisms

Characteristic	Prokaryotes (bacteria)	Eukaryotes (fungi, protozoans)
Nuclear structure	Circular DNA molecule not covered with proteins	Complex of DNA and basic proteins
Localization of nuclear structure	Dense tangle of DNA in cytoplasm; no nuclear membrane; nucleoid or nuclear equivalent	In nucleus surrounded by nuclear membrane
DNA	Nucleoid and plasmids	In nucleus and in mitochondria
Cytoplasm	No mitochondria and no endoplasmic reticulum, 70S ribosomes	Mitochondria and endoplasmic reticulum, 80S ribosomes
Cell wall	Usually rigid wall with murein layer; exception: mycoplasmas	Present only in fungi: glucans, mannans, chitin, chitosan, cellulose
Reproduction	Asexual, by binary transverse fission	In most cases sexual, possibly asexual

Mycoplasmas. Mycoplasmas are bacteria without rigid cell walls. They are found in a wide variety of forms, the most common being the coccoid cell (0.3–0.8 μm). Threadlike forms also occur in various lengths.

Fungi and Protozoa

Fungi. Fungi (*Mycophyta*) are nonmotile eukaryotes with rigid cell walls and a classic cell nucleus. They contain no photosynthetic pigments and are carbon heterotrophic, that is, they utilize various organic nutrient substrates (in contrast to carbon autotrophic plants). Of more than 50 000 fungal species, only about 300 are known to be human pathogens. Most fungal infections occur as a result of weakened host immune defenses.

Protozoa. Protozoa are microorganisms in various sizes and forms that may be free-living or parasitic. They possess a nucleus containing chromosomes and organelles such as mitochondria (lacking in some cases), an en-

doplasmic reticulum, pseudopods, flagella, cilia, kinetoplasts, etc. Many parasitic protozoa are transmitted by arthropods, whereby multiplication and transformation into the infectious stage take place in the vector.

Animals

Helminths. Parasitic worms belong to the animal kingdom. These are metazoan organisms with highly differentiated structures. Medically significant groups include the trematodes (flukes or flatworms), cestodes (tapeworms), and nematodes (roundworms).

Arthropods. These animals are characterized by an external chitin skeleton, segmented bodies, jointed legs, special mouthparts, and other specific features. Their role as direct causative agents of diseases is a minor one (mites, for instance, cause scabies) as compared to their role as vectors transmitting viruses, bacteria, protozoa, and helminths.

Host–Pathogen Interactions

The factors determining the genesis, clinical picture and outcome of an infection include complex relationships between the host and invading organisms that differ widely depending on the pathogen involved. Despite this variability, a number of general principles apply to the interactions between the invading pathogen with its aggression factors and the host with its defenses. Since the pathogenesis of bacterial infectious diseases has been researched very thoroughly, the following summary is based on the host–invader interactions seen in this type of infection.

The determinants of bacterial pathogenicity and virulence can be outlined as follows:

- Adhesion to host cells (adhesins).
- Breaching of host anatomical barriers (invasins) and colonization of tissues (aggressins).
- Strategies to overcome nonspecific defenses, especially antiphagocytic mechanisms (impedins).
- Strategies to overcome specific immunity, the most important of which is production of IgA proteases (impedins), molecular mimicry, and immunogen variability.

- Damage to host tissues due to direct bacterial cytotoxicity, exotoxins, and exoenzymes (aggressins).
- Damage due to inflammatory reactions in the macroorganism: activation of complement and phagocytosis; induction of cytokine production (modulins).

The above bacterial pathogenicity factors are confronted by the following host defense mechanisms:

- Nonspecific defenses including mechanical, humoral, and cellular systems. Phagocytosis is the most important process in this context.
- Specific immune responses based on antibodies and specific reactions of T lymphocytes (see chapter on immunology).

The response of these defenses to infection thus involves the correlation of a number of different mechanisms. Defective defenses make it easier for an infection to take hold. Primary, innate defects are rare, whereas acquired, secondary immune defects occur frequently, paving the way for infections by microorganisms known as "facultative pathogens" (opportunists).

Basic Terminology of Infectiology

Tables 1.**3** and 1.**4** list the most important infectiological terms together with brief explanations.

The terms **pathogenicity** and **virulence** are not clearly defined in their relevance to microorganisms. They are sometimes even used synonymously. It has been proposed that pathogenicity be used to characterize a particular species and that virulence be used to describe the sum of the disease-causing properties of a population (strain) of a pathogenic species (Fig. 1.**1**)

Pathogenicity and virulence in the microorganism correspond to **susceptibility** in a host species and **disposition** in a specific host organism, whereby an individual may be anywhere from highly disposed to resistant.

Determinants of Bacterial Pathogenicity and Virulence

Relatively little is known about the factors determining the pathogenicity and virulence of microorganisms, and most of what we do know concerns the disease-causing mechanisms of bacteria.

Table 1.**3** Basic Infectiological Terminology I (Pathogen)

Term	Explanation
Saprophytes	These microorganisms are nonpathogenic; their natural habitat is dead organic matter
Parasites	Unicellular or metazoan organism living in or on an organism of another species (host) on the expense of the host
– Commensals	Normal inhabitants of skin and mucosa; the normal flora is thus the total commensal population (see Table 1.**7**, p. 25)
– Pathogenic microorganisms	Classic disease-causing pathogens
– Opportunists or facultatively pathogenic microorganisms	Can cause disease in immunocompromised individuals given an "opportune" situation; these are frequently germs of the normal flora or occasionally from the surrounding environment, animals, or other germ carriers
Pathogenicity	Capacity of a pathogen species to cause disease
Virulence	Sum of the disease-causing properties of a strain of a pathogenic species
Incubation period	Time between infection and manifestation of disease symptoms; this specific disease characteristic can be measured in hours, days, weeks, or even years
Prepatency	A parasitological term: time between infection and first appearance of products of sexual reproduction of the pathogen (e.g., worm eggs in stool of a host with helminthosis)
Infection spectrum	The totality of host species "susceptible" to infection by a given pathogen
Minimum infective dose	Smallest number of pathogens sufficient to cause an infection
Mode of infection	Method or pathway used by pathogen to invade host

Tab 1.4 Basic Infectiological Terminology II (Host)

Term	Explanation
Contamination	Microbiological presence of microorganisms on objects, in the environment, or in samples for analysis
Colonization	Presence of microorganisms on skin or mucosa; no penetration into tissues; typical of normal flora; pathogenic microorganisms occasionally also show colonization behavior
Infection	Invasion of a host organism by microorganisms, proliferation of the invading organisms, and host reaction
Inapparent (or subclinical) infection	Infection without outbreak of clinical symptoms
Infectious disease (or clinical infection)	Infection with outbreak of clinical symptoms
Probability of manifestation	Frequency of clinical manifestation of an infection in disposed individuals (%)
Endogenous infection	Infection arising from the colonizing flora
Exogenous infection	Infection arising from invasion of host by microorganisms from sources external to it
Nosocomial infection	Infection acquired during hospitalization (urinary tract infections, infections of the respiratory organs, wound infection, sepsis)
Local infection	Infection that remains restricted to the portal of entry and surrounding area
Generalized infection	Lymphogenous and/or hematogenous spread of invading pathogen starting from the portal of entry; infection of organs to which pathogen shows a specific affinity (organotropism); three stages: incubation, generalization, organ manifestation
Sepsis	Systemic disease caused by microorganisms and/or their toxic products; there is often a localized focus of infection from which pathogens or toxic products enter the bloodstream continuously or in intermittent phases
Transitory bacteremia/viremia/parasitemia	Brief presence of microorganisms in the bloodstream
Superinfection	Occurrence of a second infection in the course of a first infection
Relapses	Series of infections by the same pathogen
Reinfection	Series of infections by different pathogens

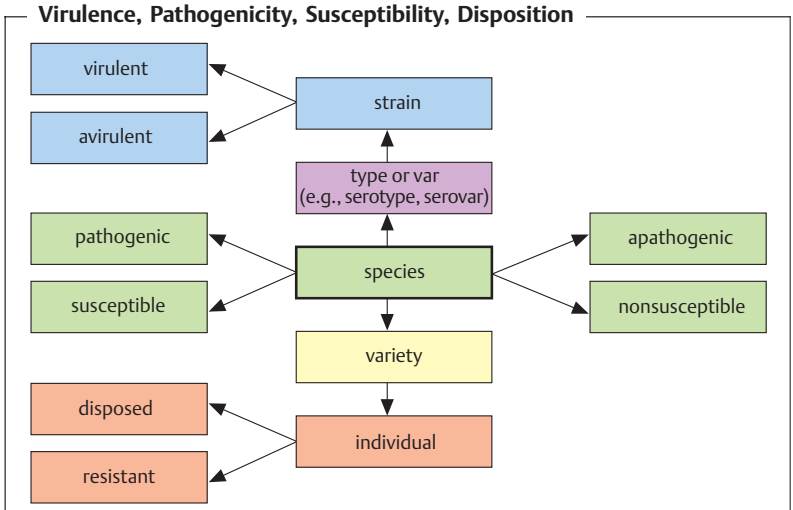


Fig. 1.1 Pathogenicity refers to pathogens, susceptibility to host species. The term virulence refers to individual strains of a pathogen species. The terms disposition and resistance are used to characterize the status of individuals of a susceptible host species.

There are five groups of potential bacterial contributors to the pathogenesis of infectious diseases:

1. **Adhesins.** They facilitate adhesion to specific target cells.
2. **Invasins.** They are responsible for active invasion of the cells of the macroorganism.
3. **Impedins.** These components disable host immune defenses in some cases.
4. **Aggressins.** These substances include toxins and tissue-damaging enzymes.
5. **Modulins.** Substances that induce excess cytokine production (i.e., lipopolysaccharides of Gram-negative bacteria, superantigens, murein fragments).

Adhesion

When pathogenic bacteria come into contact with intact human surface tissues (e.g., mucosa), they contrive to adhere to receptors on the surface of the target cells by means of various surface structures of their own (attachment

pili, attachment fimbriae, adhesion proteins in the outer membrane of Gram-negative bacteria, cell wall-associated proteins in Gram-positive bacteria). This is a specific process, meaning that the adhesion structure (or ligand) and the receptor must fit together like a key in a keyhole.

Invasion and Spread

Invasion. Bacteria may invade a host passively through microtraumata or macrotraumata in the skin or mucosa. On the other hand, bacteria that invade through intact mucosa first adhere to this anatomical barrier, then actively breach it. Different bacterial species deploy a variety of mechanisms to reach this end:

- Production of tissue-damaging exoenzymes that destroy anatomical barriers.
- Parasite-directed endocytosis, initiated by invasins on the surface of the bacterial cells, causes the cytoskeleton of the epithelial cell to form pseudopods that bring about endocytosis.
- Phagocytosis of enteropathogenic bacteria by M cells in the intestinal mucosa (cells that can ingest substances from the intestinal lumen by way of phagocytosis).

Spread.

- Local tissue spread beginning at the portal of entry, helped along by tissue-damaging exoenzymes (hyaluronidase, collagenase, elastase, and other proteases).
- Cell-to-cell spread. Bacteria translocated into the intracellular space by endocytosis cause actin to condense into filaments, which then array at one end of the bacterium and push up against the inner side of the cell membrane. This is followed by fusion with the membrane of the neighboring tissue cell, whereupon the bacterium enters the new cell (typical of *Listeria* and *Shigella*).
- Translocation of macrophage-resistant bacteria with macrophages into intestinal lymphoid tissue following their ingestion by M cells.
- Lymphogenous or hematogenous generalization. The bacteria then invade organs for which they possess a specific tropism.

Strategies against Nonspecific Immunity

Establishment of a bacterial infection in a host presupposes the capacity of the invaders to overcome the host's nonspecific immune defenses. The most important mechanisms used by pathogenic bacteria are:

■ **Antiphagocytosis** (see also Fig. 1.**6**, p. 23)**.**

– *Capsule*. Renders phagocytosis more difficult. Capsule components may block alternative activation of complement so that C3b is lacking (ligand for C3b receptor of phagocytes) on the surface of encapsulated bacteria. Microorganisms that use this strategy include *Streptococcus pneumoniae* and *Haemophilus influenzae*.

– *Phagocyte toxins*. Examples: leukocidin from staphylococci, streptolysin from streptococci.

– Macrophages may be disabled by the type III secretion system (see p. 17) of certain Gram-negative bacteria (for example salmonellae, shigellae, yersiniae, and coli bacteria). This system is used to inject toxic proteins into the macrophages.

– *Inhibition of phagosome-lysosome fusion*. Examples: tuberculosis bacteria, gonococci, *Chlamydia psittaci*.

– *Inhibition of the phagocytic "oxidative burst."* No formation of reactive O_2 radicals in phagocytes. Examples: *Legionella pneumophilia*, *Salmonella typhi*.

■ **Serum resistance.** Resistance of Gram-negative bacteria to complement. A lipopolysaccharide in the outer membrane is modified in such a way that it cannot initiate alternative activation of the complement system. As a result, the membrane attack complex (C5b6789), which would otherwise lyse holes in the outer membrane, is no longer produced (see p. 86ff.).

■ **Siderophores.** Siderophores (e.g., enterochelin, aerobactin) are low-molecular-weight iron-binding molecules that transport Fe^{3+} actively into the intracellular space. They complex with iron, thereby stealing this element from proteins containing iron (transferrin, lactoferrin). The intricate iron transport system is localized in the cytoplasmic membrane, and in Gram-negative bacteria in the outer membrane as well. To thrive, bacteria require 10^{-5} mol/l free iron ions. The free availability of only about 10^{-20} mol/l iron in human body fluids thus presents a challenge to them.

Strategies against Specific Immunity

■ **Immunotolerance.**

– *Prenatal infection*. At this stage of development, the immune system is unable to recognize bacterial immunogens as foreign.

– *Molecular mimicry*. Molecular mimicry refers to the presence of molecules on the surface of bacteria that are not recognized as foreign by the immune system. Examples of this strategy are the hyaluronic acid capsule of *Streptococcus pyogenes* or the neuraminic acid capsule of *Escherichia coli* K1 and serotype B *Neisseria meningitidis*.

Mechanism of Molecular Variation of Pilin in Gonococci

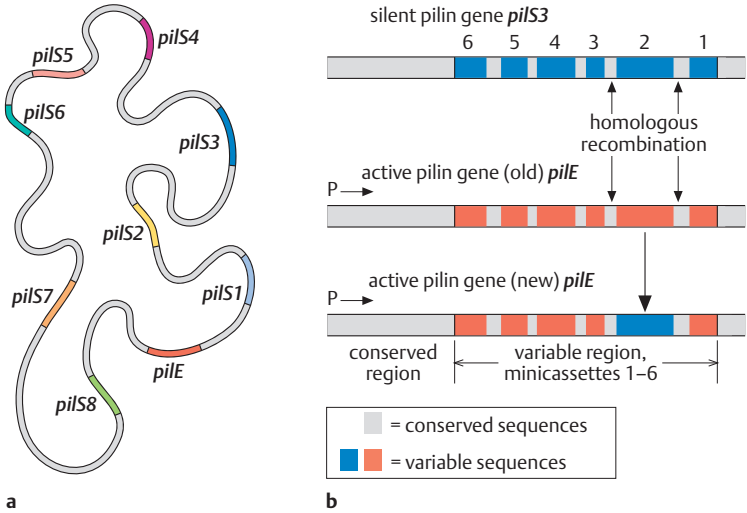


Fig. 1.2 The structural element of the attachment pili of gonococci is the polypeptide monomer pilin. Mucosal immunity to gonococci depends on antibodies in the secretions of the urogenital mucosa that attach to the immunodominant segment of the pilin, thus blocking adhesion of gonococci to the target cells.
a Model of the gonococcal genome. The primary structure of the pilin is determined by the expressed gene *pilE*. The gonococcal genome has many other *pil* genes besides the *pilE* without promoters, i.e., "silent" genes that are not transcribed (*pilS1*, *pilS2*, *pilS3*, etc.).
b *pil* genes have both a conserved and a variable region. The variable region of all *pil* genes has a mosaic structure, i.e., it consists of minicassettes. Minicassette 2 codes for the most important immunodominant segment of the pilin. Intracellular homologous recombination of conserved regions of silent *pil* genes and corresponding sequences of the expressed gene results in *pilE* genes with changed cassettes. These code for a pilin with a changed immunodominant segment. Therefore, antibodies to the "old" pilin can no longer bind to the "new" pilin.

Antigen variation. Some bacteria are characterized by a pronounced variability of their immunogens (= immune antigens) due to the genetic variability of the structural genes coding the antigen proteins. This results in production of a series of antigen variants in the course of an infection that no longer "match" with the antibodies to the "old" antigen. Examples: gonococci can modify the primary structure of the pilin of their attachment

pili at a high rate (Fig. 1.**2**). The borreliae that cause relapsing fevers have the capacity to change the structure of one of the adhesion proteins in their outer membrane (vmp = variable major protein), resulting in the typical "recurrences" of fever. Similarly, meningococci can change the chemistry of their capsule polysaccharides ("capsule switching").

IgA proteases. Mucosal secretions contain the secretory antibodies of the $sIgA_1$ class responsible for the specific local immunity of the mucosa. Classic mucosal parasites such as gonococci, meningococci and *Haemophilus influenzae* produce proteases that destroy this immunoglobulin.

Clinical Disease

The clinical symptoms of a bacterial infection arise from the effects of damaging noxae produced by the bacteria as well as from excessive host immune responses, both nonspecific and specific. Immune reactions can thus potentially damage the host's health as well as protect it (see Immunology, p. 103ff.).

Cytopathic effect. Obligate intracellular parasites (rickettsiae, chlamydiae) may kill the invaded host cells when they reproduce.

Exotoxins. Pathogenic bacteria can produce a variety of toxins that are either the only pathogenic factor (e.g., in diphtheria, cholera, and tetanus) or at least a major factor in the unfolding of the disease. One aspect the classification and nomenclature of these toxins must reflect is the type of cell affected: **cytotoxins** produce toxic effects in many different host cells; **neurotoxins** affect the neurons; **enterotoxins** affect enterocytes. The structures and mechanisms of action of the toxins are also considered in their classification (Table 1.**5**):

- *AB toxins*. They consist of a binding subunit "B" responsible for binding to specific surface receptors on target host cells, and a catalytic subunit "A" representing the active agent. Only cells presenting the "B" receptors are damaged by these toxins.
- *Membrane toxins*. These toxins disrupt biological membranes, either by attaching to them and assembling to form pores, or in the form of phospholipases that destroy membrane structure enzymatically.
- *Superantigens* (see p. 72). These antigens stimulate T lymphocytes and macrophages to produce excessive amounts of harmful cytokines.

Hydrolytic exoenzymes. Proteases (e.g., collagenase, elastase, nonspecific proteases), hyaluronidase, neuraminidase (synonymous with sialidase), lecithinase and DNases contribute at varying levels to the pathogenesis of an infection.

Table 1.**5** Examples of Bacterial Toxins; Mechanisms of Action and Contribution to Clinical Picture

Toxin	Cell specificity	Molecular effect	Contribution to clinical picture
AB toxins			
Diphtheria toxin (*Corynebacterium diphtheriae*)	Many different cell types	ADP-ribosyl transferase. Inactivation of ribosomal elongation factor eEF2 resulting from ADP-ribosylation during protein synthesis; leads to cell death.	Death of mucosal cells. Damage to heart musculature, kidneys, adrenal glands, liver, motor nerves of the head.
Cholera toxin (*Vibrio cholerae*)	Enterocytes	ADP-ribosyl transferase. ADP-ribosylation of regulatory protein G_s of adenylate cyclase, resulting in permanent activation of this enzyme and increased levels of cAMP (second messenger) (see Fig. 4.**20**, p. 298). Result: increased secretion of electrolytes.	Massive watery diarrhea; severe loss of electrolytes and water.
Tetanus toxin (*Clostridium tetani*)	Neurons (synapses)	Metalloprotease. Proteolytic cleavage of protein components from the neuroexocytosis apparatus in the synapses of the anterior horn that normally transmit inhibiting impulses to the motor nerve terminal.	Increased muscle tone; cramps in striated musculature.
Membrane toxins			
Alpha toxin (*Clostridium perfringens*)	Many different cell types	Phospholipase.	Cytolysis, resulting tissue damage.
Lysteriolysin (*Listeria monocytogenes*)	Many different cell types	Pore formation in membranes.	Destruction of phagosome membrane; intracellular release of phagocytosed listeriae.

Table 1.**5** *Continued: Examples of Bacterial Toxins*

Toxin	Cell specificity	Molecular effect	Contribution to clinical picture
Superantigen toxins			
Toxic shock syndrome toxin-1 (TSST-1) (*Staphylococcus aureus*)	T lymphocytes; macrophages	Stimulation of secretion of cytokines in T cells and macrophages.	Fever; exanthem; hypotension.

■ **Secretion of virulence proteins.** Proteins are synthesized at the ribosomes in the bacterial cytoplasm. They must then be secreted through the cytoplasmic membrane, and in Gram-negative bacteria through the outer membrane as well. The secretion process is implemented by complex protein secretion systems (I-IV) with differing compositions and functional pathways. The type III (virulence-related) secretion system in certain Gram-negative bacteria (*Salmonella*, *Shigella*, *Yersinia*, *Bordetella*, *Escherichia coli*, *Chlamydia*) is particularly important in this connection (see Fig. 1.**3**).

Needle Complex of Type III Secretion System

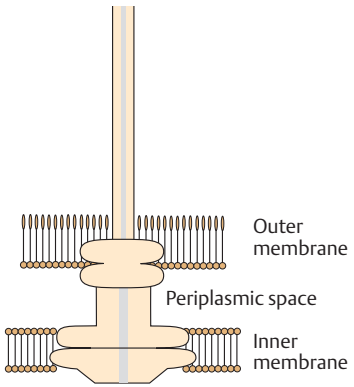


Fig. 1.**3** When certain Gram-negative rod bacteria make contact with eukaryotic target cells, a sensor molecule interacts with a receptor on the target cells. This interaction results in the opening of a secretion channel of the so-called "needle complex" (extending through both the cytoplasmic membrane and outer membrane) and in formation of a pore in the membrane of the target cell. Through the pore and channel, cytotoxic molecules are then translocated into the cytosol of the target cell where they, for example, inhibit phagocytosis and cytokine production (in macrophages), destroy the cytoskeleton of the target cell, and generally work to induce apoptosis.

Cell wall. The endotoxin of Gram-negative bacteria (lipopolysaccharide) plays an important role in the manifestation of clinical symptoms. On the one hand, it can activate complement by the alternative pathway and, by releasing the chemotactic components C3a and C5a, initiate an inflammatory reaction at the infection site. On the other hand, it also stimulates macrophages to produce endogenous pyrogens (interleukin 1, tumor necrosis factor), thus inducing fever centrally. Production of these and other cytokines is increased, resulting in hypotension, intravasal coagulation, thrombocyte aggregation and stimulation of granulopoiesis. Increased production of cytokines by macrophages is also induced by soluble murein fragments and, in the case of Gram-positive bacteria, by teichoic acids.

Inflammation. Inflammation results from the combined effects of the nonspecific and specific immune responses of the host organism. Activation of complement by way of both the classic and alternative pathways induces phagocyte migration to the infection site. Purulent tissue necrosis follows. The development of typical granulomas and caseous necrosis in the course of tuberculosis are the results of excessive reaction by the cellular immune system to the immunogens of tuberculosis bacteria. Textbooks of general pathology should be consulted for detailed descriptions of these inflammatory processes.

Regulation of Bacterial Virulence

Many pathogenic bacteria are capable of living either outside or inside a host and of attacking a variety of host species. Proliferation in these differing environments demands an efficient regulation of virulence, the aim being to have virulence factors available as required. Four different regulatory mechanisms have been described:

DNA changes. The nucleotide sequences of virulence determinants are changed. Examples of this include pilin gene variability involving intracellular recombination as described above in gonococci and inverting a leader sequence to switch genes on and off in the phase variations of H antigens in salmonellae (see p. 284).

Transcriptional regulation. The principle of transcriptional control of virulence determinants is essentially the same as that applying to the regulation of metabolic genes, namely repression and activation (see p. 169f.):

– *Simple regulation*. Regulation of the diphtheria toxin gene has been thoroughly researched. A specific concentration of iron in the cytoplasm activates the diphtheria toxin regulator (DtxR). The resulting active repressor prevents transcription of the toxin gene by binding to the promoter

region. Other virulence genes can also be activated by regulators using this mechanism.

– *Complex regulation, virulence regulon*. In many cases, several virulence genes are switched on and off by the same regulator protein. The virulence determinants involved are either components of the same operon or are located at different genome sites. Several vir (virulence) genes with promoter regions that respond to the same regulator protein form a so-called vir regulon. Regulation of the virulence regulon of *Bordetella pertussis* by means of gene activation is a case in point that has been studied in great detail. This particular regulon comprises over 20 virulence determinants, all controlled by the same vir regulator protein (or BvgA coding region) (Fig. 1.**4**).

■ **Posttranscriptional regulation.** This term refers to regulation by mRNA or a posttranslational protein modification.

■ **Quorum sensing.** This term refers to determination of gene expression by bacterial cell density (Fig. 1.**5**). Quorum sensing is observed in both

Regulation of Bacterial Virulence: Two-Component Regulator System

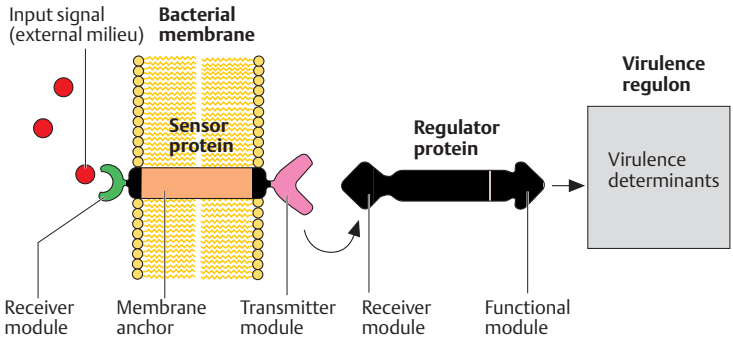


Fig. 1.**4** A sensor protein integrated in the cytoplasmic membrane receives signals from a receiver module extending into the external milieu, activating the transmitter module. These signals from the external milieu can carry a wide variety of information: pH, temperature, osmolarity, Ca^{2+}, CO_2, stationary-phase growth, hunger stress, etc. The transmitter module effects a change in the receiver module of the regulator protein, switching the functional module of the regulator to active status, in which it can then repress or activate the various virulence determinants of a virulence regulon by binding to the different promoter regions. Phosphorylation is commonly used to activate the corresponding sensor and regulator modules.

Quorum-Sensing Communication in Bacteria (Cell-to-Cell Signals)

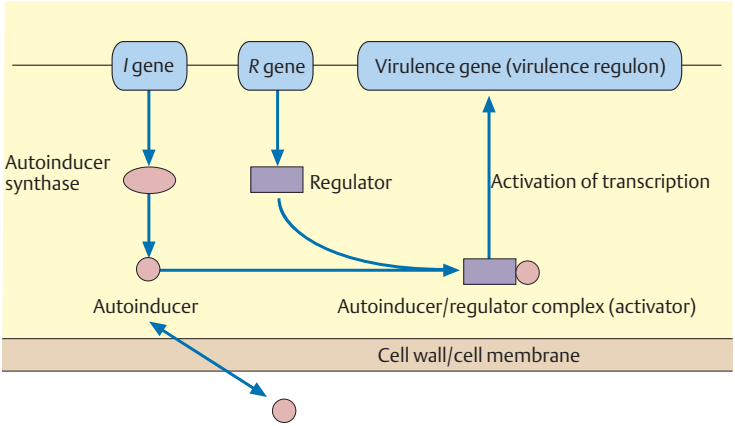


Fig. 1.5 Cell-to-cell signaling is made possible by activation of two genes. The *I* gene codes for the synthase responsible for synthesis of the autoinducer. The autoinducer (often an *N*-acyl homoserine lactone) can diffuse freely through the cell membrane. The *R* gene codes for a transcriptional regulator protein that combines with the autoinducer to become an activator for transcription of various virulence genes.

Gram-positive and Gram-negative bacteria. It denotes a mode of communication between bacterial cells that enables a bacterial population to react analogously to a multicellular organism.

Accumulation of a given density of a low-molecular-weight pheromone (autoinducer) enables a bacterial population to sense when the critical cell density (quorum) has been reached that will enable it to invade the host successfully, at which point transcription of virulence determinants is initiated.

The Genetics of Bacterial Pathogenicity

The virulence genes of pathogenic bacteria are frequently components of mobile genetic elements such as plasmids, bacteriophage genomes, or conjugative transposons (see p. 170ff.). This makes lateral transfer of these genes between bacterial cells possible. Regions showing a high frequency of virulence genes in a bacterial chromosome are called pathogenicity islands (PI).

PIs are found in both Gram-positive and Gram-negative bacteria. These are DNA regions up to 200 kb that often bear several different vir genes and have specific sequences located at their ends (e.g., IS elements) that facilitate lateral translocation of the islands between bacterial cells. The role played by lateral transfer of these islands in the evolutionary process is further underlined by the fact that the GC contents in PIs often differ from those in chromosomal DNA.

Defenses against Infection

A macroorganism manifests defensive reactions against invasion by microorganisms in two forms: as **specific, acquired immunity** and as **nonspecific, innate resistance** (see also Chapter 2, Basic Principles of Immunology, p. 43).

Nonspecific Defense Mechanisms

Table 1.**6** lists the most important mechanisms.

- **Primary defenses.** The main factors in the first line of defense against infection are mechanical, accompanied by some humoral and cellular factors. These defenses represent an attempt on the part of the host organism to prevent microorganisms from colonizing its skin and mucosa and thus stave off a generalized invasion.

- **Secondary defenses.** The second line of defense consists of humoral and cellular factors in the blood and tissues, the most important of which are the professional phagocytes.

- **Phagocytosis.** "Professional" phagocytosis is realized by polymorphonuclear, neutrophilic, eosinophilic granulocytes—also known as microphages—and by mononuclear phagocytes (macrophages). The latter also play an important role in antigen presentation (see p. 62). The total microphage cell count in an adult is approximately 2.5×10^{12}. Only 5% of these cells are located in the blood. They are characterized by a half-life of only a few hours. Microphages contain both primary granules, which are lysosomes containing lysosomal enzymes and cationic peptides, and secondary granules. Both microphages and macrophages are capable of ameboid motility and chemotactic migration, i.e., directed movement along a concentration gradient toward a source of chemotactic substances, in most cases the complement components C3a and C5a. Other potentially chemotactic substances include secretory products of lymphocytes, products of infected and damaged cells or the *N*-formyl peptides (fMet-Phe and fMet-Leu-Phe).

Table 1.**6** The Most Important Mechanisms in Nonspecific Defenses Against Infection

a	**Mechanical factors**
	Anatomical structure of skin and mucosa
	Mucus secretion and mucus flow from mucosa
	Mucociliary movement of the ciliated epithelium in the lower respiratory tract
	Digestive tract peristalsis
	Urine flow in the urogenital tract
b	**Humoral factors**
	Microbicidal effect of the dermal acidic mantle, lactic acid from sweat glands, hydrochloric acid in the stomach, and the unsaturated fatty acids secreted by the sebaceous glands
	Lysozyme in saliva and tear fluid: splitting of bacterial murein
	Complement (alternative activation pathway)
	Serum proteins known as acute phase reactants, for example C-reactive protein, haptoglobin, serum amyloid A, fibrinogen, and transferrin (iron-binding protein)
	Fibronectin (a nonspecific opsonin); antiviral interferon
	Mannose-binding protein: binds to mannose on the outer bacterial surface, thus altering the configuration and triggering alternative activation of complement
c	**Cellular factors**
	Normal flora of skin and mucosa
	Natural killer cells (large, granulated lymphocytes; null cells)
	Professional phagocytes: microphages (neutrophilic and eosinophilic granulocytes); mononuclear phagocytes (macrophages, monocytes, etc.)

Phagocytes are capable of ingestion of both particulate matter (phagocytosis) and solute matter (pinocytosis). Receptors on the phagocyte membrane initiate contact (Fig. 1.**6**). Particles adhering to the membrane are engulfed, ingested and deposited in a membrane-bound vacuole, the so-called phagosome, which then fuses with lysosomes to form the phagolysosome. The bacteria are killed by a combination of lysosomal factors:

– *Mechanisms that require no oxygen.* Low pH; acid hydrolases, lysozyme; proteases; defensins (small cationic peptides).

Phagocytosis of Bacteria

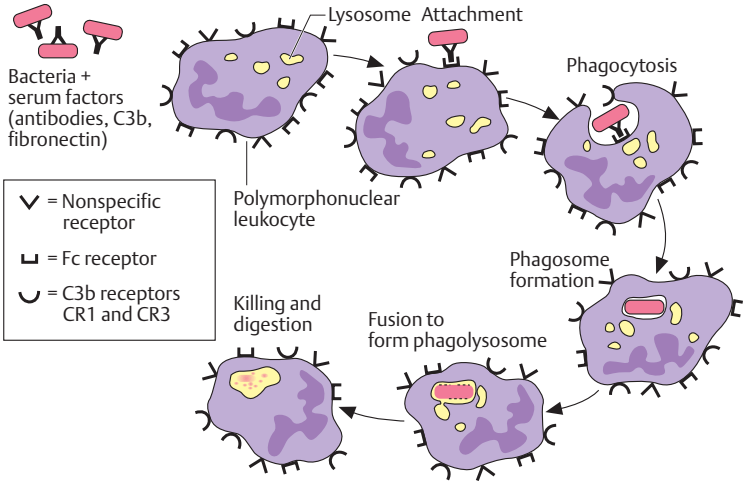


Fig. 1.6 Encapsulated bacteria can only be effectively phagocytosed if IgG-class antibodies (Fc ligand) or the complement component C3b, or both, are located on their surfaces. The Fc and C3b ligands bind to their specific receptors on the phagocyte surface.

– *Mechanisms that require oxygen.* Halogenation of essential bacterial components by the myeloperoxidase-H_2O_2–halide system; production of highly reactive O_2 radicals (oxidative burst) such as superoxide anion (O_2^-), hydroxyl radical (•OH), and singlet oxygen ($^1O_2$).

Specific Defense Mechanisms

Specific immunity, based on antibodies and specifically reactive T lymphocytes, is acquired in a process of immune system stimulation by the corresponding microbial antigens. Humoral immunity is based on antitoxins, opsonins, microbicidal antibodies, neutralizing antibodies, etc. Cellular immunity is based on cytotoxic T lymphocytes (T killer cells) and T helper cells. See Chapter 2 on the principles of specific immunity.

Defects in Immune Defenses

Hosts with defects in their specific and/or nonspecific immune defenses are prone to infection.

- **Primary defects.** Congenital defects in the complement-dependent phagocytosis system are rare, as are B and T lymphocyte defects.

- **Secondary defects.** Such effects are acquired, and they are much more frequent. Examples include malnutrition, very old and very young hosts, metabolic disturbances (diabetes, alcoholism), autoimmune diseases, malignancies (above all lymphomas and leukemias), immune system infections (HIV), severe primary diseases of parenchymatous organs, injury of skin or mucosa, immunosuppressive therapy with corticosteroids, cytostatics and immunosuppressants, and radiotherapy.

One result of progress in modern medicine is that increasing numbers of patients with secondary immune defects are now receiving hospital treatment. Such "problem patients" are frequently infected by opportunistic bacteria that would not present a serious threat to normal immune defenses. Often, the pathogens involved ("problem bacteria") have developed a resistance to numerous antibiotics, resulting in difficult courses of antibiotic treatment in this patient category.

Normal Flora

Commensals (see Table 1.**3**, p. 9) are regularly found in certain human microbiotopes. The normal human microflora is thus the totality of these commensals. Table 1.**7** lists the most important microorganisms of the normal flora with their localizations.

Bacteria are the predominant component of the normal flora. They proliferate in varied profusion on the mucosa and most particularly in the gastrointestinal tract, where over 400 different species have been counted to date. The count of bacteria per gram of intestinal content is 10^1–10^5 in the duodenum, 10^3–10^7 in the small intestine, and 10^{10}–10^{12} in the colon. Over 99% of the normal mucosal flora are obligate anaerobes, dominated by the Gram-neg. anaerobes. Although life is possible without normal flora (e.g., pathogen-free experimental animals), commensals certainly benefit their hosts. One way they do so is when organisms of the normal flora manage to penetrate into the host through microtraumas, resulting in a continuous **stimulation of the immune system**. Commensals also compete for living space with overtly pathogenic species, a function known as **colo-**

Table 1.7 Normal Microbial Flora in Humans

Microorganisms	Microbiotopes				
	Skin	Oral cavity	Intestine	Upper respiratory tract	Genital tract
Staphylococci	+++	+	+	++	++
Enterococci			++		+
α-hemolytic streptococci	+	+++	+	+	+
Anaerobic cocci		+	+		+
Pneumococci		+		+	
Apathogenic neisseriae		+		+	+
Apathogenic corynebacteria	++	+	+	+	+
Aerobic spore-forming bacteria	(+)				
Clostridia			+++		(+)
Actinomycetes		+++			+
Enterobacteriaceae	(+)	(+)	+++	(+)	+
Pseudomonas			+		
Haemophilus		+		++	(+)
Gram-neg. anaerobes		+++	+++	+++	+++
Spirochetes		++		+	(+)
Mycoplasmas		++	+	+	++
Fungi (yeast)	++	+	+	+	+
Entamoeba, *Giardia*, *Trichomonas*		+		+	

+++ = numerous, ++ = frequent, + = moderately frequent, (+) = occasional occurrence

nization resistance. On the other hand, a potentially harmful effect of the normal flora is that they can also cause infections in immunocompromised individuals.

General Epidemiology

■ Within the context of medical microbiology, epidemiology is the study of the occurrence, causality, and prevention of infectious diseases in the populace. Infectious diseases occur either sporadically, in epidemics or pandemics,

or in endemic forms, depending on the time and place of their occurrence. The frequency of their occurrence (morbidity) is described as their *incidence* and *prevalence*. The term *mortality* is used to describe how many deaths are caused by a given disease in a given population. *Lethality* is a measure of how life-threatening an infection is. The most important sources of infection are infected persons and carriers. Pathogens are transmitted from these sources to susceptible persons either directly (person-to-person) or indirectly via inert objects or biological vectors. Control of infectious diseases within a populace must be supported by effective legislation that regulates mandatory reporting where required. Further measures must be implemented to prevent exposure, for example isolation, quarantine, disinfection, sterilization, use of insecticides, and dispositional prophylaxis (active and passive immunization, chemoprophylaxis).

Epidemiological Terminology

Epidemiology investigates the distribution of diseases, their physiological variables and social consequences in human populations, and the factors that influence disease distribution (World Health Organization [WHO] definition). The field covered by this discipline can thus be defined as medical problems involving large collectives. The rule of thumb on infectious diseases is that their characteristic spread depends on the virulence of the pathogen involved, the susceptibility of the threatened host species population, and environmental factors. Table 1.**8** provides brief definitions of the most important epidemiological terms.

Transmission, Sources of Infection

Transmission

Pathogens can be transmitted from a source of infection by direct contact or indirectly. Table 1.**9** lists the different direct and indirect transmission pathways of pathogenic microorganisms.

Person-to-person transmission constitutes a **homologous chain of infection**. The infections involved are called **anthroponoses**. In cases in which the pathogen is transmitted to humans from other vertebrates (and occasionally the other way around) we have a **heterologous chain of infection** and the infections are known as **zoonoses** (WHO definition) (Table 1.**10**).

Table 1.8 Epidemiological Terminology

Term	Definition
Sporadic occurrence	Isolated occurrence of an infectious disease with no apparent connections between localities or times of occurrence
Endemic occurrence	Regular and continuing occurrence of infectious diseases in populations with no time limit
Epidemic occurrence	Significantly increased occurrence of an infectious disease within given localities and time periods
Pandemic occurrence	Significantly increased occurrence of an infectious disease within a given time period but without restriction to given localities
Morbidity	Number of cases of a disease within a given population (e.g., per 1000, 10 000 or 100 000 inhabitants)
Incidence	Number of new cases of a disease within a given time period
Prevalence	Number of cases of a disease at a given point in time (sampling date)
Mortality	Number of deaths due to a disease within a given population
Lethality	Number of deaths due to a disease in relation to total number of cases of the disease
Manifestation index	Number of manifest cases of a disease in relation to number of infections
Incubation period	Time from infection until occurrence of initial disease symptoms
Prepatency	Time between infection and first appearance of products of sexual reproduction of the pathogen (e.g., worm eggs in stool)

Tab. 1.**9** Transmission Pathways of Pathogenic Microorganisms

Direct transmission	Indirect transmission
Fecal-oral (smear infection)	Transmission via food
Aerogenic transmission (droplet infection)	Transmission via drinking water
Genital transmission (during sexual intercourse)	Transmission via contaminated inanimate objects or liquids
Transmission via skin (rare)	Transmission via vectors (arthropods)
Diaplacental transmission	Transmission via other persons (e.g., via the hands of hospital medical staff)
Perinatal transmission (in the course of birth)	

Tab. 1.**10** Examples of Zoonoses Caused by Viruses, Bacteria, Protozoans, Helminths, and Arthropods

Zoonoses	Pathogen	Reservoir hosts	Transmission
Viral zoonoses			
Rabies	*Rhabdoviridae*	Numerous animal species	Bite of diseased animals
Tickborne encephalitis (TBE)	*Flaviviridae*	Wild animals	Ticks
Bacterial zoonoses			
Brucellosis	*Brucella* spp.	Cattle, pig, goat, sheep, (dog)	Contact with tissues or secretions from diseased animals; milk and dairy products
Lyme disease	*Borrelia burgdorferi*	Wild rodents; red deer, roe deer	Ticks
Plague	*Yersinia pestis*	Rodents	Contact with diseased animals; bite of rat flea
Q fever	*Coxiella burnetii*	Sheep, goat, cattle	Dust; possibly milk or dairy products
Enteric salmonellosis	*Salmonella enterica* (enteric serovars)	Pig, cattle, poultry	Meat, milk, eggs

Tab. 1.**10** *Continued: Examples of Zoonoses*

Zoonoses	Pathogen	Reservoir hosts	Transmission
Protozoan zoonoses			
Toxoplasmosis	*Toxoplasma gondii*	Domestic cat, sheep, pigs, other slaughter animals	Postnatal toxoplasmosis: oral; prenatal toxoplasmosis: diaplacental
Cryptosporidiosis	*Cryptosporidium hominis*; *C. parvum*	Cattle (calves), domestic animals	Ingestion of oocysts
Helminthic zoonoses			
Echinococcosis	*Echinococcus granulosus, Echinococcus multilocularis*	Dog, wild canines, fox	Ingestion of eggs
Taeniosis	*Taenia saginata, Taenia solium, Taenia asiatica*	Cattle, buffalo, pigs Pigs, cattle, goat	Ingestion of metacestodes with meat
Zoonoses caused by arthropods			
Pseudo scabies	*Sarcoptes* spp.; mite species from domestic animals	Dog, cat, guinea pig, domestic ruminants, pig	Contact with diseased animals

Other Zoonoses

(For details see the corresponding chapters)

Viral zoonoses	Hantavirus and other bunyavirus infections; infections by alphavirus, flavivirus, and arenavirus.
Bacterial zoonoses	Ehrlichiosis; erysipeloid; campylobacteriosis; cat scratch disease; leptospirosis; anthrax; ornithosis; rat-bite fever; rickettsioses (variety of types); tularemia; gastroenteritis caused by *Vibrio parahaemolyticus*; gastroenteritis caused by *Yersinia enterocolitica*.
Protozoan zoonoses	African trypanosomosis (sleeping sickness); American trypanosomosis (Chagas disease); babesiosis; balantidosis; cryptosporidosis; giardiosis; leishmaniosis; microsporidosis; sarcocystosis; toxoplasmosis. ▶

Continued: **Other Zoonoses**

Helminthic zoonoses	Cercarial dermatitis; clonorchiosis; cysticercosis; dicrocoeliosis; diphyllobothriosis; echinococcosis; fasciolosis; hymenolepiosis; larva migrans interna; opisthorchiosis; paragonimosis; schistosomosis (bilharziosis); taeniosis; toxocariosis; trichinellosis.
Zoonoses caused by arthropods	Flea infestation; larva migrans externa; mite infestation; sand flea infestation.

Sources of Infection

Every infection has a source (Table 1.**11**). The **primary source of infection** is defined as the location at which the pathogen is present and reproduces. **Secondary sources of infection** are inanimate objects, materials, or third persons contributing to transmission of pathogens from the primary source to disposed persons.

Table 1.**11** Primary Sources of Infection

Source of infection	Explanation
Infected person	The most important source; as a rule, pathogens are excreted by the organ system through which the infection entered; there are some exceptions
Carriers during incubation	Excretion during incubation period; typical of many viral diseases
Carriers in convalescence	Excretion after the disease has been overcome; typical of enteric salmonelloses
Chronic carriers	Continued excretion for three or more months (even years) after disease has been overcome; typical of typhoid fever
Asymptomatic carriers	They carry pathogenic germs on skin or mucosa without developing "infection"
Animal carriers	Diseased or healthy animals that excrete pathogenic germs
Environment	Soil, plants, water; primary source of microorganisms with natural habitat in these biotopes

The Fight against Infectious Diseases

Legislation

Confronting and preventing infectious diseases can sometimes involve substantial incursions into the private sphere of those involved as well as economic consequences. For these reasons, such measures must be based on effective disease control legislation. In principle, these laws are similar in most countries, although the details vary.

The centerpiece of every disease prevention system is provision for reporting outbreaks. Basically, reporting is initiated at the periphery (individual patients) and moves toward the center of the system. Urgency level classifications of infections and laboratory findings are decided on by regional health centers, which are in turn required to report some diseases to the WHO to obtain a global picture within the shortest possible time.

Concrete countermeasures in the face of an epidemic take the form of prophylactic measures aimed at interrupting the chain of infection.

Exposure Prophylaxis

Exposure prophylaxis begins with *isolation* of the source of infection, in particular of infected persons, as required for the disease at hand. *Quarantine* refers to a special form of isolation of healthy first-degree contact persons. These are persons who have been in contact with a source of infection. The quarantine period is equivalent to the incubation period of the infectious disease in question (see International Health Regulations, www.who.int/en/).

Further measures of exposure prophylaxis include *disinfection* and *sterilization*, use of insecticides and pesticides, and eradication of animal carriers.

Immunization Prophylaxis

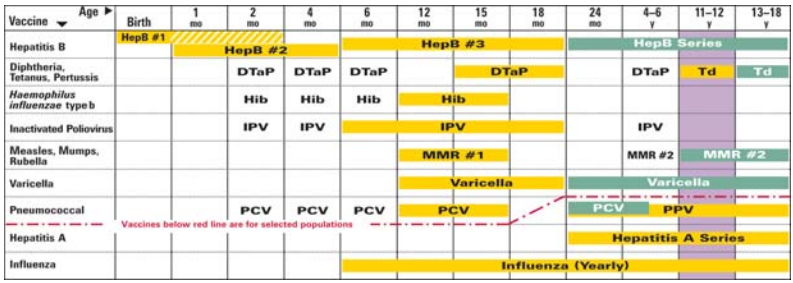
Active immunization. In active immunization, the immune system is stimulated by administration of vaccines to develop a disease-specific immunity. Table 1.**12** lists the vaccine groups used in active immunization. Table 1.**13** shows as an example the vaccination schedule recommended by the Advisory Committee on Immunization Practices of the USA (www.cdc.gov/nip). Recommended adult immunization schedules by age group and by medical conditions are also available in the National Immunization Program Website mentioned above. The vaccination calendars used in other countries deviate from these proposals in some details. For instance, routine varicella and

Table 1.12 Vaccine Groups Used in Active Immunization

Vaccine group	Remarks
Killed pathogens	Vaccination protection often not optimum, vaccination has to be repeated several times
Living pathogens with reduced virulence (attenuated)	Optimum vaccination protection; a single application often suffices, since the microorganisms reproduce in the vaccinated person, providing very good stimulation of the immune system; do not use in immunocompromised persons and during pregnancy (some exceptions)
Purified microbial immunogens	
– Proteins	Often recombinant antigens, i.e., genetically engineered proteins; well-known example: hepatitis B surface (HBs) antigen
– Polysaccharides	Chemically purified capsular polysaccharides of pneumococci, meningococci, and *Haemophilus influenzae* serotype b; problem: these are T cell-independent antigens that do not stimulate antibody production in children younger than two years of age
– Conjugate vaccines	Coupling of bacterial capsular polysaccharide epitopes to proteins, e.g., to tetanus toxoid, diphtheria toxoid, or proteins of the outer membranes of meningococci; children between the ages of two months and two years can also be vaccinated against polysaccharide epitopes
Toxoids	Bacterial toxins detoxified by formaldehyde treatment that still retain their immunogen function
Experimental vaccines	DNA vaccines. Purified DNA that codes for the viral antigens (proteins) and is integrated in plasmid DNA or nonreplicating viral vector DNA. The vector must have genetic elements—for example a transcriptional promoter and RNA-processing elements—that enable expression of the insert in the cells of various tissues (epidermis, muscle cells)
	Anti-idiotype-specific monoclonal antibodies
	Vaccinia viruses as carriers of foreign genes that code for immunogens

Table 1.**13** Recommended Childhood and Adolescent Immunization Schedule—United States, 2004

1. Hepatitis B vaccine (HepB).
   Infants born to HBs-Ag-positive mothers should receive HepB and 0.5 ml HepB Immune Globulin within 12 h of birth at separate sites.
2. Diphtheria (D) and tetanus (T) toxoids and acellular pertussis (aP) vaccine (DTaP).
   The term "d" refers to a reduced dose of diphtheria toxoid.
3. Haemophilus influenzae type b conjugate vaccine (see Table 1.**12**).
4. Measles, mumps, and rubella vaccine (MMR).
   Attenuated virus strains.
5. Varicella vaccine.
   Varicella vaccine is recommended for children who lack a reliable history of chickenpox.
6. Pneumococcal vaccine.
   The heptavalent conjugate vaccine (PCV) is recommended for all children age 2–23 months. Pneumococcal polysaccharide vaccine (PPV) can be used in elder children.
7. Hepatitis A vaccine.
   The "killed virus vaccine" is recommended in selected regions and for certain high-risk groups. Two doses should be administered at least six months apart.
8. Influenza vaccine.
   Influenza vaccine is recommended annually for children with certain risk factors (for instance asthma, cardiac disease, sickle cell disease, HIV, diabetes etc.). Children aged ≤ eight years who are receiving influenza vaccine for the first time should receive two doses separated at least four weeks.



pneumococcal vaccinations are not obligatory in Germany, Austria, and Switzerland (see www.rki.de). To simplify the application of vaccines, licensed combination vaccines may be used whenever any components of the combination are indicated and the vaccine's other components are not contraindicated. Providers should consult the manufacturers' inserts for detailed information.

Passive immunization. This vaccination method involves administration of antibodies produced in a different host. In most cases, homologous (human) hyperimmune sera (obtained from convalescent patients or patients with multiple vaccinations) are used. The passive immunity obtained by this method is limited to a few weeks (or months at most).

Principles of Sterilization and Disinfection

■ Sterilization is defined as the killing or removal of all microorganisms and viruses from an object or product. Disinfection means rendering an object, the hands or skin free of pathogens. The term asepsis covers all measures aiming to prevent contamination of objects or wounds. Disinfection and sterilization makes use of both physical and chemical agents. The killing of microorganisms with these agents is exponential. A measure of the efficacy of this process is the D value (decimal reduction time), which expresses the time required to reduce the organism count by 90%. The sterilization agents of choice are hot air (180 °C, 30 minutes; 160 °C, 120 minutes) or saturated water vapor (121 °C, 15 minutes, 2.02×10^5 Pa; 134 °C, three minutes, 3.03×10^5 Pa). Gamma rays or high-energy electrons are used in radiosterilization at a recommended dose level of 2.5×10^4 Gy.

Disinfection is usually done with chemical agents, the most important of which are aldehydes (formaldehyde), alcohols, phenols, halogens (I, Cl), and surfactants (detergents). ■

Terms and General Introduction

Terms

Sterilization is the killing of all microorganisms and viruses or their complete elimination from a material with the highest possible level of certainty.

An object that has been subjected to a sterilization process, then packaged so as to be contamination-proof, is considered **sterile**.

Killing of Prions and Thermophilic Archaea

The standard sterilization methods used in medical applications (see below) are capable of causing irreversible damage to medically relevant microorganisms such as bacteria, protozoans, fungi, and helminths including worm eggs. Much more extreme processes are required to inactivate prions, such as autoclaving at 121 °C for 4.5 hours or at 134 °C for 30 minutes. Hyperthermophilic archaea forms have also been discovered in recent years (see p. 5) that proliferate at temperatures of 100 °C and higher and can tolerate autoclaving at 121 °C for one hour. These extreme life forms, along with prions, are not covered by the standard definitions of sterilization and sterility.

Disinfection is a specifically targeted antimicrobial treatment with the objective of preventing transmission of certain microorganisms. The purpose of the disinfection procedure is to render an object incapable of spreading infection.

Preservation is a general term for measures taken to prevent microbe-caused spoilage of susceptible products (pharmaceuticals, foods).

Decontamination is the removal or count reduction of microorganisms contaminating an object.

The objective of **aseptic measures and techniques** is to prevent microbial contamination of materials or wounds.

In antiseptic measures, chemical agents are used to fight pathogens in or on living tissue, for example in a wound.

The Kinetics of Pathogen Killing

Killing microorganisms with chemical agents or by physical means involves a first-order reaction. This implies that no pathogen-killing method kills off all the microorganisms in the target population all at once and instantaneously. Plotting the killing rate against exposure time in a semilog coordinate system results in a straight-line curve (Fig. 1.**7**).

Sigmoid and asymptotic killing curves are exceptions to the rule of exponential killing rates. The steepness of the killing curves depends on the sensitivity of the microorganisms to the agent as well as on the latter's effectiveness. The survivor/exposure curve drops at a steeper angle when heat is applied, and at a flatter angle with ionizing radiation or chemical disinfectants. Another contributing factor is the number of microorganisms contaminating a product (i.e., its *bioburden*): when applied to higher organism concentrations, an antimicrobial agent will require a longer exposure time to achieve the same killing effect.

Bacterial Death Kinetics

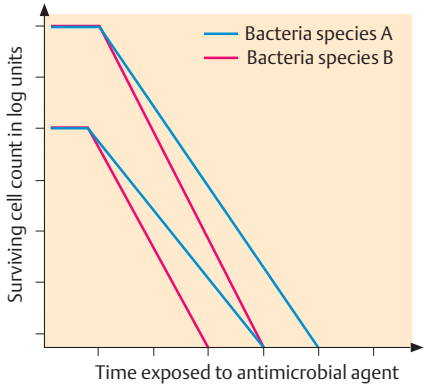


Fig. 1.**7** The death rate varies among bacterial species. The higher the initial concentration of a bacterial culture, the longer an applied antimicrobial agent will require to achieve the same effect.

Standard sterilization methods extend beyond killing all microorganisms on the target objects to project a theoretical reduction of risk, i.e., the number of organisms per sterilized unit should be equal to or less than 10^{-6}.

The D value (decimal reduction time), which expresses the time required to reduce the organism count by 90%, is a handy index for killing effectiveness.

The concentration (c) of chemical agents plays a significant role in pathogen-killing kinetics. The relation between exposure time (t) and c is called the *dilution coefficient* (n): $t \cdot c^n$ = constant. Each agent has a characteristic coefficient n, for instance five for phenol, which means when c is halved the exposure time must be increased by a factor of 32 to achieve the same effect.

The *temperature coefficient* describes the influence of temperature on the effectiveness of chemical agents. The higher the temperature, the stronger the effect, i.e., the exposure time required to achieve the same effect is reduced. The coefficient of temperature must be determined experimentally for each combination of antimicrobial agent and pathogen species.

Mechanisms of Action

When microorganisms are killed by heat, their proteins (enzymes) are irreversibly denatured. Ionizing radiation results in the formation of reactive groups that contribute to chemical reactions affecting DNA and proteins. Exposure to UV light results in structural changes in DNA (thymine dimers) that prevent it from replicating. This damage can be repaired to a certain extent

by light (photoreactivation). Most chemical agents (alcohols, phenols, aldehydes, heavy metals, oxidants) denature proteins irreversibly. Surfactant compounds (amphoteric and cationic) attack the cytoplasmic membrane. Acridine derivatives bind to DNA to prevent its replication and function (transcription).

Physical Methods of Sterilization and Disinfection

Heat

The application of heat is a simple, cheap and effective method of killing pathogens. Methods of heat application vary according to the specific application.

- **Pasteurization.** This is the antimicrobial treatment used for foods in liquid form (milk):
  - Low-temperature pasteurization: 61.5 °C, 30 minutes; 71 °C, 15 seconds.
  - High-temperature pasteurization: brief (seconds) of exposure to 80–85 °C in continuous operation.
  - Uperization: heating to 150 °C for 2.5 seconds in a pressurized container using steam injection.

- **Disinfection.** Application of temperatures below what would be required for sterilization. Important: boiling medical instruments, needles, syringes, etc. does not constitute sterilization! Many bacterial spores are not killed by this method.

- **Dry heat sterilization.** The guideline values for hot-air sterilizers are as follows: 180 °C for 30 minutes, 160 °C for 120 minutes, whereby the objects to be sterilized must themselves reach these temperatures for the entire prescribed period.

- **Moist heat sterilization.** Autoclaves charged with saturated, pressurized steam are used for this purpose:
  - 121 °C, 15 minutes, one atmosphere of pressure (total: 202 kPa).
  - 134 °C, three minutes, two atmospheres of pressure (total: 303 kPa).

In practical operation, the heating and equalibriating heatup and equalizing times must be added to these, i.e., the time required for the temperature in the most inaccessible part of the item(s) to be sterilized to reach sterilization level. When sterilizing liquids, a cooling time is also required to avoid boiling point retardation.

The significant heat energy content of steam, which is transferred to the cooler sterilization items when the steam condenses on them, explains why it is such an effective pathogen killer. In addition, the proteins of microorganisms are much more readily denatured in a moist environment than under dry conditions.

Radiation

- **Nonionizing radiation**. Ultra-violet (UV) rays (280–200 nm) are a type of nonionizing radiation that is rapidly absorbed by a variety of materials. UV rays are therefore used only to reduce airborne pathogen counts (surgical theaters, filling equipment) and for disinfection of smooth surfaces.

- **Ionizing radiation**. Two types are used:
  - Gamma radiation consists of electromagnetic waves produced by nuclear disintegration (e.g., of radioisotope ^{60}Co).
  - Corpuscular radiation consists of electrons produced in generators and accelerated to raise their energy level.

Radiosterilization equipment is expensive. On a large scale, such systems are used only to sterilize bandages, suture material, plastic medical items, and heat-sensitive pharmaceuticals. The required dose depends on the level of product contamination (bioburden) and on how sensitive the contaminating microbes are to the radiation. As a rule, a dose of 2.5×10^4 Gy (Gray) is considered sufficient.

One Gy is defined as absorption of the energy quantum one joule (J) per kg.

Filtration

Liquids and gases can also be sterilized by filtration. Most of the available filters catch only bacteria and fungi, but with ultrafine filters viruses and even large molecules can be filtered out as well. With membrane filters, retention takes place through small pores. The best-known type is the membrane filter made of organic colloids (e.g., cellulose ester). These materials can be processed to produce thin filter layers with gauged and calibrated pore sizes. In conventional depth filters, liquids are put through a layer of fibrous material (e.g., asbestos). The effectiveness of this type of filter is due largely to the principle of adsorption. Because of possible toxic side effects, they are now practically obsolete.

Chemical Methods of Sterilization and Disinfection

Ethylene oxide. This highly reactive gas (C_2H_4O) is flammable, toxic, and a strong mucosal irritant. Ethylene oxide can be used for sterilization at low temperatures (20–60 °C). The gas has a high penetration capacity and can even get through some plastic foils. One drawback is that this gas cannot kill dried microorganisms and requires a relative humidity level of 40–90% in the sterilizing chamber. Ethylene oxide goes into solution in plastics, rubber, and similar materials, therefore sterilized items must be allowed to stand for a longer period to ensure complete desorption.

Aldehydes. *Formaldehyde* (HCHO) is the most important aldehyde. It can be used in a special apparatus for gas sterilization. Its main use, however, is in disinfection. Formaldehyde is a water-soluble gas. *Formalin* is a 35% solution of this gas in water. Formaldehyde irritates mucosa; skin contact may result in inflammations or allergic eczemas. Formaldehyde is a broad-spectrum germicide for bacteria, fungi, and viruses. At higher concentrations, spores are killed as well. This substance is used to disinfect surfaces and objects in 0.5–5% solutions. In the past, it was commonly used in gaseous form to disinfect the air inside rooms (5 g/m^3). The mechanism of action of formaldehyde is based on protein denaturation.

Another aldehyde used for disinfection purposes is *glutaraldehyde*.

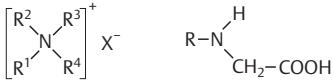
Alcohols. The types of alcohol used in disinfection are *ethanol* (80%), *propanol* (60%), and *isopropanol* (70%). Alcohols are quite effective against bacteria and fungi, less so against viruses. They do not kill bacterial spores. Due to their rapid action and good skin penetration, the main areas of application of alcohols are surgical and hygienic disinfection of the skin and hands. One disadvantage is that their effect is not long-lasting (no depot effect). Alcohols denature proteins.

Phenols. Lister was the first to use phenol (carbolic acid) in medical applications. Today, phenol derivatives substituted with organic groups and/or halogens (alkylated, arylated, and halogenated phenols), are widely used. One common feature of phenolic substances is their weak performance against spores and viruses. Phenols denature proteins. They bind to organic materials to a moderate degree only, making them suitable for disinfection of excreted materials.

Halogens. Chlorine, iodine, and derivatives of these halogens are suitable for use as disinfectants. Chlorine and iodine show a generalized microbicidal effect and also kill spores.

Chlorine denatures proteins by binding to free amino groups; hypochlorous acid (HOCl), on the other hand, is produced in aqueous solutions, then

Surfactant Disinfectants



a b

Fig. 1.**8** Quaternary ammonium compounds (**a**) and amphoteric substances (**b**) disrupt the integrity and function of microbial membranes.

disintegrates into HCl and $^1/_2$ O_2 and thus acts as a powerful oxidant. Chlorine is used to disinfect drinking water and swimming-pool water (up to 0.5 mg/l). Calcium hypochlorite (chlorinated lime) can be used in nonspecific disinfection of excretions. Chloramines are organic chlorine compounds that split off chlorine in aqueous solutions. They are used in cleaning and washing products and to disinfect excretions.

Iodine has qualities similar to those of chlorine. The most important iodine preparations are the solutions of iodine and potassium iodide in alcohol (tincture of iodine) used to disinfect skin and small wounds. Iodophores are complexes of iodine and surfactants (e.g., polyvinyl pyrrolidone). While iodophores are less irritant to the skin than pure iodine, they are also less effective as germicides.

Oxidants. This group includes ozone, hydrogen peroxide, potassium permanganate, and peracetic acid. Their relevant chemical activity is based on the splitting off of oxygen. Most are used as mild antiseptics to disinfect mucosa, skin, or wounds.

Surfactants. These substances (also known as surface-active agents, tensides, or detergents) include anionic, cationic, amphoteric, and nonionic detergent compounds, of which the cationic and amphoteric types are the most effective (Fig. 1.**8**).

The bactericidal effect of these substances is only moderate. They have no effect at all on tuberculosis bacteria (with the exception of amphotensides), spores, or nonencapsulated viruses. Their efficacy is good against Gram-positive bacteria, but less so against Gram-negative rods. Their advantages include low toxicity levels, lack of odor, good skin tolerance, and a cleaning effect.

Practical Disinfection

The objective of **surgical hand disinfection** is to render a surgeon's hands as free of organisms as possible. The procedure is applied after washing the hands thoroughly. Alcoholic preparations are best suited for this purpose, although they are not sporicidal and have only a brief duration of action.

Alcohols are therefore often combined with other disinfectants (e.g., quaternary ammonium compounds). Iodophores are also used for this purpose.

The purpose of **hygienic hand disinfection** is to disinfect hands contaminated with pathogenic organisms. Here also, alcohols are the agent of choice.

Alcohols and/or iodine compounds are suitable for **disinfecting patient's skin** in preparation for surgery and injections.

Strong-smelling agents are the logical choice for **disinfection of excretions** (feces, sputum, urine, etc.). It is not necessary to kill spores in such applications. Phenolic preparations are therefore frequently used. Contaminated hospital sewage can also be thermally disinfected (80–100 °C) if necessary.

Surface disinfection is an important part of hospital hygiene. A combination of cleaning and disinfection is very effective. Suitable agents include aldehyde and phenol derivatives combined with surfactants.

Instrument disinfection is used only for instruments that do not cause injuries to skin or mucosa (e.g., dental instruments for work on hard tooth substance). The preparations used should also have a cleaning effect.

Laundry disinfection can be done by chemical means or in combination with heat treatment. The substances used include derivatives of phenols, aldehydes and chlorine as well as surfactant compounds. Disinfection should preferably take place during washing.

Chlorine is the agent of choice for **disinfection of drinking water and swimming-pool water**. It is easily dosed, acts quickly, and has a broad disinfectant range. The recommended concentration level for drinking water is 0.1–0.3 mg/l and for swimming-pool water 0.5 mg/l.

Final room disinfection is the procedure carried out after hospital care of an infection patient is completed and is applied to a room and all of its furnishings. Evaporation or atomization of formaldehyde (5 g/m^3), which used to be the preferred method, requires an exposure period of six hours. This procedure is now being superseded by methods involving surface and spray disinfection with products containing formaldehyde.

Hospital disinfection is an important tool in the prevention of cross-infections among hospital patients. The procedure must be set out in written form for each specific case.

2 Basic Principles of Immunology

R. M. Zinkernagel

Introduction

■ Resistance to disease is based on **innate mechanisms** and adaptive or **acquired immunity**. Acquired immune mechanisms act in a specific manner and function to supplement the important **nonspecific** or **natural resistance mechanisms** such as physical barriers, granulocytes, macrophages, and chemical barriers (lysozymes, etc.). The **specific immune mechanisms** constitute a combination of less specific factors, including the activation of macrophages, complement, and necrosis factors; the early recognition of invading agents, by cells exhibiting a low level of specificity, (natural killer cells, γδ [gamma-delta] T cells); and systems geared toward highly specific recognition (antibodies and αβ [alpha-beta] T cells).

Many components of the specific immune defenses also contribute to nonspecific or natural defenses such as natural antibodies, complement, interleukins, interferons, macrophages, and natural killer cells. ■

In the strict sense, "immunity" defines an acquired resistance to infectious disease that is specific, i.e., resistance against a particular disease-causing pathogen. For example, a person who has had measles once will not suffer from measels a second time, and is thus called immune. However, such specific or acquired immune mechanisms do not represent the only factors which determine resistance to infection. The canine distemper virus is a close relative of the measles virus, but never causes an infection in humans. This kind of resistance is innate and nonspecific. Our immune system recognizes the pathogen as foreign based on certain surface structures, and eliminates it. Humans are thus born with resistance against many microorganisms (**innate immunity**) and can acquire resistance to others (**adaptive or acquired immunity**; Fig. 2.**1**). Activation of the mechanisms of innate immunity, also known as the primary immune defenses, takes place when a pathogen breaches the outer barriers of the body. Specific immune defense factors are mobilized later to fortify and regulate these primary defenses. Responses of the adaptive immune system not only engender immunity in the strict sense, but can also contribute to pathogenic processes. The terms **immunopathology, autoimmunity,** and **allergy** designate a group of immune

The Components of Anti-Infection Defense

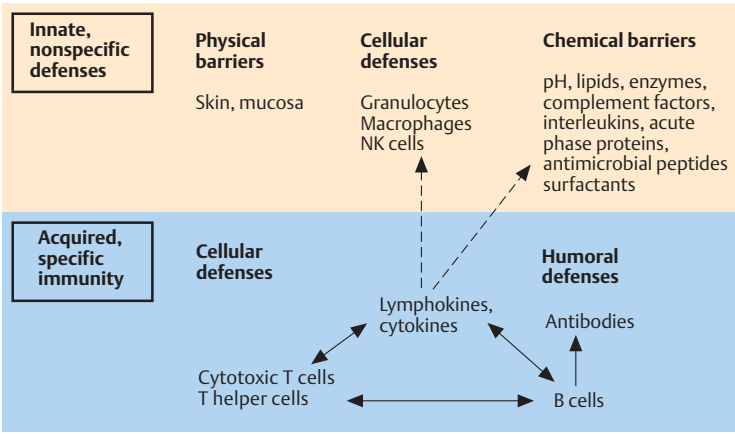


Fig. 2.1 The innate immune defense system comprises nonspecific physical, cellular, and chemical mechanisms which are distinct from the acquired immune defense system. The latter comprises cellular (T-cell responses) and humoral (antibodies) components. Specific T cells, together with antibodies, recruit non-specific effector mechanisms to areas of antigen presence.

phenomena causing mainly pathological effects, i.e., tissue damage due to inadequate, misguided, or excessive immune responses. However, a failed immune response may also be caused by a number of other factors. For instance, certain viral infections or medications can suppress or attenuate the immune response. This condition, known as **immunosuppression**, can also result from rare genetic defects causing congenital immunodeficiency.

The inability to initiate an immune response to the body's own self antigens (also termed autoantigens) is known as immunological **tolerance**.

Anergy is the term used to describe the phenomenon in which cells involved in immune defense are present but are not functional.

An immune response is a reaction to an immunological stimulus. The stimulating substances are known as **antigens** and are usually proteins or complex carbohydrates. The steric counterparts of the antigens are the **antibodies**, i.e., immunoreceptors formed to recognize segments, roughly 8–15 amino acids long, of the folded antigenic protein. These freely accessible structural elements are known as **epitopes** when present on the antigens, or as **antigen-binding sites** (ABS) from the point of view of the immunoreceptors. Presented alone, an epitope is not sufficient to stimulate an immunological response. Instead responsiveness is stimulated by epitopes con-

stituting part of a macromolecule. This is why the epitope component of an antigen is terminologically distinguished from its macromolecular carrier; together they form an **immunogen**. B lymphocytes react to the antigen stimulus by producing antibodies. The T lymphocytes (T cells) responsible for cellular immunity are also activated. These cells can only recognize protein antigens that have been processed by host cells and presented on their surface. The T-cell receptors recognize antigen fragments with a length of 8–12 sequential amino acids which are either synthesized by the cell itself or produced subsequent to phagocytosis and presented by the cellular transplantation antigen molecules on the cell surface. The T cells can then complete their main task—recognition of infected host cells—so that infection is halted.

Our understanding of the immune defense system began with studies of infectious diseases, including the antibody responses to diphtheria, dermal reactions to tuberculin, and serodiagnosis of syphilis. Characteriztion of pathological antigens proved to be enormously difficult, and instead erythrocyte antigens, artificially synthesized chemical compounds, and other more readily available proteins were used in experimental models for more than 60 years. Major breakthroughs in bacteriology, virology, parasitology, biochemistry, molecular biology, and experimental embryology in the past 30–40 years have now made a new phase of intensive and productive research possible within the field of immune defenses against infection. The aim of this chapter on immunology, in a compact guide to medical microbiology, is to present the **immune system** essentially as a system of **defense against infections** and to identify its strengths and weaknesses to further our understanding of pathogenesis and prevention of disease.

The Immunological Apparatus

The immune system is comprised of various continuously circulating cells (T and B lymphocytes, and antigen-presenting cells present in various tissues). T and B cells develop from a common stem cell type, then mature in the thymus (T cells) or the bone marrow (B cells), which are called **primary (or central) lymphoid organs**. An antigen-specific differentiation step then takes places within the specialized and highly organized **secondary (or peripheral) lymphoid organs** (lymph nodes, spleen, mucosa-associated lymphoid tissues [MALT]). The antigen-specific activation of B and/or T cells involves their staggered interaction with other cells in a contact-dependent manner and by soluble factors.

B cells bear antibodies on their surfaces (cell-bound **B-cell receptors**). They secrete antibodies into the blood (**soluble antibodies**) or onto mucosal surfaces once they have fully matured into plasma cells. Antibodies recognize

the three-dimensional structures of complex, folded proteins, and hydrocarbons. Chemically, B-cell receptors are globulins ("immunoglobulins") and comprise an astounding variety of specific types. Despite the division of immunoglobulins into classes and subclasses, they all share essentially the same structure. Switching from one Ig class to another generally requires T-cell help.

T cells recognize peptides presented on the cell surface by major histocompatibility (gene) complex (MHC) molecules. A T-cell response can only be initiated within organized lymphoid organs. Naive T cells circulate through the blood, spleen, and other lymphoid tissues, but cannot leave these compartments to migrate through peripheral nonlymphoid tissues and organs unless they are activated. Self antigens (autoantigens), presented in the thymus and lympoid tissues by mobile lymphohematopoietic cells, induce T-cell destruction (so-called **negative selection**). Antigens that are expressed only in the periphery, that is outside of the thymus and secondary lymphoid organs, are ignored by T cells; potentially autoreactive T cells are thus directed against such self antigens. T cells react to peptides that penetrate into the organized lymphoid tissues. New antigens are first localized within few lymphoid tissues before they can spread systemically. These must be present in lymphoid tissues for three to five days in order to elicit an immune response. An immune response can be induced against a previously ignored self antigen that does not normally enter lymphoid tissues if its entry is induced by circumstance, for instance, because of cell destruction resulting from chronic peripheral infection. It is important to remember that induction of a small number of T cells will not suffice to provide immune protection against a pathogen. Such protection necessitates a certain minimum sum of activated T cells.

The function of the immunological apparatus is based on a complex series of interactions between humoral, cellular, specific, and nonspecific mechanisms. This can be better understood by examining how the individual components of the immune response function.

The human immunological system can be conceived as a widely distributed organ comprising approximately 10^{12} individual cells, mainly **lymphocytes**, with a total weight of approximately 1 kg. Leukocytes arise from pluripotent stem cells in the bone marrow, then differentiate further as two distinct lineages. The **myeloid lineage** constitutes granulocytes and monocytes, which perform important basic defense functions as phagocytes ("scavenger cells"). The **lymphoid lineage** gives rise to the effector cells of the specific immune response, **T** and **B** lymphocytes. These cells are constantly being renewed (about 10^6 new lymphocytes are produced in every minute) and destroyed in large numbers (see Fig. 2.**17**, p. 88). T and B lymphocytes, while morphologically similar, undergo distinct maturation pro-

cesses (Table 2.**1**, Fig. 2.**2**). The antigen-independent phase of lymphocyte differentiation takes place in the so-called **primary lymphoid organs**: T lymphocytes mature in the **t**hymus and B lymphocytes in the **b**ursa fabricI (in birds). Although mammals have no bursa, the term B lymphocytes (or B cells) has been retained to distinguish these cells, with their clearly distinct functions and maturation in the **b**one marrow, from T lymphocytes, which mature in the thymus (Table 2.**1**). B cells mature in the fetal liver as well as in fetal and adult **b**one marrow. In addition to their divergent differentia-

Maturation of B and T cells

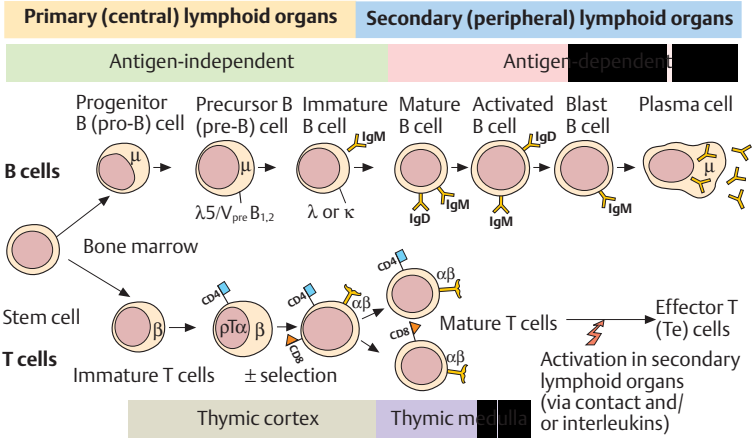


Fig. 2.**2** All lymphoid cells originate from pluripotent stem cells present in the bone marrow which can undergo differentiation into B or T cells. Stem cells that remain in the bone marrow develop into mature B cells via several antigen-independent stages; including the λ5Vpre-B cell stage, and pre-B cells with a special λ5 precursor chain. Antigen contact within secondary lymphoid organs can then activate these cells, finally causing them to differentiate into antibody-secreting plasma cells.
T cells mature in the thymus; pTα is a precursor α chain associated with TCRβ chain surface expression. The pTα chain is later replaced by the normal TCRα chain. Immature $CD4^+$ $CD8^+$ double-positive thymocytes are localized within the cortical region of the thymus; some autoreactive T cells are deleted in the cortex, whilst some are deleted in the medulla as mature single-positive T cells. The remaining T cells mature within the medulla to become $CD4^+$ $CD8^-$ or $CD4^-$ $CD8^+$ T cells. From here, these single positive T cells can emigrate to peripheral secondary lymphoid organs, where they may become activated by a combination of antigen contacts, secondary signals, and cytokines.

Table 2.1 Distribution of Lymphocyte Subpopulations and APCs in Various Organs (% of All Mononuclear Cells)

	B	T	NK, LAK, ADCC	APC
Peripheral blood	10–15	70–80	5–10	<1
Lymph	5	95–100	?	?
Thoracic duct	5–10	90–95	?	?
Thymus	1	95–100	?	0
Bone marrow	15–20	10–15	?	0
Spleen	40–50	40–60	20–30	1
Lymph nodes, tonsils, etc.	20–30	70–80	5–8	1

NK: natural killer cells; LAK: lymphokine-activated killer cells, ADCC: antibody-dependent cellular toxicity, APC: antigen-presenting cells

tion pathways, T and B cells differ with respect to their functions, receptors, and surface markers. They manifest contrasting response patterns to cytokines, and display a marked preference to occupy different compartments of lymphoid organs. T and B cells communicate with each other, and with other cell types, by means of adhesion and accessory molecules (CD antigens, see Table 2.**13**, p. 137) or in response to soluble factors, such as cytokines, which bind to specific receptors and induce the activation of intracellular signaling pathways. The antigen-dependent differentiation processes which leads to T and B cell specialization, takes place within the **secondary lymphoid organs** where lymphocytes come into contact with antigens. As a general rule the secondary lymphoid organs contain only mature T and B cells, and comprise encapsulated organs such as the **lymph nodes** and **spleen**, or non-encapsulated structures which contain lymphocytes and are associated with the skin, mucosa, gut, or bronchus (i.e. **SALT**, **MALT**, **GALT**, and **BALT**). Together, the primary and secondary lymphoid organs account for approximately 1–2% of body weight.

The B-Cell System

■ B lymphocytes produce antibodies in two forms; a membrane-bound form and a secreted form. Membrane-bound antibody forms the B-cell antigen receptor. Following antigen stimulation, B lymphocytes differentiate into **plasma cells**, which secrete antibodies exhibiting the same antigen specificity as the B-cell receptor. This system is characterized as **humoral immunity,** due to this release of receptors into the "humoral" system which constitutes vascular contents and mucous environments. The humoral

Table 2.2 Characteristics of the Various Immunoglobulin Classes

	IgM	IgD	IgG	IgE	IgA
Svedberg unit	19 S	7 S	7 S	8 S	7 S, 9 S, 11 S
Molecular weight	900 kDa	185 kDa	150 kDa	200 kDa	160 kDa
Number of dimeric units	5	1	1	1	1, 2, 3
H chain (constant domains)	μ (4)	δ (3)	γ (3)	ε (4)	α (3)
L chain	← κ or λ →				
Antigen-binding sites (ABS)	10	2	2	2	2, 4, 6
Concentration in normal serum (g/l)	0.5–2	0–0.4	8–16	0.02–0.50	1.4–4
% of Ig	6	0–1	80	0.002	13
Half-life (days)	1–2	?	7–21	1–2 in serum >200 on mast cells	3–6
Complement (C) activation:					
Classic	+	–	+	–	–
Alternative	–	–	–	–	+
Placental passage	–	–	+	+	–
Binding to mast cells and basophils	–	–	–	+	–
Binding to macrophages, granulocytes, and thrombocytes	–	–	(+)	–	(+)
Subclasses	–	–	+ (4)	–	+ (2)
IgG subclasses		**IgG1**	**IgG2**	**IgG3**	**IgG4**
% of total IgG		60–70	14–20	4–8	2–6
Reaction to *Staphlococcus* protein A		+	+	–	+
Placental passage		+	(+)	+	+
Complement (C) activation:		+++	++	++++	(+)
Binding to monocytes/macrophages		+++	+	+++	(+)
Blocks IgE binding		(–)	–	–	+
Half-life (days)		21–23	21–23	7–9	21–23

system also contains non-specific defense mechanisms, including the complement system (see "Immune response and effector mechanisms," p. 66ff.). In chemical terms, B-cell receptors are globulins (Ig or immunoglobulins). These immunoglobulins comprise a number of classes and subclasses, as well as numerous different specificities, but share a common structure (Fig. 2.**3a**).

Immunoglobulin Structure

All immunoglobulin monomers have the same basic configuration, in that they consist of two identical light chains (L) and two identical heavy chains (H). The light chains appear as two forms; **lambda** (λ) or **kappa** (κ). There are five main heavy chain variants; μ, δ, γ, α, and ε. The five corresponding immunoglobulin classes are designated as **IgM**, **IgD**, **IgG**, **IgA,** or **IgE**, depending on which type of heavy chain they use (Fig. 2.**3b**). A special characteristic of the immunoglobulin classes IgA and IgM is that these comprise a basic monomeric structure that can be doubled or quintupled (i.e., these can exist in a dimeric or pentameric form). Table 2.**2** shows the composition, molecular weights and serum concentrations of the various immunoglobulin classes (p. 49).

Fig. 2.**3** **a Immunoglobulin monomers.** The upper half of the figure shows the intact monomer consisting of two L and two H chains. The positions of the disulfide bonds, the variable *N*-terminal domains, and the antigen-binding site (ABS) are indicated. The lower half of the figure shows the monomers of the individual polypeptide chains as seen following exposure to reducing conditions (which break the disulfide bonds) and denaturing conditions; note that the ABS is lost. Papain digestion produces two monovalent Fab fragments, and one Fc fragment. Following pepsin digestion (right), the Fc portion is fragmented, but the Fab fragments remain held together by disulfide bonds. The $F(ab')_2$ arm is bivalent (with two identical ABS). Fv fragments comprise a single-chain ABS formed by recombinant technology. These consist of the variable domains of the H and L chains, joined covalently by a synthetic linker peptide.
b Classes of immunoglobulins. IgM, IgD, IgG, IgA, and IgE are differentiated by their respective heavy chains (μ, δ, γ, α, ε). IgA (α chain) forms dimers held together by the J (joining) chain; the secretory (S) piece facilitates transport of secretory IgA across epithelial cells, and impairs its enzymatic lysis within secretions. IgM (μ chain) forms pentamers with 10 identical ABS; the IgM monomers are held together by J chains. The light chains (λ and κ) are found in all classes of immunoglobulins. ▶

Immunoglobulins contain numerous domains, as illustrated by the structure of IgG. In monomeric IgG each domain consists of a protein segment which is approximately 110 amino acids in length. Both light chains possess two such domains, and each heavy chain possesses four or five domains. The domain structure was first revealed by comparison of the amino acid sequence derived from many different immunoglobulins belonging to the

Basic Immunoglobulin Structures

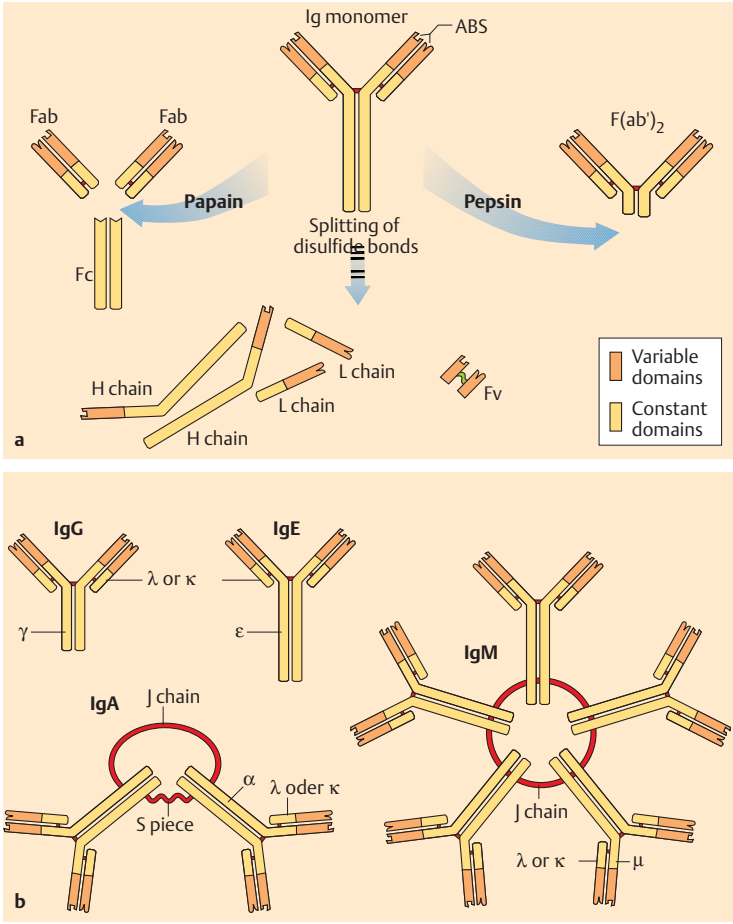


Table 2.**3** Antigen Recognition by B and T Cells

	B lymphocytes	**T helper cells ($CD4^+$)**	**Cytotoxic T cells (CTL; $CD8^+$)**
Recognition structure of B or T cell	Surface Ig (BCR)	TCR	TCR
Recognized epitope	Conformational epitopes (no MHC restriction)	Linear epitopes only (10–15 amino acids) + MHC class II	Linear epitopes (peptides) (8)–9–(10) amino acids + MHC class I
Antigen type	Proteins/carbohydrates	Peptides only	Peptides only
Antigen presentation	Not necessary	Via MHC class II structures	Via MHC class I structures
Effectors	Antibodies (+/– complement)	Signals induced by contact (T/B help) or cytokines	Cytotoxicity mediated by contact (perforin, granzyme), or release of cytokines

same class. In this way a high level of sequence variability was revealed to be contained within the N-terminal domain (**variable domain, V**), whilst such variability was comparably absent within the other domains (**constant domains, C**). Each light chain consists of one variable domain (V_L) and one constant domain (C_L). In contrast, the heavy chains are roughly 440–550 amino acids in length, and consist of four to five domains. Again, the heavy chain variable region is made up of one domain (V_H), whereas the constant region consists either of three domains (γ, α, δ chains), or four domains (μ, ε chains) (C_{H1}, C_{H2}, C_{H3}, and C_{H4}). Disulfide bonds link the light chains to the heavy chains and the heavy chains to one another. An additional disulfide bond is found within each domain.

The three-dimensional form of the molecule forms a letter Y. The two short arms of this 'Y' consist of four domains each (V_L, C_L, V_H, and C_{H1}), and this structure contains the antigen-binding fragments—hence its designation as **Fab** (**f**ragment **a**ntigen **b**inding). The schematic presented in Fig. 2.**3** is somewhat misleading, since the two variable domains of the light and heavy chains are in reality intertwined. The binding site—a decisive structure for an epitope reaction—is formed by the combination of variable domains from both chains. Since the two light chains, and the two heavy chains, contain identical amino acid sequences (this includes the variable domains), each

immunoglobulin monomer has two identical **antigen-binding sites** (**ABS**), and these form the ends of the two short arms of the 'Y'. An area within the antibody consisting of 12–15 amino acids contacts the peptide region contained within the antigen and consisting of approximately 5–800 $Å^2$ (Table 2.**3**). The trunk of the 'Y' is called the **Fc** fragment (named, "**f**raction **c**rystallizable" since it crystallizes readily) and is made up of the constant domains of the heavy chains (C_{H2} and $C_{H3,}$ and sometimes C_{H4}).

Diversity within the Variable Domains of the Immunoglobulins

The specificity of an antibody is determined by the amino acid sequence of the variable domains of the H and L chains, and this sequence is unique for each corresponding cell clone. How has nature gone about the task of producing the needed diversity of specific amino acid sequences within a biochemically economical framework? The genetic variety contained within the B-cell population is ensured by a process of continuous diversification of the genetically identical B-cell precursors. The three gene segments (variable, diversity, joining) which encode the variable domain (the VDJ region for the H chain, and the VJ region for the L chain) are capable of undergoing a process called recombination. Each of these genetic segments are found as a number of variants (Fig. 2.**4**, Table 2.**4**). B-cell maturation involves a process of genetic re-

Table 2.**4** Organization of the Genetic Regions for the Human Immunoglobulins and T-Cell Receptors (TCR)

	Immunoglobulins		**TCRαβ**		**TCRγδ**	
	H	L	α	β	γ	δ
V segments	95	150	50–100	75–100	9	6
D segments	23	–	–	2	–	3
J segments	9	12	60–80	13	5	3
Nucleotide additions	VD, DJ	VJ	VJ	VD, DJ	VJ	VD
Number of potential combinations for V (H + L)	15 000		8000		54	
Theoretical upper limit of all combinations	$>10^{12}$		$>10^{12}$		$>10^{12}$	

combination resulting in a **rearrangement** of these segments, such that one V_H, one D_H, and one J_H segment become combined. Thus the germ line does not contain *one gene* governing the variable domain, but rather gene segments which each encode fragments of the necessary information. Mature B cells contain a functional gene which, as a result of the recombination process, is comprised of one $V_HD_HJ_H$ segment. The diversity of T-cell receptors is generated in a similar manner (see p. 57).

Fig. 2.**4** explains the process of genetic recombination using examples of an immunoglobulin H chain and T-cell receptor α chain.

The major factors governing immunoglobulin diversity include:

- Multiple V gene segments encoded in the germ lines.
- The process of VJ, and VDJ, genetic recombination.
- Combination of light and heavy chain protein structures.
- Random errors occurring during the recombination process, and inclusion of additional nucleotides.
- Somatic point mutations.

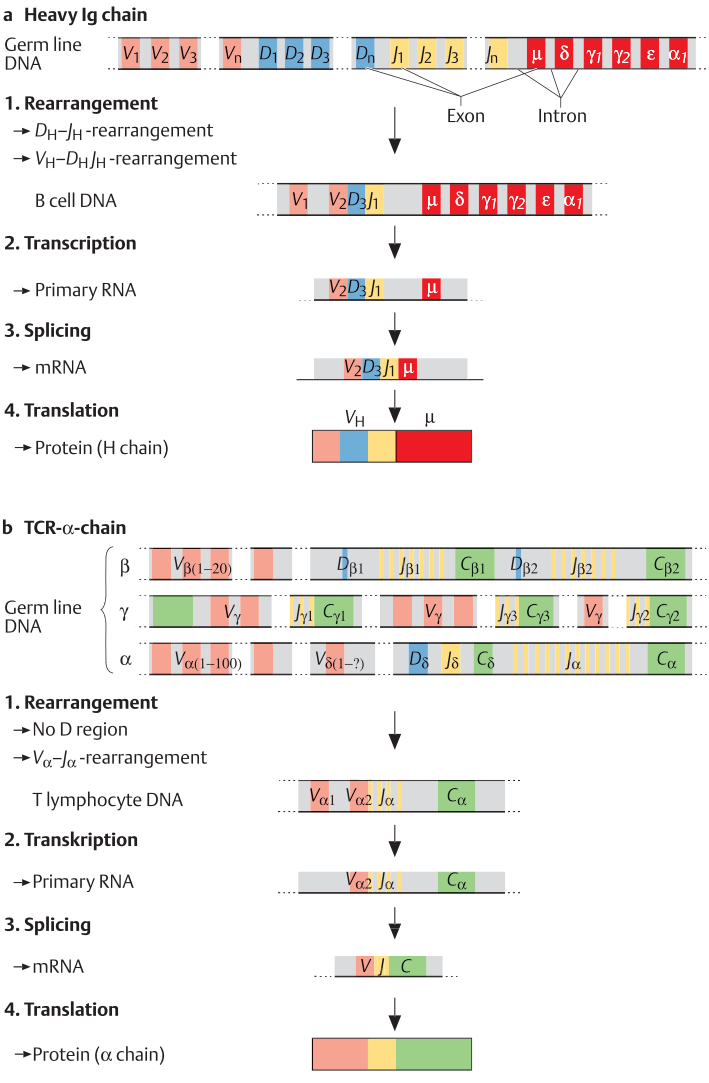
In theory, the potential number of unique immunoglobulin structures that could be generated by a combination of these processes exceeds 10^{12}, however, the biologically viable and functional range of immunoglobulin specificities is likely to number closer to 10^4.

The Different Classes of Immunoglobulins

Class switching. The process of genetic recombination results in the generation of a functional VDJ gene located on the chromosome upstream of those

Fig. 2.**4** **a Heavy chain of human IgG.** The designations for the gene segments in the variable part of the H chain are V (variable), D (diversity), and J (joining). The segments designated as μ, δ, γ, α, and ε code for the constant region and determine the immunoglobulin class. The V segment occurs in several hundred versions, the D segment in over a dozen, and the J segment in several forms. V, D, and J segments combine randomly to form a sequence (VDJ) which codes for the variable part of the H chain. This rearranged DNA is then transcribed, creating the primary RNA transcript. The non-coding intervening sequences (introns) are then spliced out, and the resulting mRNA is translated into the protein product. **b α chain of mouse T-cell receptor.** Various different V, D, and J gene segments (for β and δ), V and J gene segments (for α and γ) are available for the T-cell receptor chains. The DNA loci for the δ chain genes are located between those for the α chain. ▶

Rearrangement of the B- and T-Cell Receptor Genes



regions encoding the H chain segments Cμ, Cδ, Cγ, Cα, and Cε, in consecutive order. Thus all immunoglobulin production begins with the synthesis of IgM and IgD (resulting from transcription of the VDJ and the Cμ or Cδ gene segments). This occurs without prior antigen stimulus and is transitional in nature. Antigen stimulation results in a second gene rearrangement—during which the VDJ gene is relocated to the vicinity of Cγ, Cα, or Cε by a process of recombination involving deletion of the intervening regions. Following this event, the B cell no longer produces H chains of the IgM or IgD classes, but is instead committed to the production of IgG, IgA, or IgE—thus allowing secretion of the entire range of immunoglobulin types (Table 2.**2**). This process is known as class switching, and results in a change of the Ig class of an antibody whilst allowing its antigen specificity to be retained.

Variability types. The use of different heavy or light chain constant regions results in new immunoglobulin classes known as **isotypes**. Individual Ig classes can also differ, with such genetically determined variations in the constant elements of the immunoglobulins (which are transmitted according to the Mendelian laws) are known as **allotypes**. Variation within the variable region results in the formation of determinants, known as **idiotypes.** The idiotype determines an immunoglobulins antigenic specificity, and is unique for each individual B-cell clone.

Functions. Each different class of antibody has a specific set of functions. IgM and IgD act as B-cell receptors in their earlier transmembrane forms, although the function of **IgD** is not entirely clear. The first antibodies produced in the primary immune response are **IgM** pentamers, the action of which is directed largely against micro-organisms. IgM pentamers are incapable of crossing the placental barrier. The immunoglobulin class which is most abundant in the serum is **IgG**, with particularly high titers of this isotype being found following secondary stimulation. IgG antibodies pass through the placenta and so provide the newborn with a passive form of protection against those pathogens for which the mother exhibits immunity. In certain rare circumstances such antibodies may also harm the child, for instance when they are directed against epitopes expressed by the child's own tissues which the mother has reacted against immunologically (the most important clinical example of this is rhesus factor incompatibility). High concentrations of **IgA** antibodies are found in the intestinal tract and contents, saliva, bronchial and nasal secretions, and milk—where they are strategically positioned to intercept infectious pathogens (particularly commensals) (Fig. 2.**5**). **IgE** antibodies bind to high-affinity Fcε receptors present on basophilic granulocytes and mast cells. Cross-linking of mast cell bound IgE antibodies by antigen results in cellular degranulation and causes the release of highly active biogenic amines (histamine, kinines). IgE antibodies are produced in large quantities following parasitic infestations of the intestine, lung or skin, and play a significant role in the local immune response raised against these pathogens.

The Mucosa-Associated Lymphoid Tissue (MALT) Immune System and "Homing"

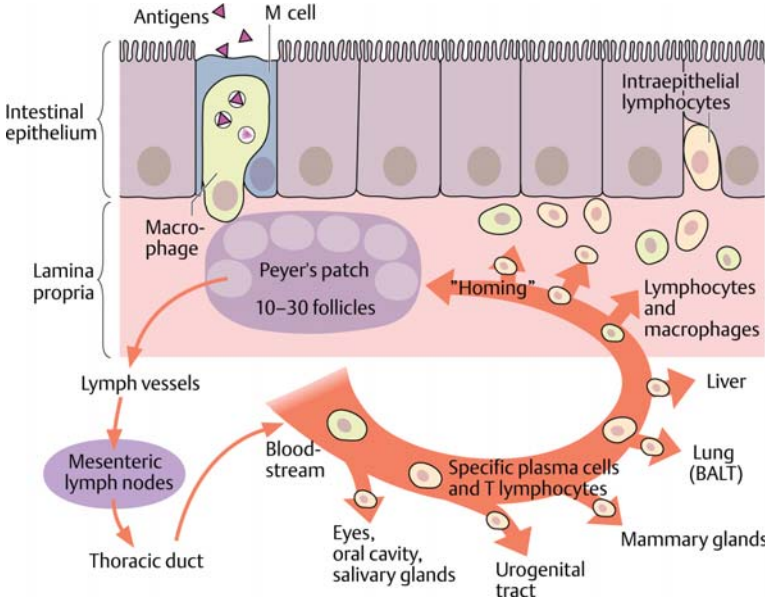


Fig. 2.**5** Specialized APCs (M cells in the intestinal wall or pulmonary macrophages in the lung) take up antigens in mucosa and present them in the Peyer's patches or local lymph nodes. This probably enhances T cell-dependent activation of IgA-producing B cells, which are preferentially recruited to the mucosal regions ("homing") via local adhesion molecules and antigen depots, resulting in a type of geographic specificity within the immune response.

The T-Cell System

T-Cell Receptors (TCR) and Accessory Molecules

Like B cells, T cells have receptors that bind specifically to their steric counterparts on antigen epitopes. The diversity of **T-cell receptors** is also achieved by means of genetic rearrangement of V, D, and J segments (Fig. 2.**4b**). However, the T-cell receptor is never secreted, and instead remains membrane-bound.

Each T-cell receptor consists of **two transmembrane chains,** of either the α and β forms, or the γ and δ forms (not to be confused with the heavy

chains of Ig bearing the same designations). Both chains have two extracellular domains, a transmembrane anchor element and a short intracellular extension. As for Ig, the terminal domains are variable in nature (i.e., Vα and Vβ), and together they form the antigen binding site (see Fig. 2.**9**, p. 65). T-cell receptors are associated with their so-called co-receptors—other membrane-enclosed proteins expressed on the T cell surface—which include the multiple-chain **CD3 complex,** and **CD4 or CD8 molecules (**depending on the specific differentiation of the T cell). CD stands for "cluster of differentiation" or "cluster determinant" and represents differentiation antigens defined by clusters of monoclonal antibodies. (Table 2.**13**, p. 135f., provides a summary of the most important CD antigens.)

T-Cell Specificity and the Major Histocompatibility Complex (MHC)

T-cell receptors are unable to recognize free antigens. Instead the T-cell receptor can only recognize its specific epitope once the antigen has been cleaved into shorter peptide fragments by the presenting cell. These fragments must then be embedded within a specific molecular groove and presented to the T-cell receptor (a process known as MHC-restricted **T-cell recognition or MHC restriction**). This "binding groove" is located on the MHC molecule. The MHC encodes for the powerful histocompatibility or transplantation antigens (also known in humans as HLA, human leukocyte antigen molecules, Fig. 2.**6**).

The designation "MHC molecule" derives from the initial discovery of the function of the complex as a cell surface structure, responsible for the

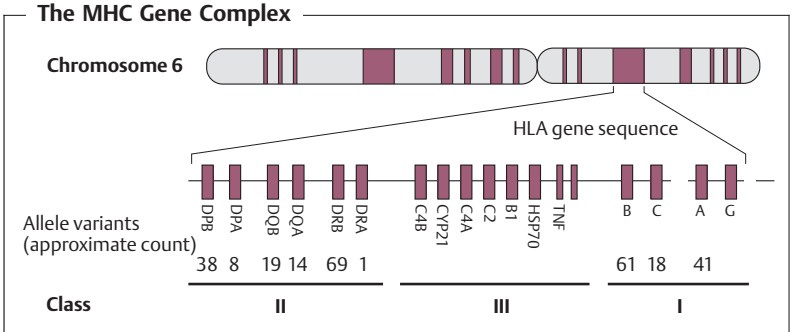


Fig. 2.**6** The human major histocompatibility gene complex (HLA genes) is located on chromosome 6. There are three different classes of MHC molecules.

immunological rejection of cell transfusions or tissue and organ transplants. Its true function as a peptide-presenting molecule was not discovered until the seventies, when its role became apparent whilst testing the specificity of virus-specific cytotoxic T cells. During these experiments it was observed that immune T cells were only able to destroy infected target cells if both cell types were derived from the same patient or from mice with identical MHC molecules. The resulting conclusion was that a T-cell receptor not only recognizes the corresponding amino acid structure of the presented peptide, but additionally recognizes certain parts of the MHC structure. It is now known that this contact between MHC on the APC and the T-cell receptor is stabilized by the co-receptors CD4 and CD8.

MHC classes. Molecules encoded by the MHC can be classified into three groups according to their distribution on somatic cells, and the types of cells by which they are recognized:

- **MHC class I molecules.** These molecules consist of a heavy α chain with three Ig-like polymorphic domains (these are encoded by 100–1000 alleles, with the α1 and α2 domains being much more polymorphic than the α3 domain) and a nonmembrane-bound (soluble) single-domain β_2 microglobulin (β_2M, which is encoded by a relatively small number of alleles). The α chain forms a groove that functions to present antigenic peptides (Fig. 2.**7**). Human HLA-A, HLA-B, and HLA-C molecules are expressed in varying densities on all somatic cells (the relative HLA densities for fibroblasts and hepatic cells, lymphocytes, or neurons are 1x, 100x and 0.1×, respectively). Additional, nonclassical, class I antigens which exhibit a low degree of polymorphism are also present on lymphohematopoietic cells and play a role in cellular differentiation.

- **MHC class II molecules.** These are made up by two different polymorphic transmembrane chains that consist of two domains each (α_1 is highly polymorphic, whilst β_1 is moderately polymorphic, and β_2 is fairly constant). These chains combine to form the antigen-presenting groove. Class II molecules are largely restricted to *lymphohematopoetic cells*, *antigen-presenting cells* (APC), *macrophages*, and so on. (see Fig. 2.**9a**, p. 65) In humans, but not in mice, they are also found on some epithelial cells, neuroendocrine cells, and T cells. The products of the three human gene regions HLA-DP, HLA-DQ and HLA-DR can additionally form molecules representing combinations of two loci—thus providing additional diversity for peptide presentation.

- **MHC class III molecules.** These molecules are not MHC antigens in the classical sense, but are encoded within the MHC locus. These include complement (C) components C4 and C2, cytokines (IL, TNF), heat shock protein 70 (hsp70), and other products important for peptide presentation.

Protein Structure of MHC Class I Molecules

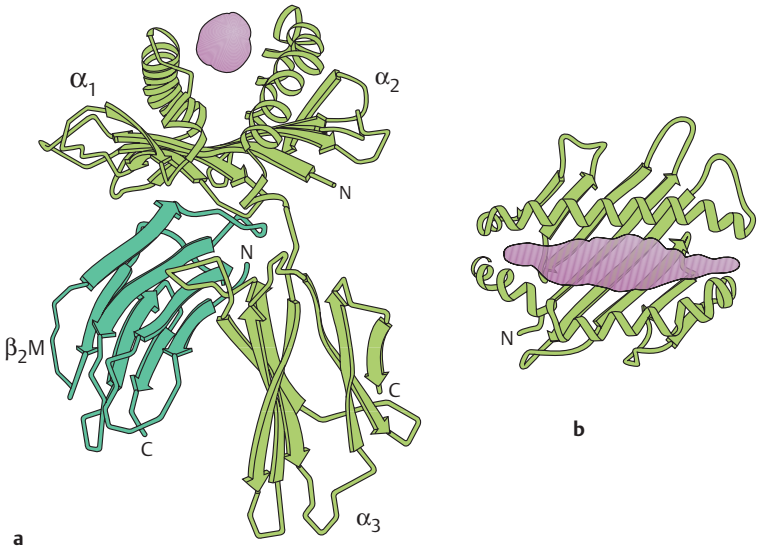


Fig. 2.**7** MHC class I translation antigen: **a** lateral view, **b** vertical view. The presenting peptide is shown in violet. The three domains of the heavy chain are α_1, α_2, and α_3. β_2 microglobulin (β_2M) functions as a light chain, and is not covalently bound to the heavy chain.

Functions of MHC molecules. MHC class I and II molecules function mainly as molecules capable of presenting peptides (Figs. 2.**7**–2.**9**). These two classes of MHC molecules present two different types of antigens:

- **Intracellular antigens**; these are cleaved into peptides within the proteasome and are usually associated with MHC class I molecules via the endogenous antigen processing pathway (Fig. 2.**8**, left side).
- **Antigens taken up from exogenous sources;** these are processed into peptides within phagolysosomes, and in most cases are then presented on MHC class II molecules on the cell surface (Fig. 2.**8**, right side). Within the phagolysosome, a fragment called the invariant chain (CLIP, class II-inhibiting protein) is replaced by an antigen fragment. This CLIP fragment normally blocks the antigen-binding site of the MHC class II dimer, thus preventing its occupation by other intracellular peptides.

The presentation groove of MHC class I molecules is closed at both ends, and only accommodates peptides of roughly 8–10 (usually 9) amino acids in

Presentation of Endogenous and Exogenous Antigens

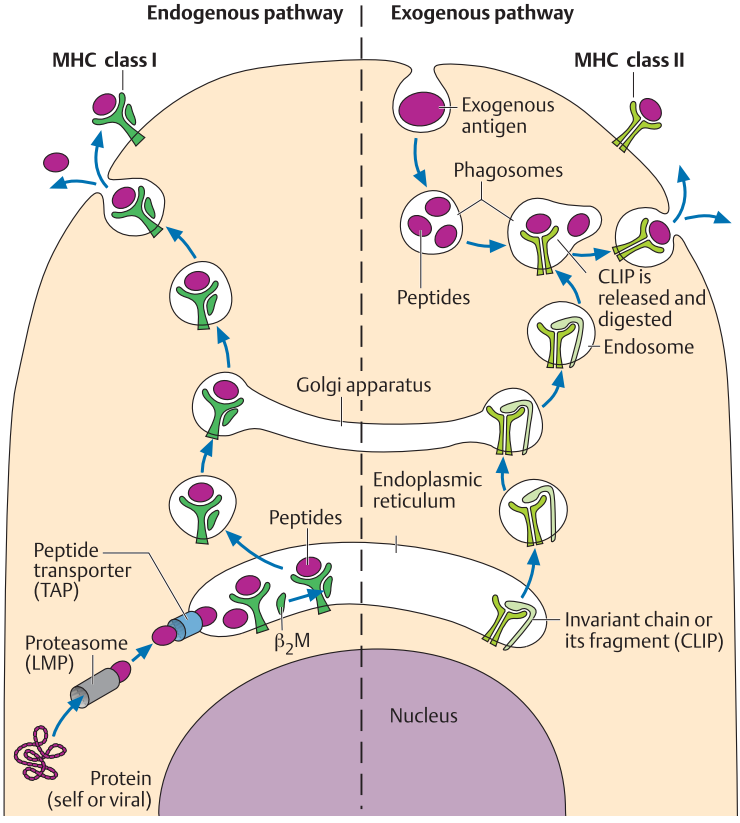


Fig. 2.**8** Intracellularly synthesized endogenous antigen peptides (left side) are bound to MHC class I molecules within the endoplasmic reticulum, fixed into the groove by β_2M, and presented on the cell surface. Antigens taken up from exogenous sources (right) are cleaved into peptides within phagosomes. The phagosome then merges with endosomes containing MHC class II molecules, the binding site of which had been protected by the so-called CLIP fragment. These two presentation pathways functionally separate MHC class I restricted $CD8^+$ T cells from MHC class II restricted $CD4^+$ T cells.

length. The groove of MHC class II molecules is open-ended, and can contain peptides of 9–15 (usually 10–12) amino acids in length.

T cells can only recognize antigenic peptides in combination with either MHC class I (which presents endogenous linear peptides, such as those derived from viruses) or MHC class II (which present exogenous linear peptides, such as those derived from bacterial toxins) (Table 2.**3**). In contrast to antibodies that recognize soluble, complex, nonlinear, three-dimensional structures—T-cell recognition is *restricted to changes on the surfaces of cells signaled via MHC plus peptide*.

T-cell specificity. T-cell recognition therefore involves two levels of specificity: first, **MHC presentation molecules** bind peptides with a certain degree of specificity as determined by the shape of the groove and the peptide anchoring loci. Second, the MHC-peptide complex will only be recognized by **specific T-cell receptors** (TCR) once a minimum degree of binding strength has been obtained. For this reason diseases associated with the HLA complex are determined largely by the quality of peptide presentation, but can also be influenced by the available TCR repertoire.

The structure of the MHC groove therefore determines which, of all the potentially recognizable, peptides will actually be presented as T-cell epitopes. Thus, the same peptides cannot function as T-cell epitopes in all individuals. Nonetheless, certain combinations of peptides and MHC are frequently observed. For example, approximately 50% of Caucasians carry the HLA-A2 antigen, although this is sometimes found in a variant form.

Antigen-presenting cells (APC). APCs belong to the lymphohematopoietic system. They attach peptides to MHC class II molecules for presentation to T cells, and induce T-cell responses. The complex mechanisms involved in this process have not yet been fully delineated. *Stromal cells* present in the **thymus and bone marrow** (i.e., connective tissue cells, dendritic cells and nurse cells in both thymus and bone marrow, plus *epithelial cells* in the thymus) can also function as APCs. The following cell types function as APCs in **peripheral secondary lymphoid organs**:

- Circulating monocytes.
- Sessile macrophages in tissues, microglia in the central nervous system.
- Bone marrow derived dendritic cells with migratory potential—these occur as cutaneous Langerhans cells, as veiled cells during antigen transfer into the afferent lymph vessels, as interdigitating cells in the spleen and lymph nodes, and as interstitial dendritic cells or as M cells within MALT.
- Follicular dendritic cells (FDC)—these are found within the germinal centers of the secondary lymph organs, do not originate in the bone marrow, and do not process antigens but rather bind antigen-antibody complexes via Fc receptors and complement (C3) receptors.

- B lymphocytes—these serve as a type of APC for T helper cells during T-B collaborations.

The consequences of MHC variation. Because every individual differs with regard to the set of polymorphic MHC molecules and self antigens expressed (with the exception of monozygotic twins and inbred mice of the same strain), the differences between two given individuals are considerable. The high degree of variability in MHC molecules—essential for the presentation of a large proportion of possible antigenic peptides for T-cell recognition—results in these molecules becoming targets for T cell recognition following cellular or organ transplantation resulting in **transplant rejection**. The term "transplantation antigens" is therefore a misnomer, and is only used because their real function was not discovered until a later time. Normally antigens are only recognized by T cells if they are associated with MHC-encoded self-structures. Transplant recognition, which apparently involves the imitation of the combination of a non-self antigen plus a self-MHC molecule, can therefore be considered an exception. The process probably arises from T-cell receptor cross-reactivity between host self-MHC antigens plus foreign peptides on the one hand, and non-self transplantation antigens associated with self-peptides from the donor on the other hand (for example, the T-cell receptor for HLA-A2 peptide X cross-reacts with HLA-A13 peptide Y). Transplant rejection is therefore a consequence of the enormous variety of combinations of antigenic peptide plus MHC, which is exhibited by each individual organism.

T-Cell Maturation: Positive and Negative Selection

Maturation of T cells occurs largely within the **thymus**. Fig. 2.**2** (p. 47) shows a schematic presentation of this process. Because the MHC-encoded presentation molecules are highly polymorphic, and are also subject to mutation, the **repertoire of TCRs is not genetically pre-determined**. One prerequisite for an optimal repertoire of T-cells is therefore the **positive selection** of T cells such that these preferentially recognize peptides associated only with self transplantation (MHC) antigens. A second prerequisite is **negative selection**, which involves the deletion of T cells that react too strongly against self MHC plus self peptide. The random processes governing the genetic generation of an array of T-cell receptors results $\alpha\beta$ or $\gamma\delta$ receptor chain combinations which are in the majority of cases are non-functional. Those T cells preserved through to maturity represent cells carrying receptors capable of effectively recognizing self-MHC molecules (positive selection). However, the T cells within this group which express too high an affinity for self-MHC plus self-peptides are deleted (negative selection).

The process of positive selection was demonstrated in experimental mice expressing MHC class I molecules of type b (MHC classI^b) from which the

thymus had been removed (and which therefore had no T cells). Implantation of a new thymus with MHC class I molecules of type a (MHC class I^a) into the MHC class I^b mice resulted in the maturation of T cells which only recognized peptides presented by MHC class I^a molecules, and not peptides presented by MHC class I^b molecules. However, recent experiments have shown that this is probable an experimental artefact and that it is not (or not solely) the thymic epithelial cells that determine the selection process, but that this process is driven by cells formed in the bone marrow. Positive selection is generally achieved by weak levels of binding affinity between the T-cell receptor and the self-MHC molecules, whereas negative selection eliminates those T cells exhibiting the highest levels of affinity (namely the self-or auto-reactive T cells) and absence of binding causes death by neglect. Thus, only T cells with moderate binding affinities are allowed to mature and exit the thymus. These T cells can potentially react to non-self (foreign) peptides presented by self MHC molecules. The enormous proliferation of immature thymocytes is paralleled by continuous cell death of large numbers of thymocytes (apoptosis, see summary in Fig. 2.**17**, p. 88). In general, the maturation and survival of lymphocytes is considered to be dependent on a continuous, repetitive, signaling via transmembrane molecules, and cessation of these signals is usually taken as a reliable indicator of cell death.

T-Cell Subpopulations

In order to recognize the presented antigen, T cells require the specific T-cell receptor and a molecule which functions to recognize the appropriate MHC molecules (i.e. CD4 or CD8 which recognize MHC class II and MHC class I, respectively). Thus T cells are classified into different subpopulations based on the CD4 or CD8 surface molecules:

$CD4^+$ T cells. These T cells recognize only MHC class II-associated antigens. They are also called **T helper cells** due to their important role in **T-B cell collaboration** (Fig. 2.**9a**), although they exhibit many other additional functions. $CD4^+$ cells can produce, or induce, the production *of cytokines* by which means they can activate macrophages and exercise a regulatory effect on other lymphocytes (see p. 75f.). Although these cells sometimes demonstrate an ability to cause cytotoxic destruction in vitro, this does not hold true in vivo.

$CD8^+$ T cells. Only MHC class I-associated antigens are recognized by the $CD8^+$ molecule. These cells are also known as **cytotoxic T cells** due to their ability to destroy histocompatible virus-infected, or otherwise altered, target cells as well as allogeneic cells. This effect can be observed both in vitro and in vivo (Fig. 2.**9b**). Costimulatory molecules are not required for this lytic

Interactions in T-Cell Antigen Recognition

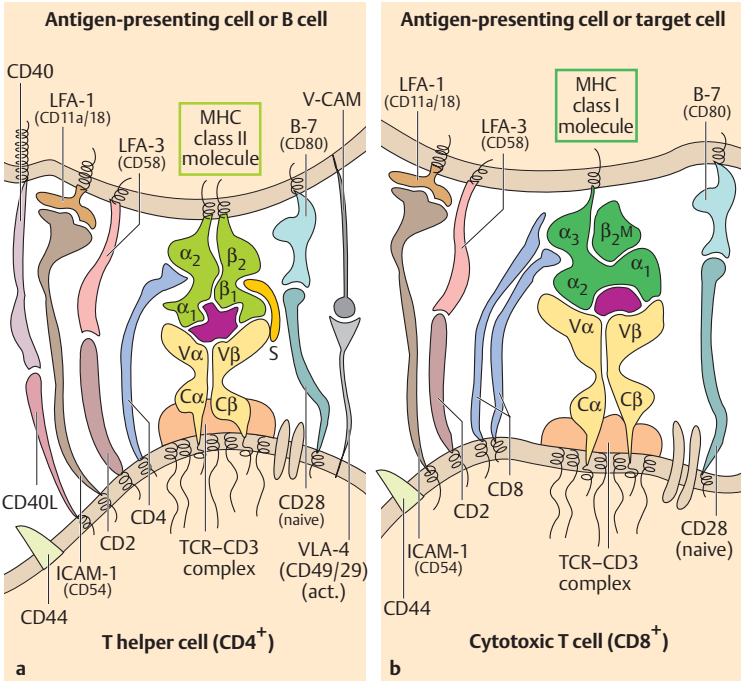


Fig. 2.9 **a** The interactions of APCs or B cells with CD4+ T cells (T helper cells) are mediated by MHC class II molecules (heterodimers). **b** Interactions between CD8+ T cells (cytotoxic T cells) and their target cells are mediated by MHC class I molecules. The presenting peptide is shown in violet. "S" indicates a superantigen, named after its capacity to activate many different T helper cells through its ability to bind to the constant regions of both the MHC and TCR molecules (naive = non-activated T cells, act. = activated T cells).

effector function. However, cytotoxicity is only one of several important functions expressed by CD8+ T cells. They also have many other non-lytic functions which they execute via the production, or induction of, cytokine release. The designation (CD8+) T suppressor cell is misleading and should not be used. It was originally coined to distinguish these cells from the function of T helper cells, mentioned above. However, plausible documentation of a suppressor effect by CD8+ T cells has only been obtained in a very small number of cases. In most cases, this suppressive effect can in fact be explained

by the direct elimination of APC (i.e., by changing the antigen kinetics), or indirectly via cytokine effects (see Fig. 2.**14**, p. 78). Thus, the name suppressor T cell suggests a regulatory function that in reality is unlikely to exist. In general, more neutral names, such as $CD4^+$ T cells or $CD8^+$ T cells, are preferable. Whereas the cytotoxic effector cells in the spleen and lymph nodes possess a heterodimeric ($\alpha + \beta$ chain) $CD8^+$ T molecule, the function of $CD8^+$ T cells found in the intestinal wall and expressing the α-homodimeric CD8 molecule remains unclear.

$\gamma\delta$ T cells. As for the homologous $\alpha\beta$ heterodimer, the $\gamma\delta$ T-cell receptor is associated with the CD3 complex within the cell membrane. The genetic sequence for the γ and δ chains resembles that of the α and β chains, however, there are a few notable differences. The gene complex encoding the δ chain is located entirely within the V and J segments of the α chain complex. As a result, any rearrangement of the α chain genes deletes the δ chain genes. There are also far fewer V segments for the γ and δ genes than for the α and β chains. It is possible that the increased binding variability of the δ chains makes up for the small number of V segments, as a result nearly the entire variability potential of the $\gamma\delta$ receptor is concentrated within the binding region (Table 2.**4**, p. 53). The amino acids coded within this region are presumed to form the center of the binding site.

T cells with $\gamma\delta$ receptors recognize certain *class I-like gene products in association with phospholipids and phosphoglycolipids.* In peripheral lymphoid tissues, only a small number of T cells express the $\gamma\delta$ and CD3 co-receptor, however, many of the T cells found within the mucosa and submucosa express $\gamma\delta$ receptors.

$\gamma\delta$ T cells can be negative for $CD4^+$ and $CD8^+$, or express two α chains (but no β chain) of the $CD8^+$ molecule. Although it is assumed that $\gamma\delta$ T cells may be responsible for early, low-specificity, immune defense at the skin and mucosa, their specificities and effector functions are still largely unknown.

Immune Responses and Effector Mechanisms

The effector functions of the immune system comprise antibodies and complement-dependent mechanisms within body fluids and the mucosa, as well as tissue-bound effector mechanisms executed by T cells and monocytes/macrophages. B cells are characterized by antigen specificity. Following antigen stimulation, specific B cells proliferate and differentiate into plasma cells that secrete antibodies into the surroundings. The type of B-cell response induced is determined by the amount and type of bound antigen recognized. Induction of an IgM response in response to antigens which are lipopolysaccharides—or which exhibit an highly organized, crystal-like

structure containing identical and repetitively arranged determinants—is a highly efficient and T cell-independent process which involves direct cross-linking of the B-cell receptor. In contrast to this process, antibody responses against monomeric or oligomeric antigens are less efficient and strictly require T cell help, for both non-self and self antigens.

Some forms of T-cell responses involve the release of soluble mediators (cytokines), which effectively expands the field of T cell function beyond individual cell-to-cell contacts to an ability to regulate the function of large numbers of surrounding cells. Other T-cell effector mechanisms are mediated in a more precise manner through cell-to-cell contacts. Examples of this include perforin-dependent cytolysis and induction of the signaling pathways involved in B-cell differentiation or Ig class switching.

B Cells

B-Cell Epitopes and B-Cell Proliferation

Burnet's **clonal selection theory**, formulated in 1957, states that every B-cell clone is characterized by an unique antigen specificity, i.e., it bears a specific antigen receptor. Accordingly, once rearrangement of the Ig genes has taken place, the corresponding protein will be expressed as a surface receptor. At the same time further rearrangement is stopped. Thus, only one ABS, or **one specificity** (one V_H plus V_L [either κ or λ]), derived from a single allele can be expressed on a single cell. This phenomenon is called **allelic exclusion**. The body faces a large number of different antigens in its lifetime, necessitating that a correspondingly large number of different receptor specificities, and therefore different B cells, must continuously be produced. When a given antigen enters an organism, it binds to the B cell which exhibits the correct receptor specificity for that antigen. One way to describe this process is to say that the antigen selects the corresponding B-cell type to which it most efficiently binds. However, as long as the responding B cells do not proliferate, the specificity of the response is restricted to a very small number of cells. For an effective response, **clonal** proliferation of the responsive B cells must be induced. After several cell divisions B cells differentiate into **plasma cells** which release the specific receptors into the surroundings in the form of soluble antibodies. B-cell stimulation proceeds with, or without, T cell help depending on the structure and amount of bound antigen.

Antigens. Antigens can be divided into two categories; those which stimulate B cells to secrete antibodies without any T-cell help, and those which require additional T-cell signals for this purpose.

Type 1 T-independent antigens (TI1). These include paracrystalline, identical epitopes arranged at approximately 5–10 nm intervals in a repetitive two-dimensional pattern (e.g., proteins found on the surface of viruses, bacteria, and parasites); and antigens associated with lipopolysaccharides (LPS). Thus TI1 antigens represent structures with a repetitive arrangement, which allows the engagement of several antigen receptors at one time and results in optimal Ig receptor cross-linking; or structures which result in sub-optimal cross-linking, but which are complemented by an LPS-mediated activation signal. Either type of antigen can induce B cell activation in the absence of T cell help.

Type 2 T-independent antigens (TI2). These antigens are less stringently arranged, and are usually flexible or mobile on cell surfaces. They can cross-link Ig receptors, but to a lesser extent than TI1 antigens. TI2 antigens require a small amount of indirectly associated T help in order to elicit a B-cell response (e.g., hapten-Ficoll antigens or viral glycoproteins on infected cell surfaces).

T help-dependent antigens. These are monomeric or oligomeric (usually soluble) antigens that do not cause Ig cross-linking, and are unable to induce B-cell proliferation on their own. In this case an additional signal, provided by contact with T cells, is required for B-cell activation (see also B-cell tolerance, p. 93ff.).

Receptors on the surface of B cells and soluble serum antibodies usually recognize epitopes present on the surface of native antigens. For protein antigens, the segments of polypeptide chains involved are usually spaced far apart when the protein is in a denatured, unfolded, state. A **conformational** or **structural epitope** is not formed unless the antigen is present in its native configuration. So-called **sequential** or **linear epitopes**—formed by contiguous segments of a polypeptide chain and hidden inside the antigen—are largely inaccessible to B cell receptors or antibodies, as long as the antigen molecule or infectious agent retains its native configuration. These epitopes therefore contribute little to biological protection. The specific role of linear epitopes is addressed below in the context of T cell-mediated immunity. B cells are also frequently found to be capable of specific recognition of sugar molecules on the surface of infectious agents, whilst T cells appear to be incapable of recognizing such sugar molecules.

Proliferation of B cells. As mentioned above, contact between one, or a few, B-cell receptors and the correlating antigenic epitope does not in itself suffice for the induction of B-cell proliferation. Instead proliferation requires either a high degree of B cell receptor cross-linking by antigen, or additional T cell-mediated signals.

Proliferation and the rearrangement of genetic material—a continuous process which can increase cellular numbers by a million-fold—occasionally

result in errors, or even the activation of oncogenes. The results of this process may therefore include the generation of **B-cell lymphomas and leukemia's**. Since the original error occurs in a single cell, such tumors are *monoclonal*. Uncontrolled proliferation of differentiated B cells (plasma cells) results in the generation of **monoclonal plasma cell tumors** known as multiple myelomas or plasmocytomas. Occasionally, myelomas produce excessive amounts of the light chains of the monoclonal immunoglobulin, and these proteins can then be detected in the urine as **Bence-Jones proteins**. Such proteins represented some of the first immunoglobulin components accessible for chemical analysis and they revealed important early details regarding immunoglobulin structure.

Monoclonal Antibodies

A normal immune response usually involves the response and proliferation of numerous B cell clones, bearing ABS with varying degrees of specificity for the different epitopes contained within the antigen. Thus the immune response is normally **polyclonal.** It is possible to isolate a single cell from such a polyclonal immune response in an experimental setting. Fusing this cell with an "immortal" proliferating myeloma cell results in generation of a **hybridoma**, which then produces chemically uniform immunoglobulins of the original specificity, and in whatever amounts are required. This method was developed by Koeler and Milstein in 1975, and is used to produce **monoclonal antibodies** (Fig. 2.**10**), which represent important tools for experimental immunology, diagnostics, and therapeutics. Many monoclonal antibodies are still produced in mouse and rat cells, making them xenogeneic for humans. Attempts to avoid the resulting rejection problems have involved the production of antibodies by human cells (which remains difficult), or the "humanization" of murine antibodies by recombinant insertion of the variable domains of a murine antibody adjacent to the constant domains of a human antibody. The generation of a transgenic mice, in which the Ig genes have been replaced by human genes, has made the production of hybridoma's producing completely human antibodies possible.

T-Independent B Cell Responses

B cells recognize antigens via the Ig receptor. However, if the antigen is in a monomeric, or oligomeric, soluble form the B cell can only mount a response if it undergoes the process of T-B collaboration. Many infectious pathogens carry surface antigens with **polyclonal activation properties** (e.g., lipopolysaccharide [LPS]) and/or crystal-like identical determinants, which are often

Production of Monoclonal Antibodies

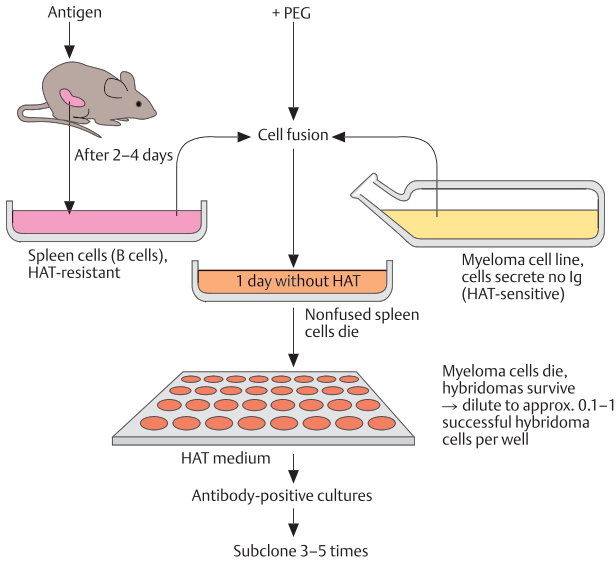


Fig. 2.**10** Monoclonal antibodies are produced with the help of cell lines obtained from the fusion of a B lymphocyte to an immortal myeloma cell. In the first instance, mice are immunized against an antigen. They then receive a second, intravenous, dose of antigen two to four days before cell fusion. Then spleen cells are removed and fused to the myeloma cell line using polyethylene glycol (PEG). Those spleen cells that fail to fuse to a myeloma cell die within one day of culture. Next, the fused cells are subjected to selection using HAT medium (hypoxanthine, aminopterin, thymidine). Aminopterin blocks specific metabolic processes, but with the help of the intermediary metabolites (hypoxanthine and thymidine) spleen cells are able to complete these processes using auxiliary pathways. The myeloma cells, on the other hand, have a metabolic defect which prevents them from utilizing such alternative pathways and resulting in the death of those cells cultured in HAT medium. However, once a spleen cell has fused with a myeloma cell, the fused spleen-myeloma product (hybridoma) is HAT-resistant. In this way only the successfully fused cells will be able to survive several days of culture on HAT medium. After this time, the cell culture is diluted such that there is, ideally, only one hybridoma within each well. Individual wells are then tested for the presence of the desired antibody. If the result is positive, the hybridoma cells are subcloned several times to ensure clonality; with the specificity of the produced antibody being checked following each round to subcloning. Production of purely human monoclonal antibodies is carried out using mice whose Ig genes have been completely replaced by human Ig genes.

repeated in a *regular pattern* (linear e.g., flagella, or two-dimensional e.g., viruses) with intervals of 5–10 nm. These paracrystalline-patterned antigens are capable of inducing B-cell responses without contact-dependent T cell help. This probably occurs by means of *maximum Ig receptor cross-linking*. Such B-cell responses are usually of the **IgM type**, since switching to different isotype classes is either impossible or very inefficient in the absence of T cell help. The IgM response is of a relatively brief duration (exhibiting a half-life of about 24 h), but can nonetheless be highly efficient. Examples of this efficiency include IgM responses induced by many viral envelope antigens which bear neutralizing ("protective") determinants accessible to the corresponding antibodies, and responses to bacterial surface antigens (e.g., flagellae, lipopolysaccharides) or parasites.

T Cells

T-Cell Activation

There are two classes of T cells; T helper cells ($CD4^+$) and cytotoxic T cells ($CD8^+$). Table 2.**5** summarizes the reliance of T-cell responses on the dose, localization, and duration of presence of antigen. T-cell stimulation via the TCR, accessory molecules and adhesion molecules results in the activation

Table 2.**5** Dependence of T-Cell Response on Antigen Localization, Amount, and Duration of Presence

Antigen			T-cell response
Localization	**Amount**	**Duration of presence**	
Thymus	Small-large	Always	Negative selection by deletion
Blood, spleen, lymph nodes (secondary lymphoid organs)	Small	Short (1 day)	No induction
	Small	Long (7 days)	Induction
	Large	Short	No induction
	Large	Long (>10 days)	Exhaustive induction/ deletion (anergy?)
Peripheral non lymphoid tissue	Large or small	Always or short	Ignorance, indifference

of various tyrosine kinases (Fig. 2.**11**) and mediates stringent and differential regulation of several signaling steps. T-cell induction and activation result from the activation of two signals. In addition to *TCR activation (signal 1 = antigen)*, a *costimulatory signal (signal 2)* is usually required. Important costimulatory signals are delivered by the binding of B7 (B7.1 and B7.2) proteins (present on the APC or B cell) to ligands on the T cells (CD28 protein, CTLA-4), or by CD40–CD40 ligand interactions. T-cell expansion is also enhanced by IL-2.

T-Cell Activation by Superantigens

In association with MHC class II molecules, a number of bacterial and possibly viral products can efficiently stimulate a large repertoire of $CD4^+$ T cells at one time. This is often mediated by the binding of the bacterial or viral product to the constant segment of certain Vβ chains (and possibly Vα chains) with a low level of specificity (see Fig. 2.**9a**, p. 65). Superantigens are categorized as either exogenous or endogenous. **Exogenous superantigens** mainly include *bacterial* toxins (staphylococcus enterotoxin types A-E [SEA, SEB, etc.]), toxic shock syndrome toxin (TSST), toxins from *Streptococcus pyogenes*, and certain retroviruses. **Endogenous superantigens** are derived from components of certain retroviruses found in mice, and which display superantigen-like behavior (e.g., murine mammary tumor virus, MMTV). The function of superantigens during T cell activation can be compared to the effect of bacterial lipopolysaccharides on B cells, in that LPS-induced B cell activation is also polyclonal (although it functions by way of the LPS receptors instead of the Ig receptors (see below)).

Interactions between Cells of the Immune System

T Helper Cells ($CD4^+$ T Cells) and T-B Cell Collaboration

Mature T cells expressing CD4 are called T helper (Th) cells (see also p. 64f.), reflecting their role in co-operating with B cells. Foreign antigens, whose three-dimensional structures are recognized by B cells, also contain linear peptides. During the **initial phase** of the T helper cell response, these antigens are taken up by APCs, processed, and presented as peptides in association with **MHC class II molecules**—allowing their recognition by Th cells (see Fig. 2.**8**, p. 61 and Fig. 2.**13**, p. 76). Prior to our understanding of MHC restriction, B-cell epitopes were known as **haptens,** whilst those parts of the antigens which bore the T-cell epitope were known as **carriers**. In order

T-Cell Activation

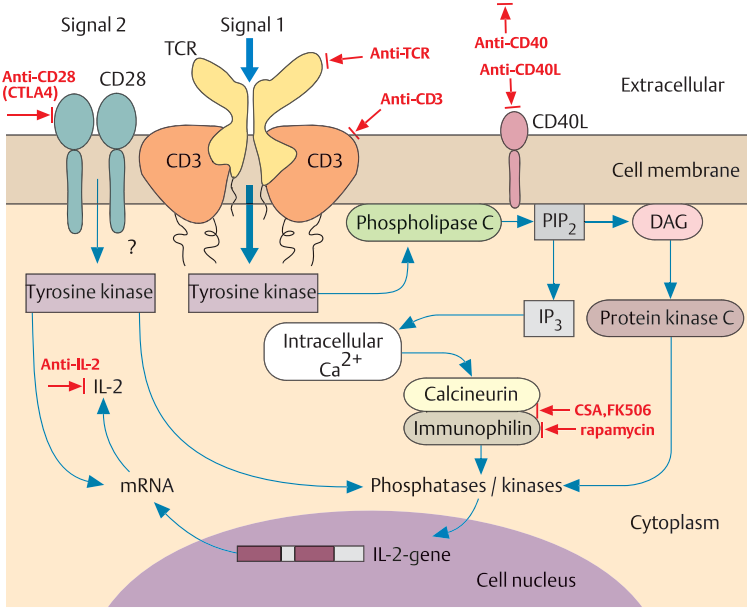


Fig. 2.**11** Regulation of T-cell activation is controlled by multiple signals, including costimulatory signals (Signal 2). Stimulation of the T cell via the T-cell receptor (TCR; Signal 1) activates a tyrosine kinase, which in turn activates phospholipase C (PLC). PLC splits phosphatidylinositol bisphosphate (PIP_2) into inositol trisphosphate (IP_3) and diacyl glycerol (DAG). IP_3 releases Ca^{2+} from intracellular depots, whilst DAG activates protein kinase C (PKC). Together, Ca^{2+} and PKC induce and activate the phosphoproteins required for *IL-2* gene transcription within the cell nucleus. Stimulation of a T cell via the TCR alone results in production of only very small amounts of IL-2. Increased IL-2 production often requires additional signals (costimulation, e.g., via CD28). Costimulation via CD28 activates tyrosine kinases, which both sustain the transcription process and ensure post-transcriptional stabilization of IL-2 mRNA. Immunosuppressive substances (in red letters) include cytostatic drugs, anti-TCR, anti-CD3, anti-CD28 (CTLA4), anti-CD40, cyclosporine A and FK506 (which interferes with immunophilin-calcineurin binding, thus reducing IL-2 production), and rapamycin (which binds to, and blocks, immunophilin and hardly reduces IL-2 at all). Anti-interleukins (especially anti-IL-2, or a combination of anti-IL-2 receptor and anti-IL-15) block T-cell proliferation.

for T cell activation to occur, antigen-transporting APCs must first reach the *organized secondary lymphoid organs* (Fig. 2.**12**), since proper contact between lymphocytes and APCs can only take place within these highly organized and

Structure of Lymph Nodes and Germinal Centers

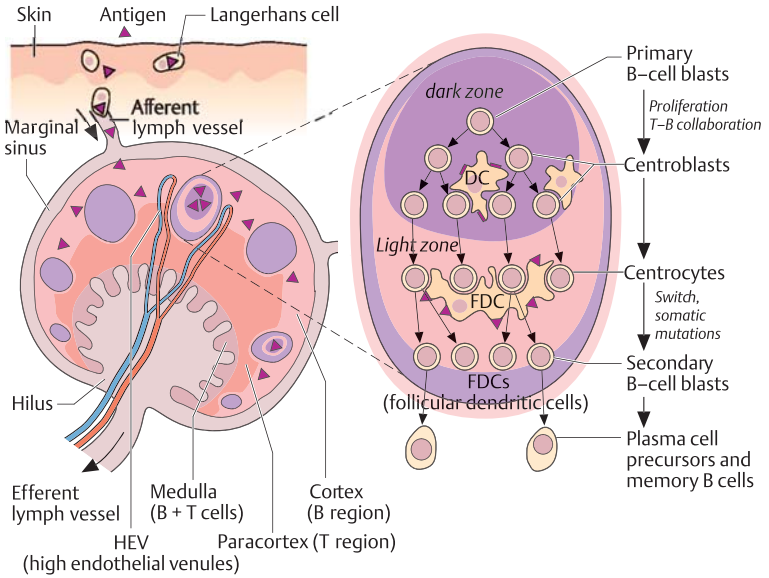


Fig. 2.**12** Antigen carried by antigen-presenting cells (e.g., Langerhans cells in the skin which have taken up local antigens), or soluble antigens enter the marginal sinus of the lymph node through afferent lymphatic vessels. In the spleen, blood-borne antigens are taken up by specialized macrophages present in the marginal zone (marginal zone macrophages, MZM). Each lymph node has its own arterial and venous vascularization. T and B cells migrate from blood vessels, through specialized venules with a high endothelium (HEV: high endothelial venules), into the paracortex which is largely comprised of T cells. Clusters of B cells (so-called primary follicles) are located in the cortex, where following antigen-stimulation, secondary follicles with germinal centers develop (right side). Active B-cell proliferation occurs at this site. Differentiation of B cells begins with the proliferation of the primary B-cell blasts within the dark zone and involves intensive interaction with antigen-presenting dendritic cells (DC). Antibody class switching and somatic mutation follows and takes place in the light zone, where FDC (follicular dendritic cells) stimulate B cells and store the antigen-antibody complexes that function to preserve antibody memory. Secondary B-cell blasts develop into either plasma cells or memory B cells. Lymphocytes can only leave the lymph nodes through efferent lymph vessels.

compartmentalized organs. The cytokines IFNγ, IL-1, IL-2, IL-4, and IL-12 play an important role in this process—as do various other factors.

During the **second phase** (Fig. 2.**13**), *activated T helper cells* recognize the same MHC class II peptide complex, but on the surface of a **B cell**. Prior to this event, the B cell must have responded to the same antigen (by virtue of its Ig surface receptor recognizing a conformational antigenic epitope), then internalized the antigen, processed it, and finally presented parts of it in the form of linear peptides bound to MHC class II molecules on the cell surface for recognition by the T helper cell. The resulting B-T cell contact results in further interactions mediated by CD4, CD40, and CD28 (see Fig. 2.**9**, p. 65)—and sends a signal to the B cell which initiates the switch from IgM to IgG or other Ig classes. It also allows induction of a process of somatic mutation, and probably enhances the survival of the B cell in the form of a memory B cell.

Subpopulations of T Helper Cells

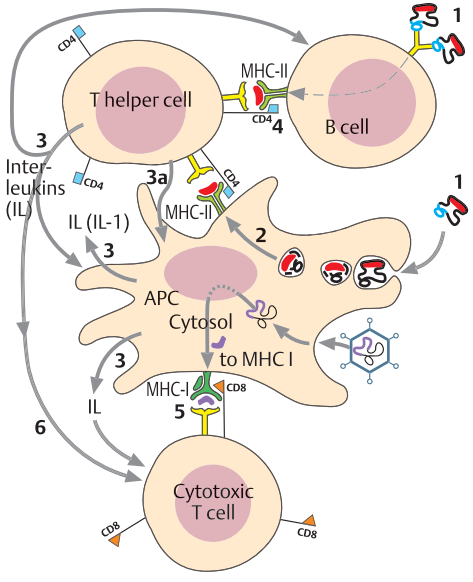
Soluble signaling substances, cytokines (interleukins), released from T helper cells can also provide an inductive stimulus for B cells. Two subpopulations of T helper cells can be differentiated based on the patterns of cytokines produced (Fig. 2.**14**). Infections in general, but especially those by intracellular parasites, induce cytokine production by natural killer (NK) cells in addition to a strong **T helper 1 (TH1) response**. The response by these cells is characterized by early gamma interferon (IFNγ) production, increased levels of phagocyte activity, elimination of the antigen by IFNγ-activated macrophages, production of IgG2a and other complement-binding (opsonizing) antibodies (see the complement system, pp. 86ff.), and induction of cytotoxic T-cell responses. IL-12 functions as the most important promoter of TH1 cell function and additionally acts as an inhibitor of TH2 cells.

In contrast, worm infections or other parasitic diseases induce the early production of IL-4, and result in the development of a **TH2 response**. TH2 cells, in turn, recruit eosinophils and induce production of *IgG1* and *IgE* antibodies. Persons suffering from allergies and atopic conditions show a pathologically excessive TH2 response potential. IL-4 not only promotes the TH2 response but also inhibits TH1 cells.

Cytotoxic T Cells ($CD8^+$ T Cells)

Mature $CD8^+$ T cells perform the biologically important function of lysing target cells. Target cell recognition involves the association of **MHC class I** structures with peptides normally derived from endogenous sources, i.e., originating in the cells themselves or synthesized within them by intracellular parasites. Induction of cytotoxic $CD8^+$ T cell response often does not require helper

Lymphocytes and APCs in the Primary Immune Response



cells—or only requires these cells indirectly. However, should the antigen stimulus and the accompanying inflammation be of a low-level nature, the quantity of cytokines secreted by the cytotoxic T cells themselves may not suffice, in which case the induction of a $CD8^+$ T cell response will be reduced unless additional cytokines are provided by helper T cells. The cytotoxic activity of $CD8^+$ T cells is mediated via *contact and perforin release* (perforin renders the membrane of the target cell permeable resulting in cellular death). $CD8^+$ T cells also function in *interleukin release* (mainly of IFNγ) by which they mediate non-cytotoxic effector functions (Fig. 2.**15**). The role of **perforin** in **contact-dependent direct cytolysis** by natural killer (NK) cells and cytotoxic T cells (see also Fig. 2.**17**, p. 88) has been investigated in gene knockout mice. In these animals the *perforin* gene has been switched off by means of homologous recombination, and as a result they can no longer produce perforin. Perforin-dependent cytolysis is important for the control of *noncytopathic viruses, tumors,* and *transformed cells*, but also plays a large role in the control of highly virulent viruses that produce syncytia (e.g., the smallpox virus). Release of **noncytolytic effector molecules** by $CD8^+$ cells, mostly IFNγ, plays a major role in control of *cytopathic viruses* and *intracellular bacteria*. Cytolytic effector mechanisms may also contribute to release of intracellular micro-organisms and parasites (e.g., tuberculosis) from cells that only express MHC class I.

◀ Fig. 2.**13** For the sake of simplicity, the principles illustrated here are based on an antigen (1) which only contains a single B epitope and a single T epitope. As an example, the structural B epitope (blue) is present on the surface of the antigen; whilst the linear T epitope (red) is hidden inside it. An antigen-presenting cell (APC), or macrophage, takes up the antigen and breaks it down in a non-specific manner. The T-cell epitope is thus released and loaded onto MHC class II molecules which are presented on the cell surface (2). A T helper cell specifically recognizes the T epitope presented by the MHC class II molecule. This recognition process activates the APC (3a) (or the macrophages). T cells, APC, and macrophages all produce cytokines (Fig. 2.**14**), which then act on T cells, B cells, and APCs (causing up-regulation of CD40, B7)(3). This in turn stimulates the T cells to proliferate, and encourages the secretion of additional signaling substances (IL-2, IFNγ, IL-4, etc.). A B cell whose surface Ig has recognized and bound a B epitope present on the intact antigen, will present the antigenic T cell epitope complexed to MHC class II on its cell surface, in a manner similar to that described for the APC (4). This enables direct interaction between the T helper cell and the specific B cell, resulting in induction of proliferation, differentiation, and B-cell class switching from IgM to other Ig classes. The B cell finally develops into an antibody-producing plasma cell. The antibody-binding site of the produced antibody thus fits the B epitope on the intact antigen. The induction of cytotoxic effector cells by peptides presented on MHC class I molecules (violet) is indicated in the lower part of the diagram (5). The cytotoxic T cell precursors do not usually receive contact-mediated T help, but are rather supported by secreted cytokines (mainly IL-2) (6). (Again, in the interest of simplicity, the CD3 and CD4 complexes and cytokines are not shown in detail; see Fig. 2.**8**, p. 61 for more on antigen presentation.)

Cytokines (Interleukins) and Adhesion

Cytokines are bioactive hormones, normally glycoproteins, which exercise a wide variety of biological effects on those cells which express the appropriate receptors (Table 2.**6**). Cytokines are designated by their cellular origin such that **monokines** include those interleukins produced by macrophages/monocytes, whilst **lymphokines** include those interleukins produced by lymphocytes. The term **interleukins** is used for cytokines which mostly influence cellular interactions. All cytokines are cyto-regulatory proteins with molecular weights under 60 kDa (in most cases under 25 kDa). They are produced locally, have very short *half-lives* (a matter of seconds to minutes), and are effective at *picomolar concentrations*. The effects of cytokines may be *paracrine* (acting on cells near the production locus), or *autocrine* (the same cell both produces, and reacts to, the cytokine). By way of interaction with highly specific cell surface receptors, cytokines can induce cell-specific or more general effects (including mediator release, expression of differen-

CD4+ T Helper Cell Subpopulations and APCs during Immune Responses

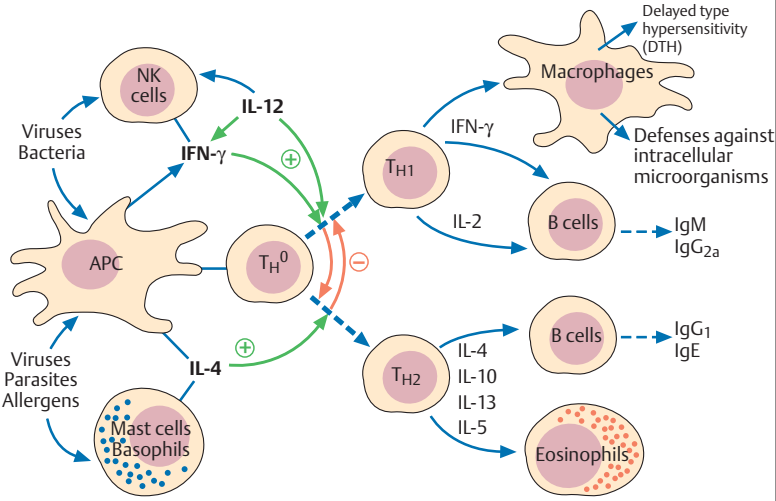


Fig. 2.**14** TH1 and TH2 cells are derived from a TH0 cell, and undergo differentiation in the presence of help derived from cytokines, DC, macrophages, and other cell types. TH1 cells are activated by IL-12 and IFNγ and inhibited by IL-4; whilst for TH2 cells the reverse is true. Viruses and bacteria (particularly intracellular bacteria) can induce a TH1 response by activating natural killer cells. In contrast, allergens and parasites induce a TH2 response via the release of IL-4. However, the strong in-vitro differentiation of CD4+ T cells into TH1–TH2 subsets is likely to be less sharply defined in vivo.

tiation molecules and regulation of cell surface molecule expression). The functions of cytokines are usually *pleiotropic*, in that they display a number of effects of the same, or of a different, nature on one or more cell types. Below is a summary of cytokine functions:

- Promotion of inflammation: IL-1, IL-6, TNFα, chemokines (e.g., IL-8).
- Inhibition of inflammation: IL-10, TGFβ.
- Promotion of hematopoiesis: GM-CSF, IL-3, G-CSF, M-CSF, IL-5, IL-7.
- Activating B cells: CD40L, IL-6, IL-3, IL-4.
- Activating T cells: IL-2, IL-4, IL-10, IL-13, IL-15.
- Anti-infectious: IFNα, IFNβ, IFNγ, TNFα.
- Anti-proliferative: IFNα, IFNβ, TNFα, TGFβ.

Antiviral Protection by T Cells

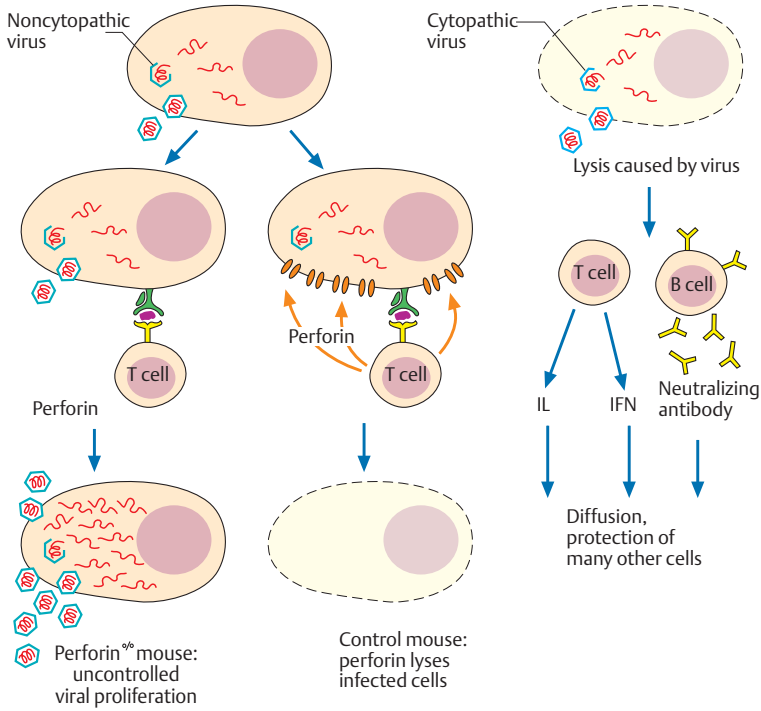


Fig. 2.**15** Certain viruses destroy the infected host cells (right), others do not (left). Cytotoxic T cells can destroy freshly infected cells by direct contact (with the help of perforin), thus inhibiting viral replication (middle). Whether the result of this lysis is clinically desirable depends on the balance between protection from viral proliferation, and the damage caused by immunologically mediated cell destruction. In perforin knockout mice (perforin$^{o/o}$), T cells are unable to produce perforin and therefore do not destroy the infected host cells. Replication of non-cytopathic viruses thus continues unabated in these mice. Soluble anti-viral interleukins (especially IFNγ and TNFα), and neutralizing antibodies, combat cytopathic viruses (which replicate comparatively rapidly) more efficiently than do cytolytic T cells; this is because interleukin and antibody molecules can readily diffuse through tissues and reach a greater number of cells, more rapidly, than can killer T cells.

Table 2.6 The Most Important Immunological Cytokines and Costimulators plus Their Receptors and Functions

Cytokines/costimulators/chemokines	Receptor	Cytokines/cytokine receptors produced by	Functions
Interleukins			
IL-1	CD121 (α)β	Macrophages Endothelial cells	Hypothalamic fever, NK cell activation, T and B stimulation
IL-2 (T-cell growth factor)	CD25 (α) CD122 (β), γc	T cells	T-cell proliferation
IL-3 (multicolony stimulating factor)	CD123, βc	T cells, B cells, thymic epithelial cells	Synergistic effect in hematopoiesis
IL-4 (BCGF-1, BSF-1) (B-cell growth factor, B-cell stimulating factor)	CD124, γc	T cells, mast cells	B-cell activation, switch to IgE
IL-5 (BCGF-2)	CD125, βc	T cells, mast cells	Growth and differentiation of eosinophilis
IL-6 (interferon/IFNβ_2, BSF-2, BCDF)	CD126, CD_w130	T cells, macrophages	Growth and differentiation of T and B cells, acute-phase immune response
IL-7	CD_w127, γc	Bone marrow stroma	Growth of pre-B and pre-T cells
IL-10		T cells	Macrophages, reduction of TH1 cytokines
IL-9	IL-9R, γc	T cells	Effect on mast cells
IL-10		T helper cells (especially mouse TH2), macrophages, Epstein-Barr virus	Efficient inhibitor for macrophage functions, inhibits inflammatory reactions
IL-11	IL-11R, CD_w130	Stromal fibroblasts	Synergistic effect with IL-3 and IL-4 in hematopoiesis

Table 2.6 *Continued: The Most Important Immunological Cytokines. . .*

Cytokines/costimulators/chemokines	Receptor	Cytokines/cytokine receptors produced by	Functions
IL-12		B cells, macrophages	Activates natural killer cells, induces differentiation of $CD4^+$ T cells into TH1-like cells, encourages IFNγ production
IL-13	IL-13R, γc	T cells	Growth and differentiation of B cells, inhibits production of inflammatory cytokines by means of macrophages
IL-15	IL-15R, γc	T cells, placenta, muscle cells	IL-2-like, mainly intestinal effects
GM-CSF (granulocyte macrophage colony stimulating factor)	CD_w116, βc	Macrophages, T cells	Stimulates growth and differentiation of the myelomonocytic lineage
LIF (leukemia inhibitory factor)	LIFR, CD_w130	Bone marrow stroma, fibroblasts	Maintains embryonal stem cells; like IL-6, IL-11
Interferons (IFN)			
IFNγ	CD119	T cells, natural killer cells	Activation of macrophages, enhances MHC expression, antiviral
IFNα	CD118	Leukocytes	Antiviral, enhances MHC class I expression
IFNβ	CD118	Fibroblasts	Antiviral, enhances MHC class I expression
Immunoglobulin superfamily			
B7.1 (CD80)	CD28 (promoter); CTLA-4 (inhibitor)	Antigen-presenting cells	Costimulation of T cell responses
B7.2 (CD86)	CD28; CTLA-4	Antigen-presenting cells	Costimulation of T cell responses

Table 2.6 *Continued: The Most Important Immunological Cytokines. . .*

Cytokines/costimulators/chemokines	Receptor	Cytokines/cytokine receptors produced by	Functions
TNF (tumor necrosis factor) family			
TNFα (cachexin)	p55, p75, CD120a, CD120b	Macrophages, natural killer cells	Local inflammations, endothelial activation
TNFβ (lymphotoxin, LT, LTα)	p55, p75, CD120a, CD120b	T cells, B cells	Endothelial activation, organization of secondary lymphoid tissues
LTβ		T cells, B cells	Organization of secondary lymphoid tissues
CD40 ligand (CD40-L)	CD40	T cells, mast cells	B-cell activation, class switching
Fas ligand	CD95 (Fas)	T cells	Apoptosis, Ca^{2+}-independent cytotoxicity
Chemokines			
IL-8 (prototype) CXCL8	CXCR1, CXCR2	Activated endothelium, activated fibroblasts	Attraction of neutrophils, degranulation of neutrophils
MCP-1 (monocyte chemoattractant protein) CCL2	CCR2	Activated endothelium, tissue macrophages, synovial cells	Inflammation
MIP-1α (macrophage inflammatory protein) CCL3	CCR5, CCR1	T cells, activated Mϕ	Proinflammatory HIVα receptor
MIP-1β CCL4	CCR5	T cells, activated Mϕ	Proinflammatory HIVα receptor
RANTES (regulated on activation, normal T cell expressed and secreted) CCL5	CCR5, CCR1, CCR3	T cells, blood platelets	Inhibits cellular entry by M-trophic HIV, proinflammatory
IP-10 (interferon gamma-inducible protein) CXCL10	CXCR3	Inflamed tissue due to effects of IFNγ	Proinflammatory

Table 2.6 *Continued: The Most Important Immunological Cytokines. . .*

Cytokines/costimulators/chemokines	Receptor	Cytokines/cytokine receptors produced by	Functions
Chemokines			
MIG (monokine induced by interferon gamma) CXCL11	CXCR3	Inflamed tissue, due to effects of IFNγ	Proinflammatory
Eotaxin CCL22	CCR3	Endothelium, epithelial cells	Buildup of infiltrate in allergic diseases, e.g., asthma
MDC (macrophage-derived chemokine)	CCR4	T-cell zone DCs, activated B cells, monocytes	Supports T-B cell collaboration during humoral immune responses
Fractalkine CXCL1	CX_3CR1	Intestinal epithelium, endothelium	Endothelial cells activation of thrombocytes
Constitutive chemokines			
LARC (liver and activation-regulated chemokine) MIP-3α	CCR6	Intestinal epithelia, Peyer's patches	Participation in mucosal immune responses
SLC (secondary lymphoid organ chemokine)	CCR7	High endothelial lymph nodes, T-cell zone	Facilitates entry of naive T cells, contact between T cells and DCs
TECK (thymus-expressed chemokine)	CCR9	Thymic and intestinal epithelia	Presumed role in T-cell selection
SDF-1α (stromal cell-derived factor)	CXCR4 (also known as fusin)	Stromal cells of bone marrow	Involved in hematopoiesis, inhibits cellular entry by T-trophic HIV
BCA-1 (B-cell attractant)	CXCR5	Follicular DCs (?)	Contact between TH and B cells, and between TH and follicular DCs
Others:			
TGFβ (transforming growth factor β)		Many cells, including monocytes and T cells	Inhibits cell growth, inhibits macrophages and production of IL-1 and TNFα, represents a switching factor for IgA

Cell adhesion molecules often play an essential role in cell-to-cell interactions. Two lympho-hematopoietic cells can only establish contact if one of them expresses surface molecules that interact with ligands expressed on the surface of the other cell. As for APC and T cell interactions, the result of such contact may be that a signal capable of inducing differentiation and functional changes will be induced. Adhesion proteins are usually comprised of several chains which can induce different effects when present in various combinations. Interaction of several cascades is often required for the final differentiation of a cell. Cell adhesion molecules normally form part of the Ig superfamily (e.g., ICAM, VCAM, CD2), integrin family (lymphocyte function antigen, LFA-1), selectin family, cadherin family, or various other families. Selectins and integrins also play an important role in interactions between leukocytes and the vascular wall, and thus mediate the migration of leukocytes from the bloodstream into inflamed tissues, or the entry of recirculating lymphocytes into the lymph node parenchyma through high endothelial venules (HEV).

Chemokines (*chemo*attractant cyto*kines*) comprise a family of over 30 small (8–12 kDa) secreted proteins. These contribute to the recruitment of "inflammatory cells" (e.g., monocytes) into inflamed tissues, and influence the recirculation of all classes of leukocytes (Table 2.**6**). Some chemokines result in the activation of their target cell in addition to exerting chemotatic properties. Chemokines can be classified into three families based on their N terminus structure: *CC chemokine*s feature two contiguous cysteine residues at the terminus; *CXC chemokine*s have an amino acid between the two residues; and *CX3C* and *C chemokine*s thus far comprise only one member each (fractalkine and lymphotactin, respectively). Although the N terminus carries bioactive determinants, using a chemokines amino acid sequence to predict its biological function is not reliable. The chemokine system forms a redundant network, or in other words, a single chemokine can often act upon a number of receptors, and the same receptor may recognize a number of different chemokines. Many of the chemokines also overlap in terms of biological function.

Chemokines can be grouped in two functional classes: *inflammatory chemokines* which are secreted by inflamed or infected tissues as mediators of the nonspecific immune response; and *constitutive chemokines* which are produced in primary or secondary lymphoid organs. Together with endothelial adhesion molecules, inflammatory chemokines determine the cellular composition of the immigrating infiltrate. In contrast, the function of constitutive chemokines is to direct lymphocytes to precise locations within lymphoid compartments. Thus, chemokines play a major role in the establishment of inflammatory and lymphoid microenvironments. Chemokine receptors are G protein-coupled membrane receptors with seven transmembrane sequences. In keeping with the above nomenclature, they are designated as CCR, CXCR, or CX3CR plus consecutive numbering. Some viruses, for instance

the cytomegaly virus, encode proteins that are functionally analogous to chemokine receptors. This allows a rapid neutralization of locally induced chemokines, and may offer an advantage to the virus. The Duffy antigen receptor for chemokines, DARC, is expressed on endothelial cells and is capable of a high-affinity binding interaction with various chemokine types. Since this receptor has no downstream signaling cascade, it is assumed to function in the presentation of chemokines to leukocytes as they flow past. DARC also functions as a receptor for *Plasmodium vivax*. CCR5 and CXCR4 are co-receptors for HIV infection of CD4+ T cells.

Antibody-Dependent Cellular Immunity and Natural Killer Cells

Lymphocytes can nonspecifically bind IgG antibodies by means of Fc receptors, then specifically attack targets cells (e.g., infected or transformed cells) using the bound antibody. This phenomenon, known as **antibody-dependent cellular cytotoxicity (ADCC)**, has been demonstrated in vitro—however its in-vivo function remains unclear. **Natural killer (NK) cells** also play a role in ADCC. The genesis of NK cells appears to be mainly thymus-independent. These cells can produce IFNγ very early following activation and do not require a specific receptor. These cells are therefore early contributors to the IFNγ-oriented TH1 immune response. NK cells can respond to cells that *do not express MHC class I* molecules, and are inactivated by contact with MHC molecules. This recognition process functions via special receptors that are not expressed in a clonal manner. NK cells probably play an important role in the *early defensive* stages of infectious diseases, although the exact nature of their role remains to be clarified (virus-induced IFNα and IFNβ promote NK activation). NK cells also appear to contribute to rejection reactions, particularly the rejection of stem cells.

Humoral, Antibody-Dependent Effector Mechanisms

The objectives of the immune response include: the inactivation (neutralization) and removal of foreign substances, microorganisms, and viruses; the rejection of exogenous cells; and the prevention of proliferation of pathologically altered cells (tumors). The systems and mechanisms involved in these effector functions are largely non-specific. Specific immune recognition by B and T cells directs these effector mechanisms to specific targets. For instance, immunoglobulins opsonize microbes (e.g., pneumococci) which are equipped with polysaccharide capsules enabling them to resist phagocyte

digestion. **Opsonization** involves the coating of such microbes with Fc-expressing antibodies which facilitates their phagocytosis by granulocytes. Many cells, particularly phagocytes (and interestingly enough also some bacteria like staphylococci), bear surface Fc receptors that interact with different Ig classes and subclasses. Mast cells and basophils bear IgE molecules, and undergo a process of degranulation following interaction with allergens against which the IgE molecules are directed. This induces the release of pharmacologically active biogenic amines (e.g., histamine). In turn, these amines represent the causative agent for physiological and clinical symptoms observed during allergic reactions (see also types I-IV, p. 108ff.).

The Complement System

The complement system (C system, Fig. 2.**16**) represents a non-specific defense system against pathogens, but can also be directed toward specific targets by antibodies. It is made up of a co-operative network of plasma proteins and cellular receptors, and is largely charged with the following tasks:

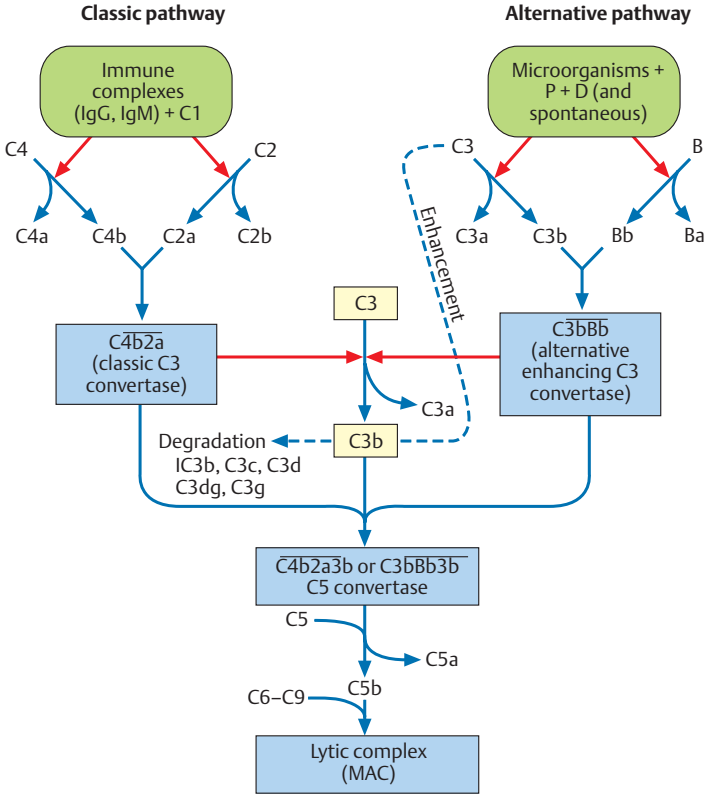
- **Opsonization** of infectious pathogens and other foreign substances, with the aim of more efficient pathogen elimination. Bound complement factors can: enhance the binding of microbes to phagocytozing cells; result in the activation of inflammatory cells; mediate chemotaxis; induce release of inflammatory mediators; direct bactericidal effects; and induce cell lysis (Fig. 2.**17**, p. 88).

Fig. 2.**16** The classic activation pathway is initiated by antigen-antibody complexes, the alternative pathway by components of microbial pathogens. The production of a C3 convertase, which splits C3 into C3a and C3b, is common to both pathways. C3b combines with C3 convertase to generate C5 convertase. C5b, produced by C5 convertase, binds to the complement factors 6–9 to form a membrane attack complex (MAC). C3b degradation products are recognized by receptors on B lymphocytes; they stimulate the production of antibodies as well as pathogen phagocytosis. The cleavage products C3a and C4a are chemotactic in their action, and stimulate expression of adhesion molecules.
Nomenclature: the components of the alternative pathway (or *cascade*) are designated by capital letters (B, D, H, I; P for properdin), those of the classical pathway (or *cascade*) plus terminal lysis are designated by "C" and an Arabic numeral (1–9). Component fragments are designated by small letters, whereby the first fragment to be split off (usually of low molecular weight) is termed "a" (e.g., C3a), the remaining (still bound) part is called "b" (e.g., C3b), the next split-off piece "c," and so on. Molecules often group to form complexes; in their designations the individual components are lined up together and are usually topped by a line. ▶

- **Solubilization** of otherwise insoluble antigen-antibody complexes.
- **Promotion of the transport** of immune complexes, and their elimination and degradation.
- **Regulation of the immune response**, achieved via their influence on antigen presentation and lymphocyte function.

Over 20 proteins of the complement system have been identified to date, and are classified as either activation or control proteins. These substances account for about 5% of the total plasma proteins (i.e., 3–4 g/l). C3 is not only present in the largest amount, but also represents a central structure for complement activation. A clear difference exists between "classic"

The Complement System: Classic and Alternative Activation



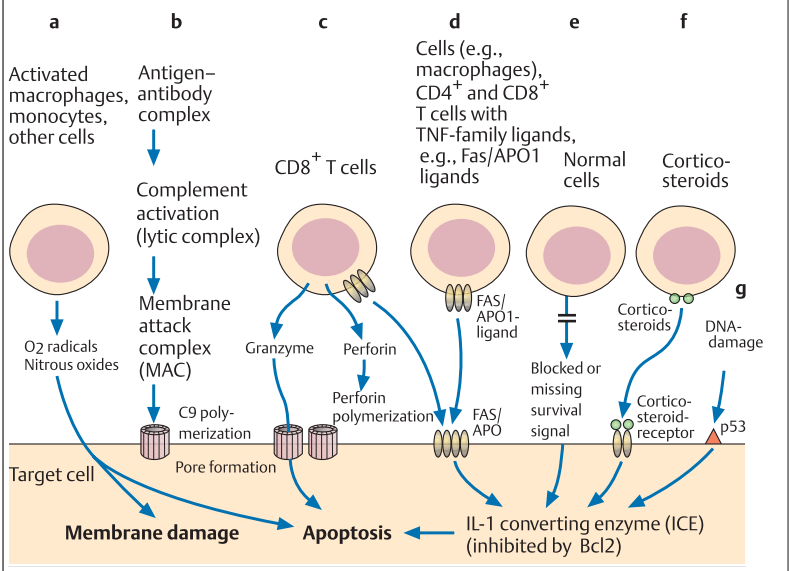


Fig. 2.**17** Oxygen radicals and nitrous oxides (**a**), MAC resulting from complement activation (**b**) and perforin (**c**) all cause membrane damage which results in cell death. Ligand binding of Fas/APO (**d**), interrupted signal receptor conduction (**e**), corticosteroid binding to receptors and intracellular structures (**f**), and DNA damage (**g**) all result in alterations of intracellular signaling cascades and lead to cellular apoptosis. (Fas = F antigen; APO = apoptosis antigen; TNF = tumor necrosis factor; Bcl2 = B-cell leukemia-2 antigen [a protein that inhibits apoptosis].)

antibody-induced complement activation and "alternative" activation via C3 (Fig. 2.**16**).

During **classic activation** of complement, C1q must be bound by at least two antigen-antibody immune complexes, to which C4 and C2 then attach themselves. Together, these three components form a C3 convertase, which then splits C3. Pentameric IgM represents a particularly efficient C activator since at least two Ig Fc components in close proximity are required for C1q binding and activation.

During **alternative activation** of complement, the splitting of C3 occurs directly via the action of products derived from microorganisms, endotoxins, polysaccharides, or aggregated IgA. C3b, which is produced in both cases, is activated by the factors B and D, then itself acts as C3 convertase. Subsequent formation of the lytic complex, C5–C9 (C5–9), is identical for both classic

and alternative activation, but is not necessarily essential since the released chemotaxins and opsonins are often alone enough to mediate the functions of microbe neutralization and elimination. Some viruses can activate the complement system without the intervention of antibodies by virtue of their ability to directly bind C1q. This appears to be largely restricted to retroviruses (including HIV). Importantly, without a stringent control mechanism complement would be activated in an uncontrolled manner, resulting in the lysis of the hosts own cells (for instance erythrocytes).

Complement Control Proteins

The following regulatory proteins of the complement system have been characterized to date:
C1 inhibitor, prevents classic complement activation.
DAF (decay accelerating factor), prevents the association of C3b with factor B, or of C4b with C2, on the cell surface. DAF can also mediate the dissolution of existing complexes, and is responsible for the regulation of classic and alternative C activities.
MCP (membrane cofactor protein), enhances the activity of the factor which degrades C3b to iC3b. Factor H and CR1 (complement receptor 1) have similar effects.
HRF (homologous restriction factor). Synonyms: MAC (membrane attack complex), inhibitory protein, C8-binding protein. HRF protects cells from C5-9-mediated lysis. This protein is lacking in patients suffering from paroxysmal nocturnal hemoglobinuria.
CD59. Synonyms: HRF20, membrane attack complex (MAC)-inhibiting factor, protectin. This is a glycolipid anchored within the cell surface which prevents C9 from binding to the C5b-8 complex, thus protecting the cell from lysis.

Those complement components with the most important biological effects include:

- **C3b**, results in the opsonization of microorganisms and other antigens, either directly or in the form of immune complexes. "C-marked" microorganisms then bind to the appropriate receptors (R) (e.g., CRI on macrophages and erythrocytes, or CR2 on B cells).
- **C3a and C5a**, contribute to the degranulation of basophils and mast cells and are therefore called anaphylatoxins. The secreted vasoactive amines (e.g., histamine) raise the level of vascular permeability, induce contraction of the smooth musculature, and stimulate arachidonic acid metabolism. C5a initiates the chemotactic recruitment of granulocytes and monocytes, promotes their aggregation, stimulates the oxidative processes, and promotes the release of the thrombocyte activating factor.
- **"Early" C factors,** in particular **C4**, interact with immune complexes and inhibit their precipitation.
- **Terminal components (C5–9),** together form the so-called membrane attack complex, MAC, which lyses microorganisms and other cells.

Some components mediate general regulatory functions on B-cell responses, especially via **CR1** and **CR2**.

Immunological Cell Death

Fig. 2.**17** summarizes the mechanisms of cell death resulting from immunological cell interactions and differentiation processes, as they are understood to date.

Immunological Tolerance

T-cell tolerance, as defined by a lack of immune reactivity can be due to a number of processes: Firstly, Negative selection in the thymus (referred to as deletion); secondly a simple lack of reactivity to antigen (self or nonself) as a result of the antigen having not been present in the secondary lymphoid organs in a sufficient quantity or for a sufficient amount of time; and thirdly an excessive stimulation of T-cells resulting from the ubiquitous presence of sufficient antigen resulting in T cell exhaustion. Finally, it may also be possible that T cells can become temporarily "anergized" by partial or incomplete antigen stimulation. As a general rule, self-reactive (autoimmune) **B cells** are not generally deleted by negative selection and can therefore be present in the periphery. Exceptions to this rule include B cells specific for membrane-bound self-determinants, some of which are deleted or anergized. B cells react promptly to antigens, even self-antigens, which are arranged repetitively. However, they only react to soluble monomeric antigens if they additionally receive T cell help. Thus, B-cell non-reactivity largely results from a lack of patterned antigen presentation structures or as a result of T-cell tolerance.

Immunological tolerance describes the concept that the immune system does not normally react to autologous structures, but maintains the ability to react against foreign antigens. Tolerance is acquired, and can be measured as the selective absence of immunological reactivity against specified antigens.

T-Cell Tolerance

A distinction can be made between **central tolerance**, which develops in the thymus and is based on the *negative selection* (deletion) of T cells recognizing self antigens present in the thymus, and **peripheral tolerance**. Peripheral tolerance results in the same outcome as central tolerance, however, this

form of tolerance involves antigen recognition by antigen-reactive peripheral T cells, followed by a process of clonal cell proliferation, end differentiation and death. The following mechanisms have been postulated, and in some cases confirmed, to account for a lack of peripheral T-cell responsiveness (Table 2.**5**, p. 71):

T-cell indifference or ignorance. Both host and foreign antigens present only within peripheral epithelial, mesenchymal or neuroectodermal cells and tissues—and which do not migrate, or are not transported by APCs, in sufficient amounts to the organized lymphoid organs—are simply ignored by T and B cells. Most self-antigens, not present in the serum or in lymphohematopoietic cells, belong to this category and are ignored despite the fact that they are potentially immunogenic. Certain viruses, and their antigens, actually take advantage of this system of ignorance. For instance, the immune system ignores the rabies virus when it is restricted to axons, and papilloma viruses as long as the antigens are restricted to keratinocytes (warts). The main reason why many self antigens, and some foreign antigens, are ignored by T cells is that immune responses can only be induced within the spleen or in lymph nodes, and non-activated (or naive) T cells do not migrate into the periphery. It has also been postulated that those naive T and B cells which do encounter antigens in the periphery will become anergized, or inactivated, due to a lack of the so-called costimulatory or secondary signals at these sites. However, the evidence supporting this theory is still indirect. Experiments seeking to understand the "indifference" of T cells are summarized in the box on p. 92f. In all probability, a great many self-antigens (as well as peripheral tumors) are ignored by the immune system in this way. These self-antigens represent a potential target for autoimmunity.

Complete, exhaustive T-cell induction. When an antigen, self or non-self, enters a lymphoid organ it encounters many APCs and T cells, resulting in the extremely efficient activation those T cells carrying the appropriate TCR. During such a scenario the responding T cells differentiate into short-lived effector cells which only survive for two to four days. This induction phase may actually correspond to the postulated phenomenon of anergy (see Table 2.**5**, p. 71). Should this be the case, anergy—defined as the inability of T cells to react to antigen stimulation in vitro—may in fact be explained by the responding cells having already entered a pathway of cell death (apoptosis) (see Fig. 2.**17**, p. 88). Once all the terminally differentiated effector T cells have died, immune reactivity against the stimulating antigen ends. Tolerance is hereafter maintained, as should the responsible antigen have entered into the thymus those newly maturing thymocytes will be subjected to the process of negative selection (e.g., as seen in chronic systemic (viremic) infections with noncytopathic viruses). Moreover, those newly matured T cells which may have escaped negative selection and emigrated into the per-

iphery will continuously be induced to undergo activation and exhaustion within the secondary lymphoid organs.

Exhaustive T-cell induction most likely occurs in responses to hepatitis C virus and HIV, and has been observed in mice experimentally infected with the noncytopathic virus causing lymphocytic choriomeningitis. Successful establishment of lymphocyte chimerism following liver transplants appears to based on the same principle. For example, a relatively short period of immunosuppression following transplantation may allow the establishment of numerous dendritic cells from the transplanted organ within the secondary lymphoid organs of the recipient, resulting in the subsequent elimination of those recipient T cells which react against the foreign MHC molecules.

Two Important Experiments addressing the induction of Immune Responses

APCs transport antigens to the peripheral lymphoid organs via the lymph vessels. *Skin flap experiment***.** To prove that antigens contacted at peripheral localizations (e.g. the skin) must first be transported on APCs *through the lymph vessels* into the local lymph node, in order to induce an immune response—an experiment was performed in which a guinea pig skin flap was prepared such that the supply vessels (lymph vessel, vein and artery) remained intact and functional.
Following sensitization of the skin flap with a contact antigen the animal reacted to a second antigenic exposure of the remaining (intact) skin with accelerated kinetics. When the lymph vessel leading from the prepared skin flap to the lymph node was interrupted, or the draining lymph node was destroyed prior to the initial sensitization, the typical secondary response was not observed—leading to the conclusion that *no T cell response was induced*. Following an initial sensitization at any other location on the skin the secondary response was observed, even on the skin flap regardless of interruption of the lymph vessel or destruction of the draining lymph node. This result indicated that the antigen-experienced effector lymphocytes reached the site of antigen via the bloodstream.
Many self antigens are ignored by $CD8^+$ cells. *A Transgenic mouse encoding a viral glycoprotein gene***.** As a comparison to the many self-antigens present in the peripheral non-lymphoid organs and cells, a gene encoding a viral glycoprotein (GP) was incorporated into mice, under the control of a regulatory gene which allowed GP expression only within the pancreatic insulin-producing β cells. This artificially integrated "self antigen" was ignored by the host's immune system, as indicated by the absence of β cell destruction or autoimmunity (diabetes). When the GP expressing transgenic mouse was infected with a virus encoding the *GP* gene, which infects lymphoid organs, GP-specific cytotoxic T cells were induced and these cells destroyed the transgenic islet cells, resulting in the onset of diabetes.
This model demonstrated that many self-antigens are ignored by the immune system simply because they are only present outside of the lymphatic system. However, should such antigens enter the immune system in a suitable form (in this case by viral infection) the host will produce an autoimmune T-cell response.

In summary, the non-responsiveness of T-cells can be achieved by: *negative selection in the thymus*; by *excessive induction in the periphery;* or by *sequestration* of the antigen outside the lymphoid organs. Persistence of the antigen within the lymphoid tissues is a prerequisite for the first two mechanisms. For the third mechanism, it is the absence of antigen within lymphatic organs which guarantees non-responsiveness. There is also a necessary role for 'second'- or 'costimulatory'-signals in the activation of T cells within lymphoid tissues, however, their role in T-cell responsiveness within solid organs remains unclear.

B-Cell Tolerance

In contrast to classic central T-cell tolerance, B cells capable of recognizing self-antigens appear *unlikely to be subjected to negative selection* (Table 2.**7**). B-cell regeneration in the bone marrow is a very intensive process, during which antigen selection probably does not play an important role. Although negative selection of bone marrow B cells can be demonstrated experimentally for highly-expressed membrane-bound MHC molecules (in antibody-transgenic mice)—this apparently does not occur for more rare membrane-bound antigens, or for most soluble self-antigens. As a general rule, these potentially self-reactive B cells are not stimulated to produce an immune response because the necessary T helper cells are not present as a result of having being subjected to negative selection in the thymus. B cell and antibody tolerance is therefore largely a result of T cell tolerance which results in the *absence of T help*.

The finding that a certain antigenic structures and sequences can activate B cells in the absence of T help indicates that autoreactive B cells which are present could be prompted to produce an IgM autoantibody response via Ig cross-linking by paracrystalline multimeric antigens. However, since self-antigens are not normally accessible to B cells in such *repetitive paracrystalline patterns*, the induction of IgM autoantibody responses is not normally observed. It is interesting to note that DNA and collagen, which often contribute to chronic autoantibody responses, exhibit repetitive antigen structures. These structures become accessible to B cells within inflamed lesions, and may therefore induce autoantibody responses in certain circumstances. A chronic autoantibody response of the IgG type, however, always requires T help arising from the presentation of self-peptides by MHC class II molecules. Ignored self-peptides, and in all likelihood infectious agents, may play a role in providing such T help. (For instance *Klebsiella* or *Yersinia* in rheumatic diseases, *Coxsackie* virus infections in diabetes, or other chronic parasitic infections.)

Table 2.7 B Cells Do Not Differentiate between Self and Nonself Antigens, but Rather Distinguish Repetitive (Usually Nonself) from Monomeric (Usually Self) Antigens

Antigen			B cells present	IgM response		
				T cell-independent	T help present	T cell-dependent
On cell membranes in the bone marrow	High concentration	Self	Unclear –	–	–	–
	Low concentration	Self	+[1]	+[1]	–	+[1]
Monomeric antigen	High concentration	Self	+[1]	not applicable	–	–
	Low concentration	Nonself	+	not applicable	+	+
Repetitive, identical 5–10 nm intervals, paracrystalline		Self (very rare)[2]	+	(+)[2]	(+)	(+)
		Nonself ("always" infectious)	+	+	+	++

[1] B cells are present and are stimulated by antigen arranged in a repetitive and paracrystalline pattern (T helper-independent type I). B-cell responses to poorly organized or monomeric antigens are not directly induced; in such cases, indirect (T helper-independent type II) or conventionally coupled T help is required.

[2] Such self antigens are not normally accessible to B cells; however collagens presents in lesions, or acetylcholine receptors, may stimulate and possibly activate B cells. When combined with T help, this activation can result in an autoimmune response.

Immunological Memory

Immunological memory is usually defined by an earlier and better immune response, mediated by increased frequencies of specific B or T cells as determined by in vitro or adoptive transfer experiments. **B-cell** immunological memory is more completely described as the ability to mediate protective immunity by means of increased antibody concentrations. Higher frequencies of specific B and T lymphocytes alone, appears to only provide limited

or no protection. Instead, immunological protection requires **antigen-dependent activation of B and T cells**, which then produce antibodies continuously or can rapidly mediate effector T functions and can rapidly migrate into peripheral tissues to control virus infections.

Usually the second time a host encounters the same antigen its immune response is both accelerated and augmented. This **secondary immune response** is certainly different from the **primary response**, however, it is still a matter of debate as to whether these parameters alone correlate with immune protection. It is not yet clear whether the difference between a primary and secondary immune response results solely from the increased numbers of antigen-specific B and T cells and their acquisition of "memory qualities", or whether immune protection is simply due to continuous antigen-induced activation (Table 2.**8**).

Table 2.**8** Characteristics of T- and B-Cell Memory

	Memory T cells		**Memory B cells**	
	Resting	Activated	Resting	Activated
Localization and migration	Blood, spleen, lymph nodes	Blood, spleen, lymph nodes, and solid tissues	Blood, spleen, lymph nodes	Germinal centers in local lymph nodes, bone marrow
Function	Secondary T-cell response	Immediate target cell lysis and interleukin release	Secondary B-cell response	Sustained IgG response
Time lapse to protective response	Slow	Fast	Slow	Immediate
Proliferation and location of proliferation	In secondary lymphoid organs	Only in secondary lymphoid organs with antigen residues	Blood, spleen	Germinal centers with antigen-IgG complexes
Antigen dependence	No	Yes	No	Yes

There is no surface marker which can unequivocally differentiate between memory T and B cells and "naive" (never before activated) cells. Instead, immunological memory is normally taken to correlate with an increased number of specific precursor T and B cells. Following an initial immunization with antigen, this increased precursor frequency of specific cells is thought to be maintained by an antigen-independent process. Yet the precursor cells can only be activated (or re-activated) by antigen, and only *activated T cells* can provide immediate protection against re-infection outside the lymphoid organs, e.g., in the solid peripheral organs. Similarly, *only antigen activated B cells can mature to become plasma cells* which maintain the increased blood antibody titers responsible for mediating protection. This indicates that residual antigen must be present to maintain protective immunological memory. As a general rule, the level of protective immunity mediated by the existence of memory T and B cells per se is minimal. Highly effective immunity and resistance to re-infection are instead provided by migratory T cells which have been recently activated (or re-activated) by antigen, and by antibody-secreting B cells. **B-cell and antibody memory** is maintained by re-encounters with antigen, or by antigen-IgG complexes which by virtue of their Fc portions or by binding to C3b are captured by-, and maintained for long periods on-, *follicular dendritic cells* present in germinal centers. **Memory T cells**, and in some cases **B cells**, can be re-stimulated and maintained in an active state by: persistent infections (e.g., tuberculosis, hepatitis B, HIV); antigen deposits in adjuvants; periodic antigen re-exposure; peptide-loaded MHC molecules with long half-lives; or possibly (but rarely) by cross-reactive antigens. Thus, secondarily activated (protective) memory T and B cells cannot easily be distinguished from primarily activated T and B cells. The antigen-dependent nature of immunological protection indeed questions the relevance of a specialized "memory quality" of B and T cells.

B-Cell Memory

It is important to differentiate between the characteristics of memory T and B cells as detected in vitro, and the salient in-vivo attributes of improved immune defenses. Following a primary immune response, increased numbers of memory B cells can of course be detected using in vitro assays or by murine experiments involving the transfer of cells into naive recipients. However, these increased B cell frequencies do not necessarily ensure immune protection against, for instance, viral re-infection. Such protection requires the existence of an increased titer of protective antibodies within the host.

Why is Immunological Memory Necessary?

A host which does not survive an initial infection obviously does not require further immunological memory. On the other hand, survival of the initial infection proves that the host's immune system can control or defeat the infection, once again apparently negating the need for immunological memory. Even assuming that better immune defenses provide a clear evolutionary advantage, especially during pregnancy, the idea of immunological memory must be understood as protection within a developmental framework:

1. Due to MHC restriction of T-cell recognition, it is not possible for a mother to pass on T-cell immunological experience to her progeny as the histoincompatibility reaction would induce mutual cellular rejection. For the same reason, a child's T cells apparently cannot mature until relatively late in its development (usually around the time of birth). This explains why newborns are almost entirely lacking in active immune defenses (Fig. 2.**18**). Newborn mice require about three to four weeks (humans three to nine months) before the T-cell immune response and the process of T-B cell collaboration which results in the generation of antibody responses become fully functional. During this period passive immune protection is essential. This type of protection is mediated by the transfer of protective, largely IgG, antibodies from mother to child through the placenta during pregnancy, and to some extent within the mother's milk. An example of this is provided by cattle where the acquisition of colostral milk by the calf is essential to its survival. Calves can only access protective IgG through the colostral milk delivered during the first 24 hours after birth (fetal calf serum contains no Ig). During the first 18 hours post partum, the calf's intestine expresses Fc receptors which allow the uptake of undigested antibodies from the mothers milk into the bloodstream. How can comprehensive, transferable, antibody-mediated protection be ensured under these conditions? During a three-week murine or 270-day human pregnancy, mothers do not normally undergo all of the major types of infection (indeed infection can be potentially life-threatening for both the embryo/fetus and the mother), and so the array of antibodies required for comprehensive protection cannot be accumulated during this period alone. Instead, an accumulation of the immunological protective antibody levels representing the **immunological life experience of infections in the mother's serum** is necessary. The female sex hormones also encourage Ig synthesis, correlating with women's higher risk level (about fivefold) for developing autoantibody diseases (e.g., lupus), and for autoimmune diseases in general.

2. Reproduction requires a relatively **good level of health** and a good nutritional status **of the mother**. However, it also requires an effective immune defense status within the population (herd), including males, since all would otherwise be threatened by repeated and severe infections. The increased frequency of specific precursor B and T cells improves immune defenses against such infections. However, this relative protection is in clear contrast to the absolute protection an immunoincompetent newborn requires to survive.

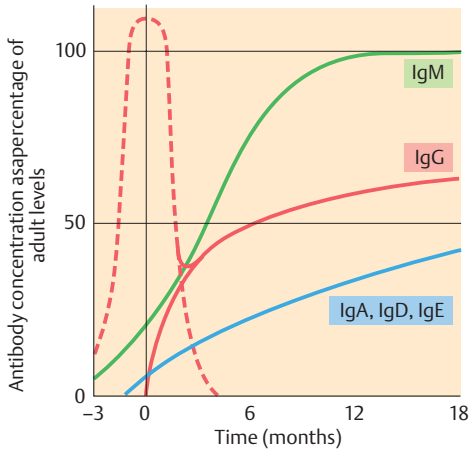


Fig. 2.**18** Synthesis of significant amounts of immunoglobulins only begins during the perinatal period (uninterrupted lines). IgG from the mother is therefore the child's main means of protective immunity before the age of three to six months (dotted line). Infections encountered during this early period are attenuated by maternal antibodies, rendering such infections vaccine-like.

T-Cell Memory

As with B cells and antibodies, enhanced defenses against intracellular pathogens (especially viruses and intracellular bacteria) does not solely depend on increased numbers of specific T cells, but rather is determined by the *activation status* of T cells. Here again it must be emphasized that protective immunological memory against most bacteria, bacterial toxins, and viruses, is mediated by *antibodies*! *Memory T cells* are nonetheless important in the *control of intracellular bacterial infections* (e.g., tuberculosis [TB], leprosy), as well as persistent *noncytopathic viruses* such as hepatitis B and HIV (see also p. 106). It has been demonstrated, at least in mouse models, that a higher number of T cells alone is often insufficient for the protection of the host against the immunopathological consequences of a defensive $CD8^+$ T-cell response. Yet such T cell responses must be activated in order to provide immunity. In the case of *tuberculosis*, sustained activation of a controlled T-cell response by minimal infection foci was postulated, and confirmed, in the 1960s as constituting **infection immunity—**i.e. the lifelong, and usually effective, immune control of the disease by an ongoing localized low-level of infection. A similar situation is observed for cell-mediated immune responses against leprosy, salmonellae, and numerous parasitic diseases (often together with antibodies). The existence of infection-immunity explains why apparently controlled, minimal, infections tend to *flare up* when the immune system is *compromised* by cytostatic drugs, age, or HIV infection. Delayed type

(dermal) hypersensitivity (DTH, see below and p. 114f.) can be applied diagnostically to determine infection immunity (for example against tuberculosis and leprosy), since the existence of continued infection continuously activates those T cells required for both pathogen control and DTH reactions.

Delayed Dermal Hypersensitivity Reaction

The classic example of a **delayed type hypersensitivity (DTH)** reaction is the **tuberculin reaction** (Mantoux test in humans). It was one of the first specific cell-mediated immune responses to be identified—as early as the 1940s in guinea pigs. The response is specific for MHC class II antigens and is $CD4^+$ T cell-dependent. In some cases, especially during active viral infections, a DTH reaction is transiently observed and is mediated by $CD8^+$ T cells. The simplest way to elicit a DTH reaction is to introduce a diagnostic protein, obtained from the pathogen, into the skin. The test reaction will only develop should continuously activated T cells be present within the host, since only these cells are capable of migrating to dermal locations within 24–48 hours. If no activated T cells are present, re-activation within the local lymph nodes must first take place, and hence migration into the dermis will require more time. By this time the small amount of introduced diagnostic peptide, or protein, will have been digested or will have decayed and thus will no longer be present at the injection site in the quantity required for induction of a local reaction.
A positive delayed hypersensitivity reaction is, therefore, an indicator of the presence of activated T cells. The absence of a reaction indicates either that the host had never been in contact with the antigen, or that the host no longer possesses activated T cells. In the case of tuberculosis, a negative skin test can indicate that; no more antigen or granuloma tissue is present, or that the systemic immune response is massive and the pathogen is spread throughout the body. In the latter case, the amount of diagnostic protein used is normally insufficient for the attraction of responsive T cells to the site of injection, and as a consequence no measurable reaction becomes evident (so that the Mantoux test may be negative in Landouzy sepsis or miliary tuberculosis). DTH reactions provide a diagnostic test for **tuberculosis** (Mantoux test), **leprosy** (lepromin test), and **Boeck's sarcoid** (Kveim test). However, these dermal reactions may disappear in those patients that are immunosuppressed or infected with measles or AIDS.

Immune Defenses against Infection and Tumor Immunity

Protection against infections can be mediated by either; non-specific defense mechanisms (interferons, NK cells), or specific immunity in the form of antibodies and T cells which release cytokines and mediate contact- and perforin-dependent cell lysis. Control of cytopathic viruses requires soluble factors (antibodies, cytokines), whilst control of noncytopathic viruses

and tumors is more likely to be mediated via perforins and cytolysis. However, cytotoxic immune responses can also cause disease, especially during noncytopathic infections. Development of an evolutionary balance between infectious agents and immune responses is an ongoing process, as reflected by the numerous mechanisms employed by pathogens and tumors to evade immune-mediated defenses.

All immune defense mechanisms (see Fig. 2.**1**, p. 44) are important in the battle against infections. Natural humoral mechanisms (antibodies, complement, and cytokines) and cellular mechanisms (phagocytes, natural killer cells, T cells) are deployed by the immune system in different relative amounts, during different phases of infection, and in varying combinations. Gross simplifications are not very helpful in the immunological field, but a small number of tenable rules can be defined based on certain model infections. Such models are mainly based on experiments carried out in mice, or on clinical experience with immunodeficient patients (Fig. 2.**19**).

General Rules Applying to Infection Defenses

- **Non-specific defenses** are very important (e.g., Toll-like receptors, IFNα/β), and 'natural immunity' (meaning not intentionally or specifically induced) represented by natural antibodies, direct complement activation, NK cell and phagocytes, plays a significant role in all infections. However, much remains to be learned about their roles.
- **Antibodies** represent potent effector molecules against acute bacterial infections, bacterial toxins, viral re-infections, and in many cases against acute cytopathic primary viral infections (e.g., rabies and influenza). Antibodies are also likely to make a major contribution to the host-parasite balance occurring during chronic parasitic infections. IgA is the most important defense mechanism at mucosal surfaces (Fig. 2.**5**, p. 57).
- **Perforin-dependent cytotoxicity in $CD8^+$ T cells** is important for defense against noncytopathic viruses, for the release of chronic intracellular bacteria, and for protection against intracellular stages of certain parasites.
- **Nonlytic T-cell responses** provide protection in the form of cytokines (very important cytokines include IFNγ and TNFα), which promote the enhanced digestion and destruction of intracellular bacteria and parasites (e.g., listeria, leishmania, etc.), and in some situations enhance immunity against complex viruses (e.g., the smallpox virus) (Fig. 2.**15**, p. 79). Infectious agents apparently induce cytokines within a matter of hours (for instance IFNγ, IL-12 , and IL-4), and this early cytokine production in turn functions to define the ensuing T cell response as type 1 or type 2 (see p. 75 and Fig. 2.**14**, p. 78).

General Schemes of Infectious Diseases

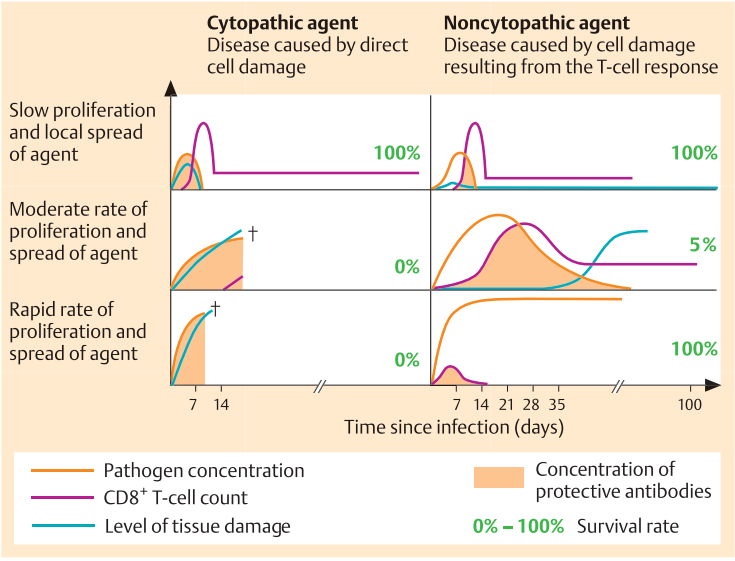


Fig. 2.**19** The degree of host survival depends on both the rate of proliferation, and the extent of spread, of an infectious agent – as well as the intensity of the host's cytotoxic T-cell response. Infection by cytopathic pathogens can only be controlled if pathogenic proliferation is slow and the pathogen remains localized; otherwise the outcome is usually fatal. In the case of noncytopathic pathogens, the cytotoxic T-cell response is the critical parameter. Pathogens which proliferate slowly are quickly eradicated. The T-cell response can be halted by pathogens which proliferate rapidly and spread widely due to the deletion of responding T cells. The degree of survival for hosts is high in both of these cases. For pathogens which exhibit moderate rates of proliferation and spread, the T-cell response may cause extensive immunopathological damage, and thus reduce the proportion of surviving hosts, some of which will controll virus, some not.
A weakened immune defense system may not progress beyond an unfavorable virus-host balance, even when confronted with a static or slowly replicating pathogen which represents an initially favorable balance.

IgE-mediated defense is important, along with IgA, in enhancing the elimination of gastrointestinal, pulmonary, and dermal parasites. Although details of the process are still sketchy, IgE-dependent basophil and eosinophil defense mechanisms have been described for model schistosomal infections.

Avoidance strategies. Infectious agents have developed a variety of strategies by which they can sometimes succeed in circumventing or escaping immune responses, often by inhibiting cytokine action.

Antibacterial Immune Effector Mechanisms

Extracellular bacteria. Capsules with carbohydrate elements render bacteria more resistant to efficient phagocytosis and digestion (mainly by granulocytes)—however, highly repetitive carbohydrate surface antigens induce efficient B cells responses which do not require T help and which are supported in part by lipopolysaccharides (LPS). *Pure carbohydrates do not induce T help!* Short-lived IgM responses can control bacteria in the blood effectively, but are usually insufficient in the control of toxins. In such cases, immunoglobulins of the IgG class are more efficient, as a result of their longer half-life and greater facility for diffusing into tissues.

Intracellular bacteria are controlled by T cells (mainly via T cell secreted IFNγ and TNFα which activate macrophages), or in some cases by the release of intracellular bacteria through $CD8^+$ T cell mediated cellular destruction.

Avoidance Mechanisms of Pathogens (with examples)

Influence on the complement system. Some pathogens prevent complement factors from binding to their surfaces:
- Prevention of C4b binding; herpes virus, smallpox virus.
- Prevention of C3b binding; herpes simplex virus (imitates DAF, see p. 86), trypanosomes.

Compartmentalization in non-lymphoid organs. Viruses can avoid confrontation with the immune defenses by restricting their location to peripheral cells and organs located outside of lymphoid tissues:
- Papilloma viruses; infect keratinocytes.
- Rabies virus; infects neurons.

Modulation and down-regulation of surface antigens. Infection agents can avoid immune defenses by mutating or reducing their expression of T- or B-cell epitopes.
- Influenza viruses; antigenic shift caused by rearrangement of genetic elements or drift resulting from mutation of hemagglutinin (at the population level).
- Gonococci; recombination of pili genes.
- Schistosoma; mutation of envelope proteins or masking by adoption of host MHC antigens.

Interference with phagocytosis and digestion. *Mycobacterium tuberculosis* uses CR1, CR2, or fibronectin as a receptor for cell entry; it does not induce efficient oxidative mechanisms in macrophages.
- Components of bacterial cell walls can impede phagosome-lysosome fusion and are resistant to digestion.
- Heat shock proteins (hsp60 and hsp70) or superoxidedismutase aid resistance.

Continued: **Avoidance Mechanisms of Pathogens (with examples)**

Influence on lymphocytes and immunosuppression.
- Direct destruction of lymphocytes, or negative regulation of their function (HIV?).
- Induction of immunopathological T-cell responses (in some cases these can be immunosuppressive, e.g. HIV).
- Induction of immunosuppressive autoantibodies.

Influence on selection, induction, and deletion of T cells.
- Negative selection of T cells; if viral antigens are present in the thymus responsive T cells will be deleted.
- Exhaustive activation, and subsequent deletion, of peripheral T cells; in some overwhelming peripheral virus infections all of the responding T cells are deleted (HBV, HCV).

Interference with cytokines, cytokine and chemotaxin receptors (R), etc. Many viruses produce substances that block or inhibit receptors for the humoral components of the immune defense system, for instance:
- IL-1βR, TNFαR, IFNγR; herpesvirus, smallpox virus.
- Chemotaxin receptor; cytomegalovirus.
- IL-10R; the Epstein-Barr virus produces B-cell receptor factor I, which binds to the IL-10R thus preventing activation of TH2 cells.
- Viral-induced inhibition of interleukin production.

Impairment of MHC antigen expression. Down-regulation of MHC class I and/or class II expression:
- Adenovirus; E19 protein reduces expression of MHC class I on infected cells.
- Murine cytomegalovirus; prevents transport of MHC class I to the Golgi apparatus.

Immune Protection and Immunopathology

Whether the consequences of an immune response are protective or harmful depends on the balance between infectious spread and the strength of the ensuing immune response. As for most biological systems, the immune defense system is optimized to succeed in 50–90% of cases, not for 100% of cases. For example, immune destruction of virus-infested host cells during the eclipse phase of a virus infection represents a potent means of preventing virus replication (Fig. 2.**15**, p. 79). From this point of view, **lytic CD8⁺ T-cell responses** make good sense as the host will die if proliferation of a *cytopathic virus* is not halted early on. If a *noncytopathic virus* is not brought under immediate control, the primary illness is not severe—however, the delayed cytotoxic response may then lead to the destruction of very large numbers of infected host cells and thus exacerbate disease (Tables 2.**9** and 2.**10**). Since an infection with noncytopathic viruses is not in itself life-threatening to the

Table 2.9 Balance between Infection and Host Immunity: Effect on the Disease

Infectious agent	Cytopathogenicity of agent	Efficiency of immune response		
		Early start	*Later start*	*No immune response*
Extracellular bacteria				
Meningococci Staphylococci	High	Recovery	Death	Death
Facultatively intracellular bacteria				
Listeria	High	Recovery	Death	Death
Tuberculosis bacilli	Moderate	Recovery	Immunopathological inflammation	Miliary tuberculosis (early death) Landouzy sepsis (very early death)
Leprosy bacilli	Very low	Recovery	Tuberculoid leprosy	Lepromatous leprosy (late death)
Viruses				
Smallpox virus	High	Recovery	Death	Death (early)
LCMV (lymphocytic choriomeningitis)	Very low	Recovery	Immunopathological disease	Healthy carrier
Hepatitis B virus	Very low	Recovery	Aggressive hepatitis	Carrier (very late liver carcinoma)
HIV	Low (?)	Recovery	AIDS	Healthy carrier (occult infection) (?)
Unrecognized and unknown infections, viruses, bacteria, and endogenous retroviruses	Low	?	Autoimmunity	"Healthy" or occult carrier (although infectious agent is unknown)
Clinical symptoms		None	Chronic disease	Variable disease symptoms, sometimes delayed or asymptomatic

Table 2.**10** Hepatitis B Virus (HBV) Infection. Inter-relations between Efficient Antigen Presentation by MHC Molecules, T-Cell Responses, Course of Infection, and Clinical Picture. Decreased Immunocompetence or Enhanced HBV Proliferation Shifts the Balance Towards an Unfavorable Outcome; Vaccination Shifts the Balance Towards a Favorable Outcome

Presentation of HBV antigen by MHC	T-cell response	Kinetics of infection	Clinical phenotype
+++	*Early*	HBV proliferation is halted	Acute hepatitis with or without icterus, due to hepatocyte damage being minimal
+/-	*Late*	HBV proliferation is halted too late. Liver cells are lysed by $CD8^+$ T cells	Acute to chronic aggressive hepatitis
–	*None*	HBV proliferation is not halted, but there is no immunopathology	Healthy HBV carrier (late liver cell carcinoma)

host, it is paradoxically the immune response that is responsible for pathology and illness due to its ability to destroy infected host tissue.

Hepatitis B viral infections in humans (Table 2.**10**), and LCMV infections (lymphocytic choriomeningitis) in mice, are amongst the most thoroughly studied examples of this potentially negative consequence of protective immune responses. A similar situation is also observed for the cellular immune response against facultative intracellular tuberculosis and leprosy bacilli which themselves have relatively low levels of pathogenicity (Table 2.**9**). A healthy immune system will normally bring such infectious agents under control efficiently, and the immunological cell and tissue damage (which occurs in parallel with the elimination of the pathogen) will be minimal, ensuring that there is little by way of pathological or clinical consequence. However, should the immune system allow these agents to spread further, the result will be a chronic immunopathological response and resultant tissue destruction—as seen during hepatitis B as *chronic or acute aggressive hepatitis* and in leprosy as the *tuberculoid form*. Should a rapidly spreading infection result in exhaustion of the T cell response, or should an insufficient level of immunity be generated, the infected host will become a carrier. This carrier state, which only occurs during infections characterized by an absent or low-level of cytopathology, is convincingly demonstrated in hepatitis B carriers and sufferers of lepromatous leprosy.

Immunopathological Damage and AIDS

Could it be that immunopathological damage resulting from T cell immune responses play a role in AIDS?

The general assumption at the present time is that the causal HIV virus destroys those T helper cells it infects, yet no unequivocal in-vivo proof of this assumption has been obtained. T helper cells do disappear, but how and why they disappear remains unclear. Animal models employing viruses similar to HIV suggest that AIDS might also develop by alternative means:

Assuming that HIV is a *noncytopathic, or only mildly cytopathic*, virus—infection of macrophages, dendritic cells, and/or T helper cells will not cause an immediate outbreak of disease. Soon, however, the virus-infected macrophages and T helper cells will be destroyed by specifically reacting cytotoxic $CD8^+$ T cells. Because the immune response also acts to inhibit virus proliferation, the process of cellular destruction is generally a gradual process. However, over time the immune system itself may become damaged and weakened. Paradoxically, the process of immunological cell destruction would help the virus survive for longer periods in the host and hence facilitate its transmission. From the point of view of the virus this would be an astounding, and highly advantageous, strategy—but one with tragic consequences for the host following, in most cases, a lengthy illness. If proliferation of HIV could be slowed or even halted, the virus would infect fewer lymphocytes, and thus fewer cells would be destroyed by the cytotoxic T-cell response. Prevention or reduction of HIV proliferation, either by pharmacological means or by bolstering the early immune defenses through other means, therefore represents an important objective despite the likelihood that HIV is not very cytopathic.

Influence of Prophylactic Immunization on the Immune Defenses

Vaccines provide protection from diseases, but in most cases cannot entirely prevent re-infection. Vaccination normally results in a limited infection by an attenuated pathogen, or induces immunity through the use of killed pathogens or toxoids. The former type of vaccine produces a very *mild infection or illness* capable of inducing an immune response and which subsequently protects the host against re-infection. The successful eradication of smallpox in the seventies so far represents the greatest success story in the history of vaccination. The fact is that vaccinations never offer absolute security, but instead improve the chances of survival by a factor of 100 to 10 000. A special situation applies to infections with noncytopathic agents in which disease results from the immune response itself (see above). Under certain circumstances, and in a small number of vaccinated persons, the vaccination procedure may therefore shift the balance between immune defense and infection towards an unfavorable outcome, such that the vaccination will actually *strengthen the disease*. Rare examples of this phenomenon may include the

use of inactivated vaccines against the respiratory syncytial virus (RSV) in the sixties, and experience with certain so-called subunit vaccines and recombinant vaccines against noncytopathic viral infections in rare model situations. Generally, it should be kept in mind that most of the successful immunization programs developed to date have mediated *protection via antibodies*. This particularly applies to the classic protective vaccines listed in Table 1.**13** (p. 33) for children, and explains why antibodies not only are responsible for the protection of neonates during the immuno-incompetent early postnatal period where immunological experience is passed on from the mother via antibodies, but also attenuate early childhood infections to become vaccine-like. This explains why successful vaccines all protect via neutralizing antibodies, because this pathway has been selected by co-evolution. As mentioned earlier, with regard to immunological memory, memory T cells appear to be essential to host immune protection, particularly in those situations when antigen persistence is controlled efficiently by means of infection-immunity (e.g., tuberculosis, HIV).

Tumor Immunity

Our knowledge concerning the immune control of tumors is still modest. Some tumor types bear defined tumor-associated, or tumor-specific, antigens. However this is apparently not sufficient for induction of an efficient immune defense. There is also the problem of tumor diagnosis; the presence of tumors is sometimes confirmed using a functional or immunological basis, yet the tumor cannot be located because conventional examinations are often unable to discover them until they reach a size of about 10^9 cells (i.e., about 1 ml) of tumor tissue.

Factors important in immune defense reactions include the location and rate of proliferation, vascularization or the lack thereof, and necrosis with phagocytosis of disintegrating tumor tissue. We never actually get to see those rare tumors against which immune control might have been successfully elicited, instead we only see those clinically relevant tumors that have unfortunately become successful tumors which have escaped immune control.

Evidence of the immune system's role in tumor control includes:

- Greater than 85% of all tumors are carcinomas and sarcomas, that is nonlymphohematopoietic tumors which arise in the periphery, outside of organized lymphoid tissues. The immune system, in a manner similar to that seen for many strictly extra-lymphatic self antigens, ignores such tumors at first.

- Lymphohematopoietic tumors often present immunological oddities such as unusually low, or entirely absent, MHC and/or low tumor antigen concentrations, plus they frequently lack accessory molecules and signals.

- Congenital or acquired immunodeficiency—whether caused by anti-lymphocytic sera, cytostatic drugs, gamma irradiation, UV irradiation, or infection—usually encourages tumor growth, especially for lymphohematopoietic tumors. Carcinomas and sarcomas show little or no increased susceptibility. Interestingly, experimental carcinogens are frequently also immunosuppressive.
- Surgical removal of a large primary tumor may result in the disappearance (or rarely in rapid growth) of metastases within the lymph nodes.
- Tumor cells often display modulated MHC expression—some tumors lack MHC class I molecules entirely—or in some cases tumors selectively down-modulate the only MHC allele capable of presenting a specific tumor-associated peptide (e.g., the colon adenocarcinomas). Other tumors side-step immune defenses by down-regulating tumor-specific antigens.
- The immune response may fail if tumor differentiation antigens are expressed, against which the host exhibits an immunological tolerance (e.g., carcinoembryonic antigen [CEA], T-cell leukemia antigen).
- Blockade of the reticuloendothelial system may encourage the development of lymphohematopoietic tumors. For instance, chronic parasitic infections or infection by malaria can result in the development of Burkitt lymphoma, a B-cell malignancy.

The Pathological Immune Response

An immune response can also cause disease. Such responses can be classified into the following types: **Type I:** allergic IgE-dependent diseases; **Type II:** antibody-dependent responses to cell membranes, blood group antigens or other auto-antigens; **Type III:** immune complex-initiated diseases whereby surplus antigen-antibody complexes are deposited on basement membranes, resulting in development of chronic disease via complement activation and inflammatory reactions; **Type IV:** cellular immunopathology resulting from excessive T-cell responses against infections that otherwise exhibit low cytopathogenicity, or against allogenic organ transplants.

Type I: IgE-Triggered Anaphylaxis

This type of immediate hypersensitivity reaction occurs within minutes in allergically sensitized individuals. Although serum IgE has a short half-life (one to two days), IgE antibodies bound to the Fc_{ε} receptor on basophils

and mast cells have a half-life of several months and when bound by the specific allergen mediate cellular degranulation and the release of biogenic amines (e.g., histamine, serotonin). These mediators can influence the smooth musculature, and mainly result in the constriction of the pulmonary- and broncho-postcapillary venules, together with arteriole dilation. The local manifestations of IgE-triggered anaphylaxis include whealing of the skin (urticaria), diarrhea for food allergies, rhinitis or asthma for pollen allergies, or a generalized anaphylactic shock. IgE reactions are usually measured in vitro using RIA (radioimmunoassay), RIST (radioimmunosorbent test) or RAST (radioallergosorbent test) (see Fig. 2.**28** and Fig. 2.**29**, p. 131f.) Frequent causal agents of IgE allergies in humans include pollen, animal hair, house dust (mites), insect bites and stings, penicillin, and foods. Examples of allergic diseases include local allergic rhinitis and conjunctivitis, allergic bronchial asthma, systemic anaphylactic shock, insect toxin allergies, house dust (mite) and food allergies, urticaria, and angioedemas.

Degranulation of mast cells and basophils can be induced by factors other than the cross-linking of specific IgE antibodies. Such factors include the complement factors C3a and C5a, and pharmacological inducers ("pseudo-allergy!").

Atopic patients suffer severely from allergies. Atopia is genetically conditioned, with a child exhibiting a 50% risk of developing atopy if both parents are allergic, or a 30% risk if only one parent is allergic. The incidence level of atopy within the general population is roughly 10–15%. Atopia correlates with high levels of IgE production, and desensitization refers to attempts to change a TH2 (IgE-producing) response into a TH1 (IgG-favoring) response by means of repeated inoculations or oral doses of allergens (see Fig. 2.**14**, p. 78). It is likely that increased production of IgG—as opposed to IgE—antibodies plays a major role in the success of desensitization. IgE no doubt has an important biological function, probably against ectoparasites, with allergic reactions representing nothing more than an unfortunate side effect of this biological system. Little research has been performed on the nature of the protective function of IgE during parasitic infections (or on the role of eosinophils). However, we do know that mediators released by IgE-triggering of mast cells and basophils cause the smooth intestinal musculature to contract, and in this way facilitate the elimination of intestinal parasites.

Type II: Cytotoxic Humoral Immune Responses

These are pathological immune responses induced by the binding of IgM or IgG antibodies to antigens present on a cell surface (including viral products or haptens), or within tissue components. The mediators responsible for such tissue damage are usually components of the complement system,

Table 2.**11** Examples of Antibody–Related Type II Immunopathologies

Antibody	Autoimmune pathology or immunopathology
Anti-cell membrane	– Rhesus incompatibility – Blood transfusion complications – Autoimmune hemolytic anemia – Immune neutropenia, idiopathic thrombocytopenia
Anti-basement membrane	– Goodpasture syndrome
Anti-collagen	– Sclerodermia – Pemphigoid (anti-epidermal basal membrane)
Anti-desmosome	– Pemphigus vulgaris
Anti-receptor	– Anti-acetylcholine receptors: myasthenia gravis – Anti-TSH receptors: Basedow disease
Anti-hormone	– Anti-thyroid hormone (Hashimoto thyroiditis) – Anti-intrinsic factor (pernicious anemia)
Anti-medication	– Chemical groups (haptens) bound to cell surface (cytolysis, agranulocytosis)
Anti-cell component	– Anti-DNA (lupus erythematosus, LE) – Anti-mitochondrial (LE, Hashimoto thyroiditis)

or granulocytic digestive enzymes. The most important diseases resulting from cytotoxic humoral immune responses are listed in Table 2.**11**.

Autoantibody Responses

Some clinically important autoantibodies are directed against hormone receptors, for example thyrotoxicosis in Basedow's disease is caused by autoantibodies that stimulate the TSH receptor, and myasthenia gravis is caused by blockage of the acetylcholine receptor by specific autoantibodies. Other antibody-induced diseases mediated by antibodies, directed against hormones and other cellular self antigens, include Hashimoto thyroiditis (induced by anti-thyroglobulin and anti-mitochondrial autoantibodies), pernicious anemia (anti-intrinsic factor), pemphigus vulgaris (anti-desmosome) Guillain-Barré syndrome (ascending paralysis caused by specific myelin autoantibodies), and scleroderma (involving anti-collagen antibodies). Other immunopathologies involving autoantibodies include transplant rejection as a result of endothelial damage (especially in xenogeneic transplants), and tumor rejection caused by antibodies against tumor-associated antigens present on neoplastic cells (especially relevant for lymphohematopoietic

Table 2.**12** Mechanisms Of Autoantibody Induction

Possible mechanisms	Autoimmune pathology or immunopathology
Polyclonal B-cell activation	Lipopolysaccharides, viruses, chronic parasitic infection
Molecular mimicry (overall very rare)	Anti-tat (HTLV-1), anti-H. pylori, or anti-streptococcus crossreacting with self-antigens
Exposure of hidden autoantigens	Cytopathic effects of infectious agents
Adjuvant effects	In the presence of granuloma formation and chronic inflammatory reactions lymphoid tissue may form in peripheral organs (e.g., during Hashimoto's thyroiditis)
Breakdown of tolerance	Due to coupling of T helper epitopes to autoantigens, possible in connection with virus infections of cells

tumors). However, in general the detection of autoantibodies does not necessarily correlate with evidence of pathological changes or processes. In fact, our detection methods often measure low-avidity autoantibodies that may have no direct disease-causing effects.

Exactly how autoantibody responses are induced remains to be clarified. As explained earlier (in the discussion of immunological tolerance) such IgG responses cannot be induced without T help. Thus, intensive research is currently focused on those mechanisms by which T cell help for autoreactive B cells is regulated; Table 2.**12** sums up some of the possible mechanisms.

Anti-blood Group Antibody Reactions

ABO system. These B-cell epitopes consist of *sugar groups present in the membranes of red blood cells*. The four classic blood groups are determined by one gene with three alleles. This gene controls glycosylation. The O allele codes only for a basic cell surface structure (H substance) with the terminal sugars galactose and fucose. The A allele adds *N*-acetylgalactosamine to this basic structure, the B allele adds galactose. This results in epitopes, which are also seen frequently in nature largely as *components of intestinal bacteria*. Individuals who carry the A allele are tolerant to the A-coded epitope, whilst individuals with the B allele are tolerant to the B epitope. Individuals who carry both of these alleles (genotype AB) are tolerant to both epitopes, whereas persons who are homozygotes for the O allele are not tolerant to either A or B. Following birth, the intestinal tract is colonized by bacteria con-

taining large numbers of epitopes similar to the A and B epitopes. During the first months of life, people with blood group O (homozygous for the O allele) produce both anti-A and anti-B antibodies, people with blood group A (genotype AO or AA) produce only anti-B antibodies, people with blood group B (genotype BO or BB) produce only anti-A antibodies, and people with blood group AB produce neither anti-A or anti-B antibodies.

These so-called "natural" antibodies (meaning these antibodies are produced without a recognizable immunization process) are of the IgM class; there is usually no switch to IgG, probably resulting from a lack of necessary helper T-cell epitopes. The presence of the blood group antibodies makes blood transfusions between non-matched individuals extremely risky, necessitating that the blood group of both the donor and recipient is determined before the blood transfusion takes place. Nevertheless, the *antibodies in the donor blood are not so important* because they are diluted. The O genotype is therefore a universal donor. Note that IgM antibodies to blood groups present no danger to the fetus since they cannot pass through the placental barrier.

Rhesus factor. This system is also based on genetically determined antigens present on red blood cells, although as a general rule there is no production of "natural" antibodies against these. IgM and IgG antibodies are not induced unless an *immunization* (resulting from blood transfusion or pregnancy) takes place. During the birth process, small amounts of the child's blood often enter the mother's bloodstream. Should the child's blood cells have paternal antigens, which are lacking in the mother's blood, his or her blood will effectively 'immunize' the mother. Should IgG antibodies develop they will represent a potential risk during *subsequent pregnancies* should the fetus once again present the same antigen. The resulting clinical picture is known as *morbus hemolyticus neonatorum* or *erythroblastosis fetalis* ("immune hydrops fetalis").

Once immunization has occurred, *thus endangering future pregnancies*, genetically at risk children can still be saved by means of cesarean section and exchange blood transfusions. Should the risk of rhesus immunization be recognized at the end of the first pregnancy, immunization of the mother can be prevented by means of a *passive infusion* of antibodies against the child's antigen, immediately following the birth. This specific immunosuppressive procedure is an empirical application of immunological knowledge, although the precise mechanism involved is not yet been completely understood.

Other blood group systems. There are other additional blood group systems against which antibodies may be produced, and which can present a risk during transfusions. Thus, the crossmatch test represents an important measure in the avoidance of transfusion problems. Immediately prior to a planned transfusion, serum from the prospective recipient is mixed with erythrocytes from the prospective donor, and serum from the prospective donor is mixed

with erythrocytes from the prospective recipient. To ensure no reaction following transfusion, there should be no agglutination present in either mixture. Some potentially dangerous serum antibodies may bind to the erythrocytes causing opsonization, but not necessarily inducing agglutination. To check for the presence of such antibodies, anti-human immunoglobulin serum is added and should it crosslink such antibodies agglutination will result.

Type III: Diseases Caused by Immune Complexes

Pathologies initiated by immune complexes result from the deposition of **small, soluble, antigen-antibody complexes** within tissues. The main hallmark of such reactions is *inflammation* with the involvement of complement. Normally, large antigen-antibody complexes (that is, those produced in equivalence) are readily removed by the phagocytes of the reticuloendothelial system. Occasionally, however—especially in the presence of *persistent bacterial, viral, or environmental, antigens* (e.g., fungal spores, vegetable or animal materials), or during *autoimmune diseases* directed against autoantigens (e.g., DNA, hormones, collagen, IgG) where autoantibodies to the body's own antigens are produced continuously—deposition of antigen-antibody complexes may become widespread often being present on active secretory membranes and within smaller vessels. Such processes are mainly observed within infected organs, but can also occur within kidneys, joints, arteries, skin and lung, or within the brain's plexus choroideus. The resulting inflammation causes local tissue damage. Most importantly, activation of complement by such complexes results in production of inflammatory C components (C3a and C5a). Some of these *anaphylatoxins* cause the release of vasoactive amines which increase vascular permeability (see also p. 103f.). Additional chemotactic activities attracts *granulocytes* which attempt to phagocytize the complexes. When these phagocytes die, their lysosomal hydrolytic enzymes are released and cause further tissue damage. This process can result in long-term chronic inflammatory reactions.

There are two basic patterns of immune complex pathogenesis:

- **Immune complexes in the presence of antigen excess.** The acute form of this disease results in *serum sickness*, the chronic form leads to the development of arthritis or glomerulonephritis. Serum sickness often resulted from serum therapy used during the pre-antibiotic era, but now only occurs rarely. Inoculation with equine antibodies directed against human pathogens, or bacterial toxins, often induced the production of host (human) antibodies against the equine serum. Because relatively large amounts of equine serum were administered for such therapeutic purposes, such therapy would result

in the induction of antigen-antibody complexes—some of which were formed in the presence of antigen excess—and occasionally induced a state of shock.

Immune complexes in the presence of antibody excess. The so-called *Arthus reaction* is observed when an individual is exposed to repeated small doses of an antigen over a long period of time, resulting in the induction of complexes and an antibody excess. Further exposure to the antigen, particularly dermal exposure, induces a typical reaction of edema and erythema which peaks after three to eight hours and disappears within 48 hours, but which sometimes leads to necrosis. Arthus-type reactions often represent occupational diseases in people exposed to repeated doses of environmental antigens: farmer's lung (thermophilic *Actinomyces* in moldy hay), pigeon breeder's lung (protein in the dust of dried feces of birds), cheese worker's lung (spores of *Penicillium casei*), furrier's lung (proteins from pelt hairs), malt-worker's lung (spores of *Aspergillus clavatus* and *A. fumigatus*).

Type IV: Hypersensitivity or Delayed Type, Cell-Mediated Hypersensitivity

Intracutaneous injection of a soluble antigen derived from an infectious pathogen induces a delayed dermal thickening reaction in those people who have suffered a previous infection. This *delayed skin reaction* can serve as a test to confirm immunity against intracellular bacteria or parasites.

For most cases, the time between administration of the antigen and the swelling reaction is 48–72 hours—as described above for cellular delayed type hypersensitivity (DTH) reactions in the skin (p. 99). As observed for antibody-dependent hyper-reactions of types I-III, the type IV response is pathogenic and *differs from protective immune responses only in terms of the extent and consequences of the tissue damage, but not in terms of the mechanism of action*. The balance between autoimmune disease and type IV immunopathology in such cases is readily illustrated by type IV reactions (e.g., aggressive hepatitis in humans or lymphocytic choriomeningitis in mice). Should the causal infectious pathogen be known, the response is termed a type IV reaction, if the causal agent is unknown (or not yet determined) the same condition may be termed "autoimmune disease." The reader is referred to the many examples of type IV responses already discussed within various chapters (DTH [p. 99], immune protection and immunopathology [Tables 2.**9** and 2.**10**, pp. 104 and 105], transplantation immunology [see below], and autoimmunity [p. 110ff.]).

Autoimmune T cells are usually directed against autoantigens that would otherwise be ignored (since they are only expressed in the extralymphatic periphery). Autoaggressive $CD4^+$ T cells apparently respond against myelin

basic protein in *multiple sclerosis*, against collagen determinants in *polyarthritis*, and against islet cell components in *diabetes*.

Transplantation Immunity

■ Transplant rejection within the same species is largely a consequence of MHC-restricted T-cell recognition of foreign MHC antigens. Interspecies rejection is additionally contributed to by antibodies, and intolerance between complement activation mechanisms. Methods for reducing, or preventing, rejection include general immunosuppression, tolerance induction by means of cell chimerism, and sequestering of the transplanted cells or organ. ■

The *strong* transplantation antigens are *encoded within the MHC complex* (see p. 58ff.), whilst the *weak* antigens constitute the MHC-presented allelic differences of *non MHC-encoded host proteins or peptides*. It is possible to differentiate between the **host-versus-graft** (HVG) reaction of the recipient against a genetically foreign tissue or organ, and the **graft-versus-host** (GVH) reaction.

The GVH reaction. This type of reaction results when *immunologically responsive donor T cells* are transferred to an allogeneic recipient who is unable to reject them (e.g., following a bone marrow transplant into an immuno-incompetent or immuno-suppressed recipient). The targets against which the transplanted T cells generate an immune response include the MHC class I and II molecules of the recipient. The recipient's transplantation antigens also present allelic variants of recipient self-peptides, which can be recognized by donor T cells as weak transplantation antigens when presented by common MHC alleles (it is conceivable that strong recipient transplantation antigens could be accepted and processed by donor APCs, however even if this did occur it would be of limited functional consequence as they would not be presented by the recipient APC in the correct antigen configuration). Weak histocompatibility antigens—for instance those peptide variants recognized as nonself when presented in combination with essentially histocompatible MHC molecules—play a more significant role in bone marrow transplants. The existence, and pathological role, of weak transplantation antigens has only been demonstrated in completely histocompatible siblings or within inbred animal strains with identical MHC. The *wide variety of alloreactive T cells* can be explained by cross-reactivity, as well as by the enormous number of different combinations of MHC molecules and cellular peptides. It must be emphasized that allogeneic MHC antigens on APCs and lymphocytes (so-called passenger lymphocytes) derived from the donor organ are particularly immunogenic since they express high levels of antigens and can traffic to

secondary lymphatic organs. Indeed the same foreign transplantation antigens are hardly immunogenic when expressed on fibroblasts or on epithelial or neuroendocrine cells, unless these cells are able to reach local lymphoid tissue.

To avoid a GVH reaction in immunoincompetent or suppressed bone marrow recipients, immunocompetent T cells must first be eliminated from the transplanted bone marrow. This can be achieved by using anti-T-cell antibodies, anti-lymphocyte antisera, and complement or magnetic bead cell-separation techniques. However, it is noteworthy that complete elimination of mature T cells leads to a reduction in the acceptance rate for bone marrow transplants, and that it may also weaken the anti-tumor effect of the transplant (desirable in leukemia). It seems that the small number of T cells transplanted with the bone marrow can mediate a subclinical GVH reaction, thus preventing rejection of the transplant but retaining the ability to destroy the recipient's leukemia cells and preventing tumor re-emergence.

Bone Marrow Transplants Today

- Reconstitution of immune defects involving B and T cells
- Reconstitution of other lymphohematopoietic defects
- Gene therapy via insertion of genes into lymphohematopoietic stem cells
- Leukemia therapy with lethal elimination of tumor cells and reconstitution with histocompatible, purified stem cells, either autologous or allogenic.

HVG reactions, that is immune responses of the recipient against transplanted cells or organs, are not generated in autotransplants (for instance transplantation of skin from one part of the body to another on the same individual). This also applies to transplants between monozygotic twins or genetically identical animals (**syngeneic transplants**). However, transplants between non-related or non-inbred animals of the same species (**allogeneic transplants**), and transplants between individuals of different species (**xenogeneic transplants**) are immunologically rejected. Because T cells recognition is subject to MHC restriction, cellular rejection within a species is even more pronounced than between different species, although the latter procedure involves other transplantation complications. These include the occurrence of natural *cross-reactive antibodies,* and a *lack of complement inactivation* by anti-complement factors (which are often species-incompatible and therefore absent in xenogeneic transplants), which together often results in hyperacute rejection within minutes, hours, or a few days—that is before any specific immune responses can even be induced.

Three types of transplant rejection have been characterized:

- **Hyperacute rejection** of vascularized transplants, occurring within minutes to hours and resulting from preformed recipient antibodies reacting

against antigens present on the donor endothelium, resulting in coagulation, thromboses, and infarctions with extensive necrosis.

- **Acute rejection**, occurring within days or weeks. This is accompanied by a perivascular and prominent occurrence of T lymphocyte infiltrates. Acute rejection can be prevented by immunosuppression.

- **Chronic rejection,** occurring within months to years. This is caused by low-level chronic T-cell responses, and can be mediated by cellular and humoral mechanisms. This can include obliterative vascular intima proliferation, vasculitis, toxic, and immune complex glomerulonephritis.

Antigenicity and Immunogenicity of MHC in Organ Transplants

A thyroid gland from donor "a," freshly transplanted under the renal capsule of an MHC (H-2)-incompatible recipient mouse "b" is acutely rejected (within seven to nine days). If the organ is treated in such a way as to kill the migratory APCs and leukocytes before it is transplanted, then transplant "a" will be accepted by recipient "b" (often permanently). However, should fresh spleen cells (APCs) from donor "a" be transferred by infusion 100 days later into the recipient "b," the previously accepted transplant "a," can sometimes be acutely rejected (i.e., within 10 days).

This experiment demonstrates that it is not the MHC antigens per se that are potently immunogenic, but rather that they only show this immunogenicity when they are located on cells capable of migration to local lymph nodes. Methods of implanting foreign tissue cells or small organs strictly extralymphatically, without inducing immune responses, are currently undergoing clinical trials (i.e., with islet cells in diabetes and neuronal cells in parkinsons disease).

Methods of measurement. The main methods used for follow-up analysis of HVG and GVH reactions are biopsies and histological evaluation, evaluation of blood cells and in-vitro mixed lymphocyte reactions (p. 132).

Immune Defects and Immune Response Modulation

- **Immune defects** are frequently acquired by therapy or viral infections, or as a consequence of advanced age. In rare cases immune defects can also result from congenital defects, these include severe combined immunodeficiency's (SCID) or transient partial immune defects (mainly involving IgA responses). **Immunomodulation** can be attempted using interleukins or monoclonal antibodies directed against lymphocyte surface molecules or antigenic peptides. **Immunostimulation** is achieved using adjuvants or

the genetically engineered insertion of costimulatory molecules into tumor cells. **Immunosuppression** can be induced globally using drugs, or specifically using antibodies, interleukins or soluble interleukin receptors; this can also be achieved by means of tolerance induction with proteins, peptides, or cell chimerism.

Immune Defects

The most important and frequent immune defects are *acquired*, e.g., iatrogenic (cytostatics, cortisone, irradiation, etc.), age-induced, or the result of viral infections (above all HIV). *Congenital defects* are rare; examples include Bruton's X-chromosome-linked B-cell defect, thymic hypoplasia (DiGeorge), and combined T- and B-cell deficiency resulting from MHC defects (bare lymphocyte syndrome) or from enzyme defects (adenosine deaminase [ADA] deficiency or purine nucleoside phosphorylase [PNP] deficiency). These defects can also be repaired by reconstitution (thymic transplants), or in some cases through the use of stem cells (gene therapy; one of the very first successful gene therapies was the treatment of ADA deficiency). More frequent congenital defects involve selective deficiencies, for example a relative-to-absolute IgA deficiency, normally being more prominent in infants than later in life. Children with such deficiencies are more susceptible to infection with *Haemophilus influenzae*, pneumococci, and meningococci. *General consequences of immune defects* include recurring and unusual infections, eczemas, and diarrhea.

Immunoregulation

This area of immunology is difficult to define and remains elusive. Antigens represent the most important positive regulator of immunity; since there is simply no immune stimulation when antigens have been eliminated or are absent. Other important regulators include interferon gamma (IFNγ) for TH1 responses, and IL-4 for TH2 responses. Further IL-dependent regulatory functions are in the process of being defined. The existence of specific $CD8^+$ T suppressor cells, capable of downregulating immune responses, has been postulated and their role was assumed to be that of counteracting the inflammatory $CD4^+$ T cell response. However, to date there has been no convincing proof of their existence. The term $CD8^+$ T suppressor cells, which is used frequently, is therefore misleading and inaccurate. In relatively rare cases, cyto-

toxic $CD8^+$ T cells do exercise a regulatory effect by lysing infected APCs or B cells (see also p. 106). It is unclear whether $CD4^+$ T cells could have similar effects. Regulation via *idiotypic/anti-idiotypic antibody networks* (i.e., antibodies directed against the ABS of other antibodies), or anti-TCR networks, have also been postulated—but remain hypothetical. Although attractive hypothesis, for most cases such regulatory pathways have only proved disappointing theoretical concepts, and as such should no longer be employed in the explanation of immunoregulation. In isolated cases, anti-idiotypic, or anti-TCR peptide-specific feedback, mechanisms can be modeled under forced experimental conditions. However such conditions probably fail to model normal situations, therefore they cannot accurately indicate whether these feedback mechanisms have a role in regulating the immune system as a whole.

Immunostimulation

The aim of immunological treatment of infections and tumors is to enhance immune responsiveness via the use of thymic hormones (thymopoietin, pentapeptides), leukocyte extracts, or interferons. Derivatives or synthetic analogs of microorganisms such as BCG, components of *Corynebacterium parvum* and peptidoglycans (e.g., muramyl peptide), or oligonucleic acids (CpG), are used as *adjuvants*. Components of streptococci and *Streptomyces*, eluates and fractions of bacterial mixtures, and the related synthetic substance levamisole are also used. The role of Toll-like receptors in these adjuvant effects is becoming increasingly understood, with a major role of these molecules being to link non-specific innate resistance to specific immunity. .

Recently developed immune therapy strategies aim to improve antigen presentation. For instance *interleukins,* or *costimulatory molecules* such as B7 or CD40, have been inserted into tumor cells by means of transfection. Hybrid antibodies have been constructed in an attempt to improve antigen recognition and phagocytosis (one such example is the coupling of an anti-CD3 antibody with tumor antigen-specific antibodies). Other ideas tested successfully in model experiments include systemic treatment with interleukins (this presents with frequent toxicity problems) or targeted insertion of GM-CSF, TNF, or IL-2. Alternatively, the production of IFNγ or IFNβ by cells, or the use of molecules capable of polyclonal T- and B-cell stimulation has been employed. This concept utilizes local chronic or acute infections with the aim of achieving inflammation surrounding, or direct infection of, tumor cells resulting in their cytolytic destruction. Such concepts have also been used to force phagocytosis and uptake of antigens by APCs with the aim of inducing or enhancing tumor immunity (e.g., BCG infections in bladder carcinoma treatment).

Immunosuppression

Various methods are employed to inhibit, or suppress, the immune response:

- Generalized immunosuppression; glucocorticoids (inhibition of inflammatory cells), cytostatic drugs (endoxan, DNA alkylating agents, methotrexate, antimetabolites), and more specific immunosuppressants, e.g., cyclosporine A, FK506, rapamycin (inhibition of signal transduction in T cells, see Fig. 2.**11**, p. 73).

- Immunosuppression by antibodies, soluble cytokine receptors, deletion of T cells or T-cell sub-populations (anti-CD4, anti-CD8, anti-CD3, anti-Thy1, etc.). Administration of monoclonal antibodies directed against adhesion molecules and accessory molecules or cytokines and cytokine receptors. Administration of soluble cytokine receptors, or soluble CTLA4, in order to block B7-1 and B7-2 (important costimulators, see p. 71ff.).

- Specific tolerance induction or "negative immunization." Massive and depletive T-cell activation brought about by systemic administration of large amounts of peptides, proteins (risk of immunopathology), or cells (chimerism).

- Complete neutralization and elimination of the antigen with the purpose of preventing induction of an antibody response. Example; rhesus prophylaxis with hyperimmune serum.

Adaptive Immunotherapy

This involves in-vitro antigen stimulation, and consequent proliferation, of patient T-cell effector clones or populations ($CD8^+$ T cells or less specific **lymphokine-activated killer cells, LAK cells**), followed by transfusion of these cells back into the patient. This method is sometimes used as a means of limiting cytomegaly or Epstein-Barr virus infection of bone marrow recipients. The LAK cells also include less specific NK-like cells, which can be expanded with IL-2 in the absence of antigen stimulation.

Toxic antibodies are monoclonal antibodies to which toxins have been coupled. These are used as specific toxin transporters, administered directly, or with liposomes bearing anchored antibodies and containing a toxin or cytostatic drug.

Immunological Test Methods

Antigen and Antibody Assays

Immunoprecipitation in Liquids and Gels

Immunoprecipitate. Maximum precipitation results when both reaction partners are present in an approximately *equivalent ratio* (Fig. 2.**20**). In antibody excess, or antigen excess, the amount of precipitate is considerably reduced.

Double diffusion according to Ouchterlony. This technique allows for a *qualitative* evaluation of whether certain antibodies or antigens are present or not, plus determination of the degree of relationship between antibodies and antigens. It also provides information on whether different antigenic de-

Immunoprecipitation

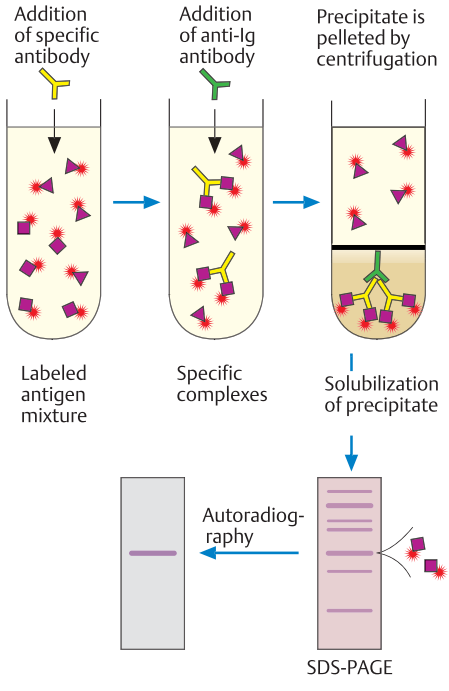


Fig. 2.**20** To identify the unknown antigen, a known specific antibody is added to an antigen mixture which is radio-, or otherwise-, labeled. The immune complexes are precipitated with the help of co-precipitating reagents (e.g., anti-immunoglobulin antibodies). The precipitate is thoroughly washed to remove unbound antigen, then dispersed into solution once again (e.g., in SDS), after which the components are separated using SDS polyacrylamide gel electrophoresis (SDS-PAGE). The labeled antigen is then rendered visible by means of autoradiography.

terminants are localized on the same, or on different, antigens; or whether different antibodies can bind to the same antigen (Fig. 2.**21**).

Radial immunodiffusion according to Mancini. This is a *quantitative* antigen assay based on a predetermined standard curve (Fig. 2.**22**).

Nephelometry. This method measures the amount of light scatter as a quantification of precipitation turbidity.

Immunoprecipitation Combined with Electrophoresis. Antigens are separated in an agarose gel by applying an electric current. The antibodies react by migrating in the gel, either without an electric field, or simultaneously within the electric field; and either in the same dimension as the antigens or in a second vertical step ("rocket" electrophoresis).

Immunoelectrophoresis according to Grabar and Williams. In the first instance serum proteins are electrophoretically separated within a thin agarose gel layer. A trough is then cut into the agar, next to the separated sample and parallel to the direction of migration along the entire migration distance, and anti-serum is applied to the trough. The antibodies diffuse into the gel, and precipitation lines are formed wherever they encounter their antigens. The

Double Diffusion According to Ouchterlony

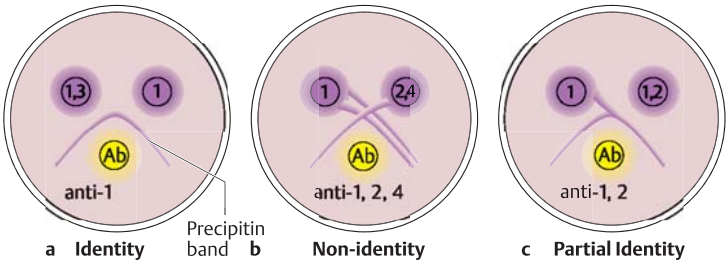


Fig. 2.**21** This technique facilitates assignment of antigens (violet) to a certain test antibody (yellow), or vice versa. The antigens and antibodies are pipetted into troughs within the gel and diffuse through this medium (the numbers designate the epitopes present). Where they meet lines of precipitation (known as precipitin bands) develop, indicating immune complex formation. **a** The antibodies precipitate identical epitopes (epitope 1) of both antigens, resulting in formation of precipitin bands which flow together to form an arch, mutually inhibiting their migration. In **b**, three independent precipitin bands form, indicating that the antibodies differentiate three different epitopes on three different antigens. **c** Epitope 1 of both antigen samples forms precipitin bands which flow together. Anti-2 migrates beyond the line of confluence into the area in which it precipitates with free antigen 1, 2 and forms a spur.

Radial Immunodiffusion According to Mancini

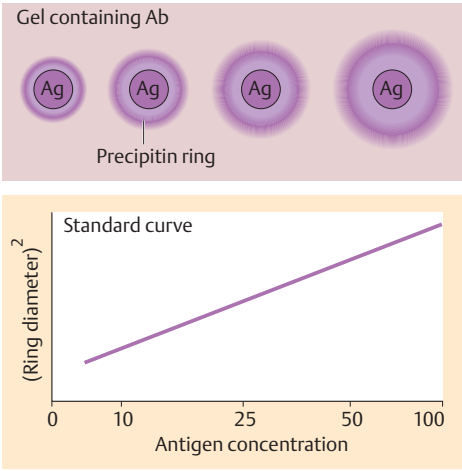


Fig. 2.**22** Quantitative assay of an antigen using a monospecific anti-serum which is mixed with agar and poured into a plate. The antigen is then diluted to different concentrations, and pipetted into wells that have been previously punched into the plate. Antigen-antibody complexes precipitate in the form of a ring around the well, the diameter of which is proportional to the antigen concentration. The result is a standard curve from which unknown test antigens can be quantified. Analogously, antibodies can also be quantified by mixing antigens into the gel.

precipitate can then be stained and evaluated. This older method is still used to identify paraproteins, monoclonal immunoglobulins, etc. (Fig. 2.**23**).

Electrophoresis plus antibody reaction: Western blotting. This method involves electrophoresis of proteins in a gel, coupled with detection by specific antibodies. The separated proteins are transferred to nitrocellulose, where they are identified with the help of specific antibodies (Fig. 2.**24**). Polyclonal sera is normally used for this purpose as monoclonal antibodies only rarely bind to denaturated and separated proteins.

Agglutination Reaction

Antibodies can agglutinate antigen-loaded particles (Fig. 2.**25**), whilst antigens can agglutinate antibody-loaded particles. Application: agglutination of bacteria or erythrocytes (e.g., blood group tests).

Immunoelectrophoresis According to Grabar and Williams

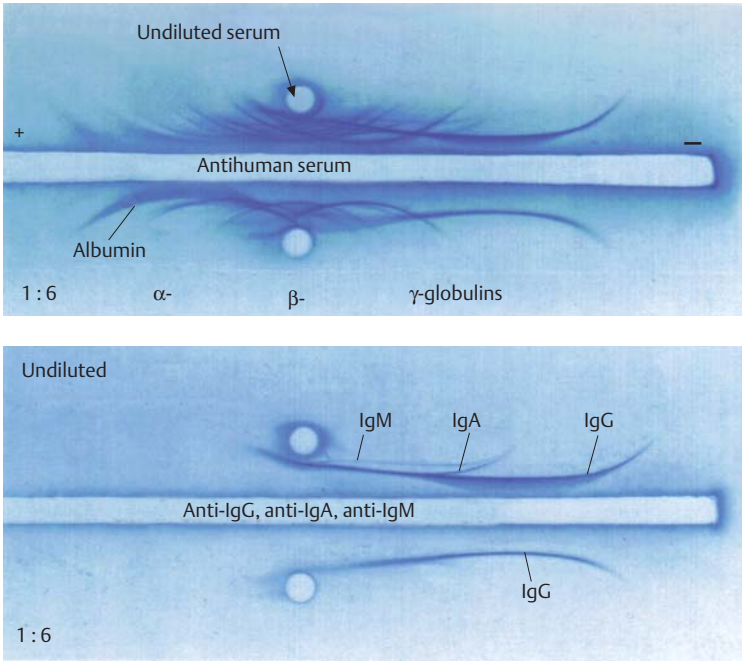


Fig. 2.**23** Serum is separated within agarose by an electric field, and rendered visible with anti-serum directed against human serum (above), or with selected specific antibodies (below).

Indirect hemagglutination. An antigen is fixed on the surface of erythrocytes and the antigen-loaded erythrocytes are then agglutinated using specific antibodies.

Hemagglutination inhibition test. The ability of a sample containing antigen to inhibit hemagglutination between antigen-loaded erythrocytes and antiserum is measured. This test is frequently used to quantify antibodies against hemagglutinating viruses (mainly influenza and parainfluenza viruses).

Antiglobulin tests according to Coombs. The direct Coombs test determines antibody binding directly to erythrocytes (e.g., anti-Rh antibodies agglutinate Rh^+ erythrocytes of neonates). The indirect Coombs test is suitable for detection of antibodies that have already bound to the Rh^+ erythrocytes of newborns (second pregnancy or sensitized mother), or which have been in-

Western Blotting

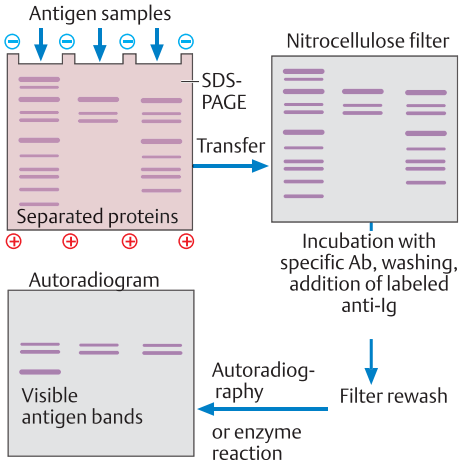


Fig. 2.**24** Antigen samples separated in a gel are transferred to nitrocellulose. Non-specific binding of the antibodies to the filter is then prevented with serum albumin or irrelevant proteins that do not cross-react with any of the antibodies used. Antibodies specific for the antigens being sought are then added. Once immune complexes have formed, the unbound antibodies are thoroughly washed away and the remaining bound antibodies are labeled using anti-immunoglobulin antibodies. These are in turn rendered visible by the autoradiographic procedure.

cubated in vitro with erythrocytes or antigenic particles. In all cases agglutination is detected using anti-Ig antibodies. Antigens can also be adsorbed to latex.

Complement Fixation Test (CFT)

CFT was formerly used to measure complement consumption by preformed antigen-antibody complexes. The unused complement is then detected by addition of a known amount of antibody-loaded erythrocytes. Should all of the erythrocytes be lysed, this indicates that no complement had been consumed and the CFT is negative. This method is no longer used very frequently, with the newer immunosorbent tests being preferred (RIA, ELISA, RAST, see below).

Direct and Indirect Immunofluorescence

Direct immunofluorescence. Immunofluorescence can be used for in-vivo detection of antibodies, complement, viruses, fungi, bacteria, or other im-

Hemagglutination

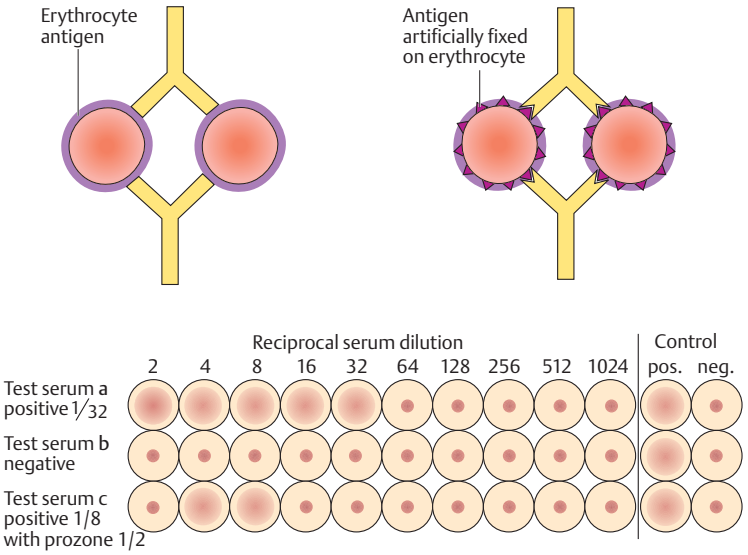


Fig. 2.**25** The hemagglutination test is based on the principle that erythrocytes cross-linked by antibodies settle to the bottom of the microtiter plate wells in mat-like aggregates (test sera **a** and **c**), whereas non-agglutinated erythrocytes collect at the lowest point of the wells to form a single "button" in the middle (test serum **b**). The test sera are first pipetted into the wells at the indicated dilutions, then the erythrocyte suspension is added. Non-specific agglutination is prevented by addition of an irrelevant protein. The test can be carried out using erythrocyte antigens (above left). Alternatively, other antigens can be fixed to the erythrocyte surface and the agglutination monitored (above right). The so-called "prozone" phenomenon results from non-specific blocking mechanisms present in sera which has not been sufficiently diluted.

mune factors present within patient cells and tissues. For this purpose tissue sections, or cell preparations, are treated with specific antibodies (anti-sera) which have been labeled with a fluorochrome (Fig. 2.**26a**). Antigen-antibody reactions can thus be detected using a fluorescence microscope. The fluorochrome absorbs light of a certain wavelength (e.g., UV light), and emits the light energy in the form of light at a different (visible) wavelength. The fluorochrome fluorescein isothiocyanate (FITC), which absorbs UV light and emits it as green light, is used most frequently (caution: bleaches out quickly!).

Antigen Detection Methods

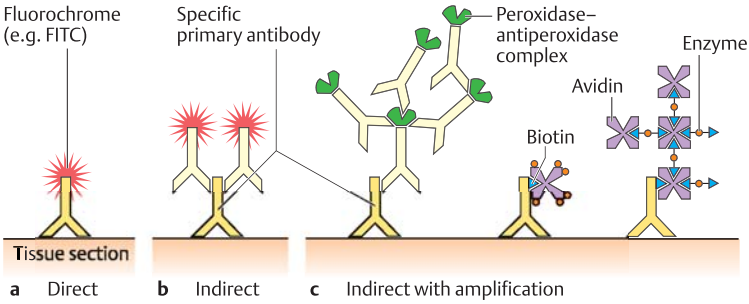


Fig. 2.**26** Immunofluorescence (**a**, **b**) is particularly suitable for the detection of antigens, or specific antibodies, fixed on plastic (solid phase) (ELISA) or present within a tissue section (immunohistology). For direct immunofluorescence (**a**) the specific primary antibody is labeled with a fluorochrome, or an enzyme (ELISA = enzyme-linked immunosorbent assay). The term indirect immunofluorescence is used when it is not the primary antibody being detected, but a secondary antibody which is directed against the unlabeled primary antibody and has also been labeled with a fluorochrome or enzyme (**b**). In most cases, this method achieves a certain degree of amplification. However, an even higher level of amplification can be achieved using preformed complexes of secondary antibody and enzyme (**c**). For the peroxidase method the detector enzyme is bound directly to the secondary antibody (peroxidase catalyzes a color reaction). In the biotin-avidin method the detector enzymes are coupled to either biotin or avidin.

Indirect immunofluorescence and enzyme histology. In this technique the specific or "first" antibody can be unlabeled. The antigen–antibody complexes that form are then detected using a labeled or "second" antibody, directed against the first antibody (Fig. 2.**26b**). Instead of fluorochromes, enzyme-labeled antibodies are now frequently used for tissue sections. The enzyme catalyzes the formation of a color signal following addition of a previously colorless detector substance. This color precipitate allows the direct observation of signals using a light microscopic, and exhibits little bleaching.

Indirect immunofluorescence can be used for the qualitative and quantitative analysis of antibodies directed against particular microbial antigens, or self-tissue antigens, within a patients serum. In the quantitative test, the antigen is fixed in a well or to a tissue section on a slide. The patient sample is repeatedly diluted by a factor of two and added to the antigen or section then rendered visible with a labeled anti–antibody.

There are two main methods of amplifying the immunohistological color signal:

The direct 'primary' antibody, or the detected 'secondary' antibody, is labeled with peroxidase. Following the antigen-antibody reaction, large preformed peroxidase-antiperoxidase complexes are added to the tissue section; these complexes can attach to the peroxidase-labeled antibodies, which are already specifically bound, thus amplifying the signal considerably (Fig. 2.**26c**).

Similarly, biotinylated antibodies can be used. The vitamin biotin is bound with strong affinity by avidin, a basic glycoprotein. Various colorants or enzymes coupled to avidin thus facilitate the color reactions. Such reactions can be amplified on the tissue section by adding preformed biotin-avidin-peroxidase complexes that bind to those biotin-coupled antibodies which have already been bound.

Radioimmunological and Enzyme Immunological Tests

Radioimmunoassay (RIA) and enzyme immunoassay (EIA), also known as ELISA (enzyme-linked immunosorbent assay) (Fig. 2.**28**), are now used very frequently to test for antigens and antibodies. All absorbency tests involve the fixation of antigens or antibodies to a plastic surface. The lower detection limit is a few nanograms. This method forms the basis of modern hepatitis serology, HIV tests, and tests for autoantibodies, lymphokines, cytokines, etc. All of these assays can be performed in a direct form (different sandwich combinations of antigen, antibody and anti-antibody, Fig. 2.**27**)

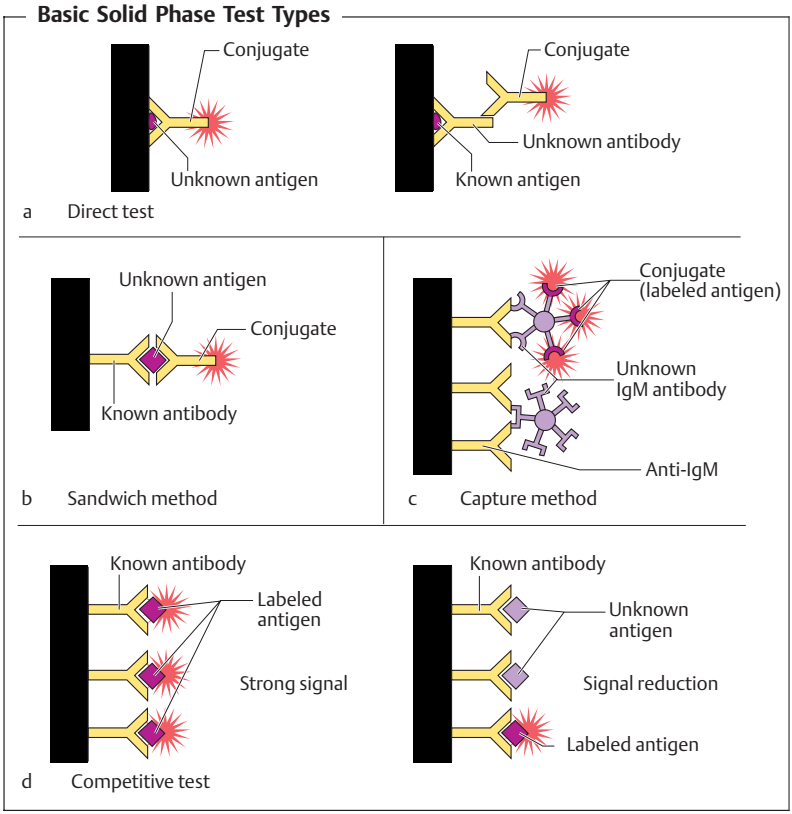
Fig. 2.**27** For solid phase tests both the antigen and antibody are bound to a solid phase (e.g., plastic surface). Various methods are then used to detect any interaction between the antigen and antibody. In the direct test (**a**) an immobilized, unknown, antigen can be detected using a fluorescent-labeled antibody. If the immobilized antigen is known, this test method can also be used to detect an antibody bound to the antigen. In the sandwich method (**b**) a known antibody is immobilized. Detection of antibody-antigen binding is then performed using a second, labeled antibody which interacts with the antigen at a different site. The capture method (**c**) can be used to detect any antigen, for instance IgM antibodies. First, anti-IgM antibodies are immobilized, then serum containing IgM is added to them. The bound IgM can then bind a foreign antigen (e.g., a virus). The detection procedure next makes use of either the labeled foreign antigen or a specific, additionally labeled, antibody which binds to the bound antigen but not to the plastic bound antibody. In the competition or competitive inhibition test (**d**) antibodies are immobilized, and labeled antigens are then bound to them. An unlabeled (unknown) antigen is added, which competes with the labeled antigen. The level of interaction between the antibody and the unknown antigen is then determined by measuring attenuation of the signal. ▶

or as competition assays. Fig. 2.**28** illustrates the quantitative IgE assay, Fig. 2.**29** the procedure for detection of specific IgE in patient sera. Analogous procedures are used to detect specific antibody-binding cells or cytokine-releasing T cells (Fig. 2.**30**).

In-Vitro Cellular Immunity Reactions

Isolation of Lymphocytes

The methods used to measure cellular immunity are experimentally complex. The first step is to isolate human lymphocytes from blood, which can be achieved using Ficoll density gradient centrifugation. Certain lymphocyte



Radioimmunosorbent Test (RIST)

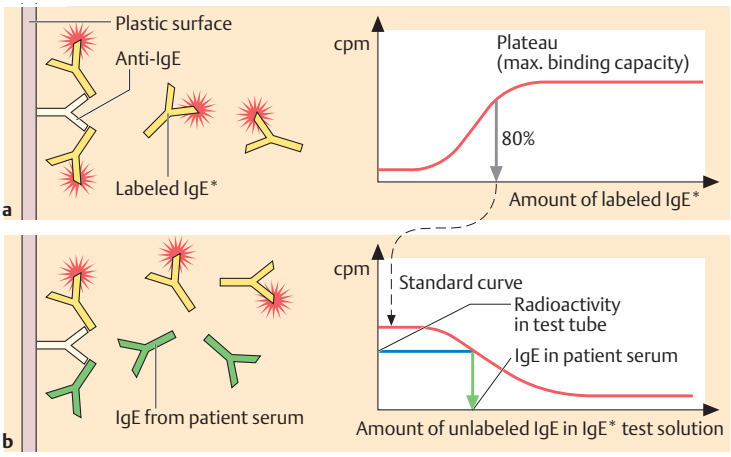


Fig. 2.**28** RIST is a competitive radioimmunoassay (RIA) used for quantitative measurement of antibodies of any given Ig class (in this example total IgE) within patient serum. Anti-IgE antibodies are allowed to adsorb to the solid phase (plastic surface). In the first instance, defined concentrations of radiolabeled IgE (IgE*) are used to determine the maximum binding capacity of these antibodies (**a**). The actual test (**b**) is then performed using the IgE* concentration determined to result in 80% saturation of the fixed antibodies: The IgE* test solution is added to the fixed anti-IgE antibodies and the patient serum is then added by pipette. The more IgE the serum contains, the more IgE* will be displaced by the patients antibodies, and the lower the radioactivity level will be in the test tube. The IgE concentration in the patient serum is then calculated based on a standard curve established previously by progressively "diluting" the IgE* test solution with unlabeled IgE.

populations can be coated with magnetic beads, or sheep erythrocytes loaded with specific antibodies, then purified using a magnet or a Ficoll gradient. The fluorescence-activated cell sorter (FACS, Fig. 2.**31**, p. 133) is now regularly used for this purpose. In this assay, monoclonal antibodies labeled with various fluorochromes directed against cell surface antigens (such as CD4, CD8), or against intracellular cytokines (which involves the use of detergents to increase the permeability of the cell membrane), are incubated with the isolated blood lymphocytes. Alternatively, antigen-specific T lymphocytes can be labeled with MHC class I or II plus peptide tetramers (see below). Following incubation, and several washing steps, the equipment identifies and counts the antibody-loaded lymphocytes, employing magnetic pulse sorting as required.

Radioallergosorbent Test (RAST)

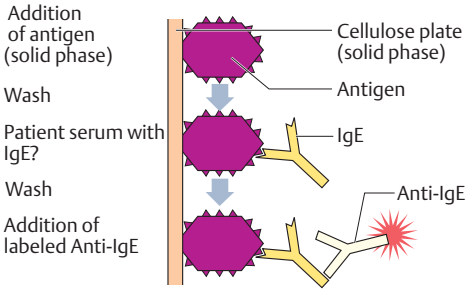


Fig. 2.**29** This test is a highly sensitive detection method for the presence of specific IgE in patient serum. Antigen is bound covalently to a cellulose plate (solid phase). Any IgE in the serum that binds to the antigen is then detected using radiolabeled anti-IgE antibodies.

Tetramer test for detection of specific T cells (Fig. 2.**32**, p. 134): recombinant MHC class I antigen coupled to biotin, labeled with avidin, and correctly folded together with peptide and β_2 microglobulin, forms tetramers; which are recognized by specific TCRs. Subsequent analysis of tetramer binding using FACS equipment is based on the color indicator of the avidin (fluorescein, phycoerythrin, etc.). Tetramers specific for MHC class II antigens plus

ELISPOT Assay

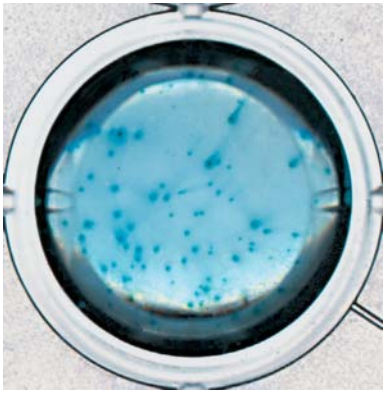


Fig. 2.**30** In the ELISPOT assay antigens, or specific anti-IL antibodies, are applied to the plastic surface. It is then possible to determine the number of immune cells releasing antibodies specific for the applied antigen, or releasing interleukins that are recognized by the applied anti-IL antibodies. Following incubation at 37 °C, the immune complexes which form around these cells can be visualized using a covering agarose layer which includes an enzyme-coupled antibody. These enzymes catalyze a color reaction, resulting in the formation of color spots, each of which will correspond to a single cell producing the specific antibody or interleukin.

peptide can theoretically be used to assay specific $CD4^+$ T cells, but are still difficult to manufacture. Using the tetramer test, specific T cells can be detected directly from blood or lymphoid organs. Histological applications are feasible, but still difficult.

Lymphocyte Function Tests

Certain functions of isolated lymphocyte populations can be determined by a number of methods:

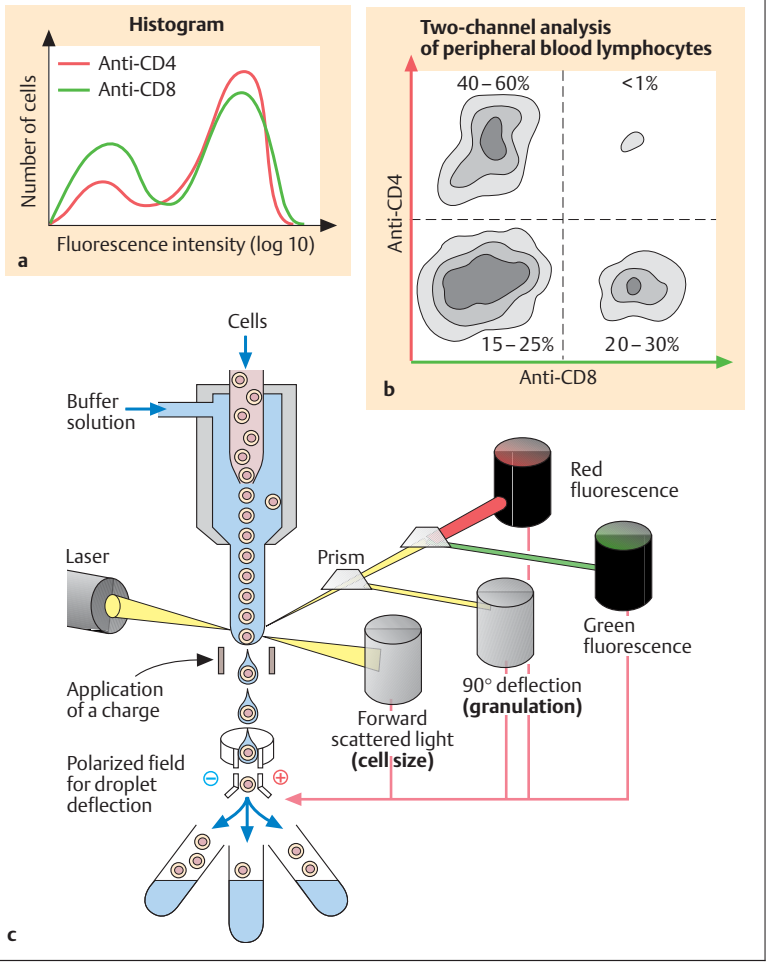
- Determination of the *number of cells producing antibodies*, e.g., the hemolytic plaque assay in which antibody production is tested by adding antigen-coupled erythrocytes. In the vicinity of antibody-secreting cells, the erythrocytes are covered with antibodies and can be lysed by addition of complement. Today, ELISA methods are more often used than erythrocytes (ELISPOT).
- *ELISPOT ASSAY*: used to measure antibody-producing, or IL-releasing, lymphocytes. The antigen or anti-IL antibody is fixed on a plastic surface. Lymphocytes are then placed over this, within a thin layer of agar medium. When the cells are incubated at 37 °C, they may secrete the antibodies or IL recognized by the corresponding test substances. After a certain period of time, the cell layer is shaken off and the preparation is thoroughly washed. The bound material can then be developed using an overlaid semisolid agar, as for the ELISA method. The enzyme reaction generates spots of color, each of which corresponds to a cell, and which can be counted (Fig. 2.**30**).
- Measurement of the *release capacity of cytokines*, or detection of mRNA, is also possible with the ELISPOT assay.
- *Lymphocyte stimulation assay*: isolated lymphocytes are incubated with antigen in culture medium. Measuring the ^{3}H-thymidine incorporation,

Fig. 2.**31** This device analyzes cells by means of fluorescent-labeled antibodies directed against cell surface antigens – or for permeabilized cells, directed against internal cell antigens. In the example shown, peripheral blood lymphocytes (PBL) are incubated with monoclonal antibodies specific for CD4 or CD8, resulting in the distribution of fluorescence intensity as indicated in **a**. In **b** the labeling of different cell populations with anti-CD4 or anti-CD8 is shown. By this means, the percentages of the subpopulations in the total population can be determined. The fluorescence-activated cell sorter shown in (**c**) makes use of this data. By means of vibration, the cell stream is broken up into fine droplets which, depending on the fluorescence and sorting settings used, are charged just before they are separated and ideally contain one cell each. Certain parameters are measured for each cell with the help of a laser beam, where-upon the droplets are deflected into the intended containers by the + and – plate fields. ▶

interleukin release, or a pH transition, can determine whether antigen-specific lymphocytes are present or whether polyclonal T-cell responses (concanavalin [ConA], phytohemagglutinin [PHA]) or B-cell responses (lipopolysaccharide [LPS], pokeweed mitogen [PMA]) were induced.

■ Mixed lymphocyte reactions are used to measure *alloreactivity* (proliferation, cytotoxicity), mainly between recipients and donors of organ or bone

Fluorescence-Activated Cell Sorter (FACS)



Tetramer Assay

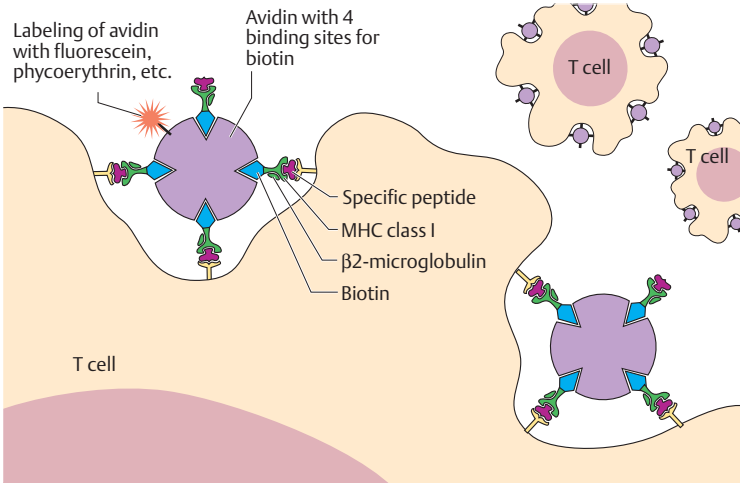


Fig. 2.**32** The tetramer assay is an antigen-specific binding test for living T cells. Complexes comprising biotin-coupled MHC class I heavy chains, β_2 microglobulin, and specific peptide are properly folded, washed, then bound to avidin (which contains four binding sites for biotin). The resulting tetrameric complexes are then incubated with a population of T cells. Those T cells expressing the appropriate T-cell receptor will bind to two or three of the exposed MHC class I-peptide complexes present on each tetramer. Labeling of the avidin component with fluorescein, phycoerythrin, or other fluorescent substances then permits FACS analysis of tertramer binding T cells.

marrow transplants. This test is based on the principle that T lymphocytes are stimulated to proliferate by nonself MHC class I or II antigens and to develop into cytotoxic T cells directed against class I.

- Chromium release assay measures *cytolytic activity*, mainly by $CD8^+$ T cells, directed against allogeneic, virus-infected, or peptide-loaded target cells. The target cells are incubated with ^{51}Cr which the cells incorporate. They are then cultivated with effector cells for 4–6 hours. When the target cells are lysed chromium is released into the culture medium, following which it can be quantitatively measured.

- Assay of intracellular cytokines. Following a brief stimulatory culture (six hours), the cells are rendered permeable using a mild detergent so that specifically labeled antibodies can diffuse into the cells. Labeled cells can then be analyzed by FACS equipment (or by a microscope).

Table 2.**13** Important CD Antigens

Designation (alternatives)	Cells which express the antigen	Functions
CD1	Cortical thymocytes, Langerhans cells, dendritic cells, B cells, intestinal epithelium	MHC class I-like molecule, associated with β_2 microglobulin. Possible special significance in specialized antigen presentation
CD2 (LFA-2)	T cells, thymocytes, natural killer cells	Adhesion molecule which binds to CD58 (LFA-3) and can activate T cells (LFA = lymphocyte function antigen)
CD3	Thymocytes, T cells	Associated with the antigen receptor of T cells, and is necessary for T-cell receptor surface expression and signal transduction
CD4	Several groups of thymocytes, T helper cells, and inflammatory T cells (about two-thirds of the peripheral T cells), monocytes,macrophages	Co-receptor for MHC class II molecules. Binds signal transducers via cytoplasmic portion. Receptor for gp120 in HIV-1 and HIV-2
CD5	Thymocytes, T cells, a subgroup of B cells	Binds to CD72
CD8	Several groups of thymocytes, cytotoxic T cells (about one-third of the peripheral T cells)	Co-receptor for MHC class I molecules, binds signal transducer via cytoplasmic portion
CD10	B and T precursor cells, bone marrow stroma cells	Zinc metal proteinase, and marker for acute lymphoid leukemia of pre-B cells
CD11a (α chain)	Lymphocytes, granulocytes, monocytes, and macrophages	α subunit of β_2 integrin LFA-1 (associated with CD18). Binds to CD54 (ICAM-1), CD102 (ICAM-2), and ICAM-3 (CD50)
CD19	B cells	Forms a complex with CD21 (CR2) and CD81 (TAPA-1), Co-receptor for B cells

Table 2.13 *Continued: Important CD Antigens*

Designation (alternatives)	Cells which express the antigen	Functions
CD21 (CR2)	Mature B cells, follicular dendritic cells	Receptor for the complement components (CR) C3d and the Epstein-Barr virus. Forms a co-receptor for B cells together with CD19 and CD81
CD22	Mature B cells	Adhesion of B cells to monocytes and T cells
CD23 (FcεRII)	Mature B cells, activated macrophages, eosinophils, follicular dendritic cells, blood platelets	Low-affinity receptor for IgE, and ligand for the CD19:CD21:CD81 coreceptor
CD25 (Tac)	Activated T cells, B cells, and monocytes	α chain of the IL-2 receptor, associated with CD122 and the IL-2Rγ chain
CD26	Activated B and T cells, macrophages	A protease which may be involved in HIV entry into host cells
CD28	Subgroup of T cells, activated B cells	Activation of naïve T cells. A receptor for costimulatory signal (signal 2); binds CD80 (B7.1) and B7.2
CD29	Leukocytes	β_1 subunit of γ_1 integrins, associated with CD49a in VLA-1 integrin
CD34	Hematopoietic precursor cells, capillary endothelium	Ligand for CD62L (L-selectin)
CD35 (CR1)	Erythrocytes, B cells, monocytes, neutrophils and eosinophils, follicular dendritic cells	Complement receptor 1; binds C3b and C4b. Also mediates phagocytosis
CD38	Early B and T cells, activated T cells, germinal center B cells, plasma cells	B-cell proliferation?
CD39	Activated B cells, activated natural killer cells, macrophages, dendritic cells	Unknown fuction, but may mediate adhesion of B cells

Table 2.13 *Continued: Important CD Antigens*

Designation (alternatives)	Cells which express the antigen	Functions
CD40	B cells, monocytes, dendritic cells	Receptor for the costimulatory signal for B cells; binds CD40 ligand (CD40-L)
CD40L	Activated CD4 T cells	Ligand for CD40
CD44 (Pgp-1)	Leukocytes, erythrocytes	Binds hyaluronic acid and mediates adhesion of leukocytes
CD45, RO, RA, RB (leukocyte common antigen, LCA), T200, B220	Leukocytes	A tyrosine phosphatase which enhances signal mediation via the antigen receptors of B and T cells; alternative splicing results in many isoforms (see below)
CD54	Hematopoietic and nonhematopoietic cells	Intercellular adhesion molecule (ICAM-1); binds the CD11a/CD18 integrin (LFA-1) and the CD11b/CD18 integrin (MAC-1); receptor for rhinoviruses
CD55 (DAF)	Hematopoietic and nonhematopoietic cells	Decay accelerating factor (DAF); binds C3b and cleaves C3/C5 convertase
CD62E (ELAM-1, E-selectin)	Endothelium	Endothelial leukocyte adhesion molecule (ELAM); binds sialyl-Lewis x and mediates rolling of neutrophilic cells along endothelium
CD64 (FcγRI)	Monocytes, macrophages	High-affinity receptor for IgG
CD80 (B7.1) CD86 (B7.2)	Subgroup of B cells	Costimulators which act as ligands for CD28 and CTLA-4
CD88	Polymorphonuclear leukocytes, macrophages, mast cells	Receptor for the complement component C5a
CD89	Monocytes, macrophages, granulocytes, neutrophils cells, subgroups of B and T cells	IgA receptor?
CD95 (APO-1, Fas)	Many different cell lines; unclear distribution in vivo	Binds TNF-like Fas ligands; induces apoptosis

Table 2.**13** *Continued: Important CD Antigens*

Designation (alternatives)	Cells which express the antigen	Functions
CD102 (ICAM-2) (intercellular cell adhesion molecule)	Resting lymphocytes, monocytes, endothelial cells (in which expression is most pronounced)	Binds CD11a/CD18 (LFA-1), but not CD11b/CD18 (MAC-1)
CD106 (VCAM-1) (vascular cell adhesion molecule)	Endothelial cells	Adhesion molecule; ligand for VLA-4 (very late antigen)
CD115	Monocytes, macrophages	Receptor for the macrophage colony-stimulating factor (M-CSF)
CD116	Monocytes, neutrophils, and eosinophils, endothelium	α chain of the receptor for the granulocyte-macrophage colony-stimulating factor (GM-CSF)
CD117	Hematopoietic precursor cells	Receptor for stem cell factor (SCF)
CD118	Widespread	Receptor for alpha/beta interferons (IFNα/β)
CD119	Macrophages, monocytes, B cells, endothelium	Receptor for gamma interferon (IFNγ)
CD120a	Hematopoietic and nonhematopoietic cells	Most pronounced on epithelial cells

Acknowledgment
The expert editing and translating help of Nicola Harris PhD is gratefully acknowledged.

Glossary

ABC: Antigen-binding cell.
ABS: Antigen-binding site on an antibody.
ADCC: Antibody-dependent cell-mediated cytotoxicity.
Adjuvant: A substance which intensifies the immune response against an antigen, in a immunologically non-specific manner.
AFC: Antibody-forming cell.
Affinity: A measure of binding strength between an antigen determinant (epitope) and the binding site of an antibody (paratope).
Affinity maturation: An increase in the average antibody affinity acquired during the course of a secondary and following immune responses.
AFP: α-fetoprotein.
Allele: Gene locus variations within a species.
Allergy: An altered response following secondary contact with the same antigen, also defined as type I hypersensitivity.
Allogeneic: Refers to the genetic variety contained within a species.
Allotransplant: Transplanted allogeneic tissue.
Allotype: Different forms of a protein product, usually Ig, recognized as an antigen by another individual of the same species.
Alternative pathway: Activation of the complement system via C3 or other factors, but not via C1q.
ANA (anti-nuclear antibodies)**:** Autoantibody directed against DNA contained within the cell nucleus.
Anaphylatoxins: Complement fragments (C3a and C5a), responsible for mediating mast cell degranulation.
Anaphylaxis: An antigen-specific, primarily systemic IgG- or IgE-mediated-immune response.
Antigens: Molecules which are usually characterized by complex folding, and which can be recognized by antibodies.
Antibody: A molecule which binds to a specific antigen.
APC: Antigen-presenting cell.
Atopic: Increased susceptibility to the clinical manifestations associated with type I hypersensitivity (e.g. eczema, asthma, and rhinitis).
Autologous: Derived from the same individual (or inbred strain).
Autosomes: All chromosomes other than the X or Y sex chromosomes.
Avidity: A measure of the functional binding strength between an antibody and its antigen; dependent on affinity and valences (number of binding sites).
BCA-1: B-cell attractant.
BCG: Bacillus Calmette-Guerin. An attenuated form of *Mycobacterium tuberculosis*.
BCGF: B-cell growth factor.
Bence-Jones proteins: Free light chains of Ig present in the serum and urine of multiple myeloma patients.
Bursa fabricii: Lymphoepithelial organ adjacent to the cloaca of birds, in which B cells mature.
C: Complement (C1–9).
C domain: Constant component of Ig.
C3b inactivator: A component of the complement system, known as factor I.
Capping: Aggregation of surface molecules on the cell membrane.
Carrier: The part of a molecule which is recognized by T cells during an immune response.
CBR: Complement-binding reaction.
CCR: A receptor for those chemokines which contain adjacent cysteine-cysteines (CXC or CXXC, cysteines separated by one or two amino acids).
CD marker: Cluster determinant or cluster of differentiation, characteristic of distinct lymphocyte subpopulations.
CDR: Complementarity determining regions (hypervariable antibody regions).
Chemokines: Chemoattractant cytokines

Chimera: A single host bearing cells derived from genetically distinct individuals.

CLIP: A protein which blocks the binding groove of MHC class II prior to its inclusion in the phagolysosome (class II-inhibiting protein).

CMI: Cell-mediated immunity.

Cobra venom factor: A component of cobra venom which exhibits enzymatic activity corresponding to the activity of mammalian C3b.

Combining site: The configuration on an antibody which forms a link with antigen determinants (ABS).

Complement system: A group of serum proteins that are activated in cascades; usually via antibodies, but in some cases directly by infectious agents. Plays an important role during inflammation, chemotaxis, cytolysis, and phagocytosis.

Con A (concanavalin A): A T-cell mitogen.

Cryoglobulin: Antibodies in immune complexes that can be precipitated at 4 °C.

CSF: Colony-stimulating factor (also: cerebrospinal fluid).

CTL: Cytotoxic $CD8^+$ T cell.

CXCR: Receptor for those chemokines which contain a cysteine-x-cysteine motif.

Cyclophosphamide: A toxic substance frequently used to induce immunosuppression.

Cyclosporine A: An immunosuppressant used for the prevention of rejection reactions.

Cytophilic: Exhibiting an affinity towards cells (i.e., binds to cells).

Cytostatic: Exhibiting an inhibitory effect on cell proliferation.

Cytotoxic: Exhibiting a destructive effect towards target cells.

DARC: Duffy antigen receptor for chemokines.

Dendritic cells: Professional APCs derived from the bone marrow. Dendritic cells are mobile and function to transport antigen into lymphoid organs. In the skin they are known as *Langerhans* cells, on the way to the lymph nodes *veiled* cells, and in the lymph nodes *interdigitating* cells.

Desensitization: Repeated exposure to small amounts of an antigen, against which the host shows an allergic reaction; the aim being to downregulate IgE production and upregulate IgG production.

DiGeorge syndrome: Congenital thymic hypoplasia.

DNP: Dinitrophenol, a frequently used small hapten.

Domain: A peptide region with a stable tertiary structure. Immunoglobulins (Ig), MHC class I, and MHC class II molecules all contain comparable Ig domains.

DTH: Delayed type hypersensitivity; A delayed cellular type IV response.

EAE: Experimental allergic encephalitis.

ELISA: Enzyme-linked immunosorbent assay.

ELISPOT: A modified ELISA method used for the detection of specific cell secretion products.

Endotoxins: Bacterial toxins; largely comprised by lipopolysaccharides (LPS) from Gram-negative bacteria.

Eotaxin: A chemokine which regulates eosinophil migration.

Epitope: A special region within an antigen, which is recognized by an antibody binding site.

Epstein-Barr virus: A herpes virus capable of transforming human B cells, and for which B cells possess a special receptor (EBVR). The causative agent of infectious mononucleosis (Pfeiffer disease).

Exon: A protein-coding gene fragment.

Fab: The part of the antibody molecule which contains the antigen-binding site following treatment with papain; comprises a light chain and the first two domains of the heavy chain.

FACS: Fluorescence-activated cell sorter.

Fc: Antibodies use the Fc fragment to bind to cellular receptors (FcR) and C1q complement components.

FcR: Fc receptor.

Fractalkine: A chemokine expressed by endothelial cells; has effects on inflammation and other processe.

Freund's adjuvant (FA): A water-in-oil emulsion. Complete FA contains killed *Mycobacterium tuberculosis*, whilst incomplete FA does not.

GALT: Gut-associated lymphoid tissue.

Gammaglobulins: The serum fraction which migrates most rapidly towards the anode during electrophoresis. Contains all five classes of immunoglobulins.

Gel diffusion test: Immunoprecipitation test; Antigens and antibodies diffuse towards one another, forming a stainable precipitate at the equivalence zone (Ouchterlony test).

Germ line: The genetic material of gametes. Mutations in the germ line, unlike somatic mutations, are inherited by progeny.

GVH: Graft-versus-host reaction; rejection of host tissue by transplanted cells.

H-2: Main histocompatibility complex of mice.

Haplotype: The set of genetic determinants present on one chromosome or chromosome set.

Hapten: A small molecule which can function as an epitope by itself, without being coupled to a carrier, but which alone does not elicit an antibody response.

Helper cells: The $CD4^+$ subclass of T cells which are functionally important for B cells, and which release cytokines; a single helper T-cell clone is specific for one peptide presented by a specific MHC class II molecule.

Hereditary angioedema: Result of congenital C1 inhibitor deficiency.

Heterologous: Belonging to another species.

HEV: High endothelial venules; these are specialized to allow the movement of lymphocytes from the blood into the lymph nodes.

High responder: Individuals (or inbred strains) which exhibit a strong immune response against a defined antigen.

Hinge region: The segment of an immunoglobulin heavy chain which lies between the Fc and Fab regions.

Histocompatibility: Quality which determines rules of acceptance or rejection of a transplant.

HLA: Human leukocyte antigen coded for by the human major histocompatibility gene complex (MHC).

Homologous: Belonging to the same species.

Humoral: Any factor present within extracellular body fluids (e.g. serum, lymph).

HVG: Host-versus-graft reaction. Rejection of transplanted cells by host tissue.

Hybridoma: An antigen-specific B cell that has been successfully fused with a myeloma cell.

Hypervariable region: The three most variable segments present within the V domains of immunoglobulins and T-cell receptors.

Idiotype: The antigenic characteristic of the ABS region of an antibody.

IFN: Interferons; cellular derived substances which contribute to nonspecific cellular resistance, particularly with regard to viral infections.

IL: Interleukins; short-lived substances which mediate the transfer of information between distinct cells (both of the immune system and other tissues).

Immune complex: The product of an antigen-antibody reaction; may also contain components of the complement system.

Immune paralysis: Temporary inability to produce a specific immune response usually resulting from the presence of excessive antigen.

Immunity: Actively or passively acquired immune protection against pathogens and other antigens.

Immunoconglutinins: Auto-antibodies directed against complement components.

Immunofluorescence: Rendering certain antigens visible by binding of a specific fluorescence-labeled antibody.

Immunogen: Any substance which can elicit a specific immune response.

Immunological memory: The ability to produce a faster and stronger immune response following a second, or subsequent, encounter with the same antigen.

Intron: The gene segment present between two exons.

Ir genes: Immune response genes; an early designation used for MHC genes. These code for MHC molecules which control peptide presentation, and thus directly determine the specificity and strength of an immune response.

Isologous: Of identical genetic constitution.

Isotype: The "isotypic" variants of certain proteins coded within the genome, which are identical for all individuals of a species (e.g., immunoglobulin classes).

J genes: Joining genes; a set of gene segments contained within the genetic loci of the heavy and light immunoglobulin chains, or T-cell receptor chains.

K cells: Killer cells; a group of lymphocytes bearing Fc receptors which can destroy their target cells by means of antibody-dependent cell-mediated cytotoxicity (ADCC).

Kupffer cells: Phagocytic cells present in the hepatic sinusoids.

LAK: Lymphokine-activated killer cells (lymphocytes).

LARC: Liver and activation-regulated chemokine.

LCM: Lymphocytic choriomeningitis; an non-bacterial, viral, meningitis.

LGL: Large granular lymphocyte.

Low responder: Individuals (or inbred strains) which exhibit a weak immune response against a given antigen.

LPS: Lipopolysaccharide; a component of the cell wall of certain Gram-negative bacteria, which acts as a B-cell mitogen.

MALT: Mucosa-associated lymphoid tissue.

MBP: Myelin basic protein; functions as an antigen in experimental allergic encephalitis (and probably in multiple sclerosis).

MCP: Monocyte chemoattractant protein.

MDC: Macrophage-derived chemokine.

MHC: Major histocompatibility complex; the main genetic complex responsible for determining histocompatibility. This gene complex codes for the most important transplantation antigens (HLA antigens) in humans. MHC class I molecules are associated with β_2 microglobulin, class II molecules consist of two noncovalently bound transmembrane molecules. The actual function of MHC I, and MHC II, molecules is to present antigenic peptides on the cell surface. Class III molecules comprise complement components, cytokines, and so on.

MHC restriction: Resulting from the interaction of T lymphocytes with other cells, and being controlled by recognition of MHC-presented peptides by the TCR.

MIF: Migration inhibition factors; a group of peptides produced by lymphocytes which inhibit macrophage migration.

MIG: A monokine induced by interferon gamma.

β_2 microglobulin: A protein component of MHC class I molecules.

MIP: Macrophage inflammatory protein.

Mitogen: Any substance which can alone stimulate cells, particularly lymphocytes, to undergo cell division.

MLC: Mixed lymphocyte culture. An in-vitro assay which measures the stimulation response of lymphocytes as alloreactive cytotoxic T-cell reactivity.

MLR: Mixed lymphocyte reaction. An in-vitro assay which measures the stimulation response of lymphocytes as alloreactive proliferation (determined by ^{3}H-thymidine incorporation).

Monoclonal: Any substance derived from a single cell clone, for example monoclonal antibodies.

Myeloma: A B-cell lymphoma, which produces antibodies (plasmocytoma).

NK cells: Natural killer cells. Non-MHC-restricted lymphocytes capable of recognizing and destroying certain cells

that are either virally infected or tumorous.

Nude mice: A mouse strain which carries a spontaneous mutation resulting in the animals having no hair, and usually being athymic.

NZB/W: A strain of mouse bred as an animal model for systemic lupus erythematosus.

Opsonization: Depositions of proteins on an infectious pathogen, that facilitate phagocytosis of the pathogen (e.g., antibodies and C3b).

Paratope: The part of an antibody molecule which contacts the antigenic determinant (epitope); the antigen-binding site (ABS) on the antibody.

PC: Phosphorylcholine; a commonly used hapten found on the surface of a number of microorganisms.

PCA: Passive cutaneous anaphylaxis; a classic detection reaction for antigen-specific IgE.

PFC: Plaque forming cell; an antibody-producing cell which can be detected by the hemolysis plaque test.

PHA: Phytohemagglutinin; a mitogen for T cells.

Plasma cell: An antibody-producing B cell which has reached the end of its differentiation pathway.

PMN: Polymorphonuclear neutrophilic granulocytes.

Pokeweed mitogen: A mitogen for B cells.

Polyclonal: A term describing products derived from a number of different cell clones (e.g., polyclonal antibodies).

Primary lymphoid tissues: Thymus, bursa of Fabricius (in birds), bone marrow.

Primary response: The immune response which follows an initial encounter with a particular antigen (see priming, secondary response).

Priming: Following an initial contact with an antigen, an immunocompetent cell becomes sensitized or "primed."

Prozone phenomenon: Lack of a measurable response at high-test substance concentrations.

Pseudoalleles: Tandem variants of a gene, which do not occupy a homologous position on the chromosome.

Pseudogenes: Genes containing structures which are homologous to other genes, but which cannot be expressed.

RANTES: Regulated on activation; this is normally expressed and secreted by T cells.

Reagin: Historical term for IgE.

Rearrangement: For instance the rearrangement of genetic information in somatic B and T cells.

Recombination: A process by which genetic information is rearranged during meiosis.

Reticuloendothelial system (RES): Phagocytic cells distributed within the supportive connective tissue of the liver, spleen, lymph nodes, and other organs (e.g. sinus endothelial cells, Kupffer cells, histiocytes).

Rhesus (Rh) antigens: Antigenic proteins present on the surface of erythrocytes in approximately 85% of all humans.

Rheumatoid factor (RF): Autoantibodies—these are usually IgM but can also be of the IgG and IgA isotypes—which are specifically directed against the body's own IgG molecules.

SCID: Severe combined immunodeficiency disease; a congenital deficiency of the humoral and cellular immune system, resulting from a lack of both T and B cells. The animal model is the SCID mouse and is a spontaneous mutant.

SDF-1alpha: Stromal cell-derived factor.

Secretory piece: An IgA-associated polypeptide produced by epithelial cells, and which facilitates the transmembrane transport of IgA.

Secondary response: The immune response which follows a second encounter with a specific antigen.

Serum sickness: An inflammatory type III reaction, occurring after repeated injection of a foreign protein.

SLC: Secondary lymphoid organ chemokine.

SLE: Systemic lupus erythematosus.

Somatic mutation/recombination: Rearrangements of genes in somatic cells (as opposed to germ line cells), resulting in a newly combined DNA sequence which is not heritable.

Splenomegaly: Splenic enlargement; often observed in cases of hematopoietic cell tumors, vascular circulatory problems, or following various parasitic infections. Can also be used as a measure of GVH reactions.

SRBC: Sheep red blood cells (erythrocytes).

Stripping: The process by which antibodies remove antigen determinants from target cells.

Suppressor cell: A proposed antigen-specific T-cell subpopulation which acts to reduce the immune responses of other T cells or B cells. This suppression can also be of a nonspecific nature.

Syngeneic: Animals produced by repeated inbreeding, or monozygotic twins, which are considered syngeneic when each pair of autosomes within the individuals is identical.

TATA: Tumor-associated transplantation antigens.

Tc: Cytotoxic T cell ($CD8^+$ T cell) or CTL.

TCGF: T-cell growth factor; identical with interleukin 2 (IL-2).

T-dep/T-ind: T cell-dependent/T cell-independent; an antibody response to T-dependent antigens is only possible if (MHC-restricted) T-cell help is also available.

T-DTH: A T cell that contributes to delayed type hypersensitivity reactions.

TECK: Thymus-expressed chemokine.

Tetramer: Biotinylated MHC class I, or class II, molecule complexed to peptide and bound to labeled avidin. Used to determine the presence of peptide-specific T cells.

TGF: Transforming growth factor.

TH: T helper cells ($CD4^+$ T cell; see also Helper cells).

Thy: A cell surface antigen of mouse T cells; there are several allelic variants of this marker.

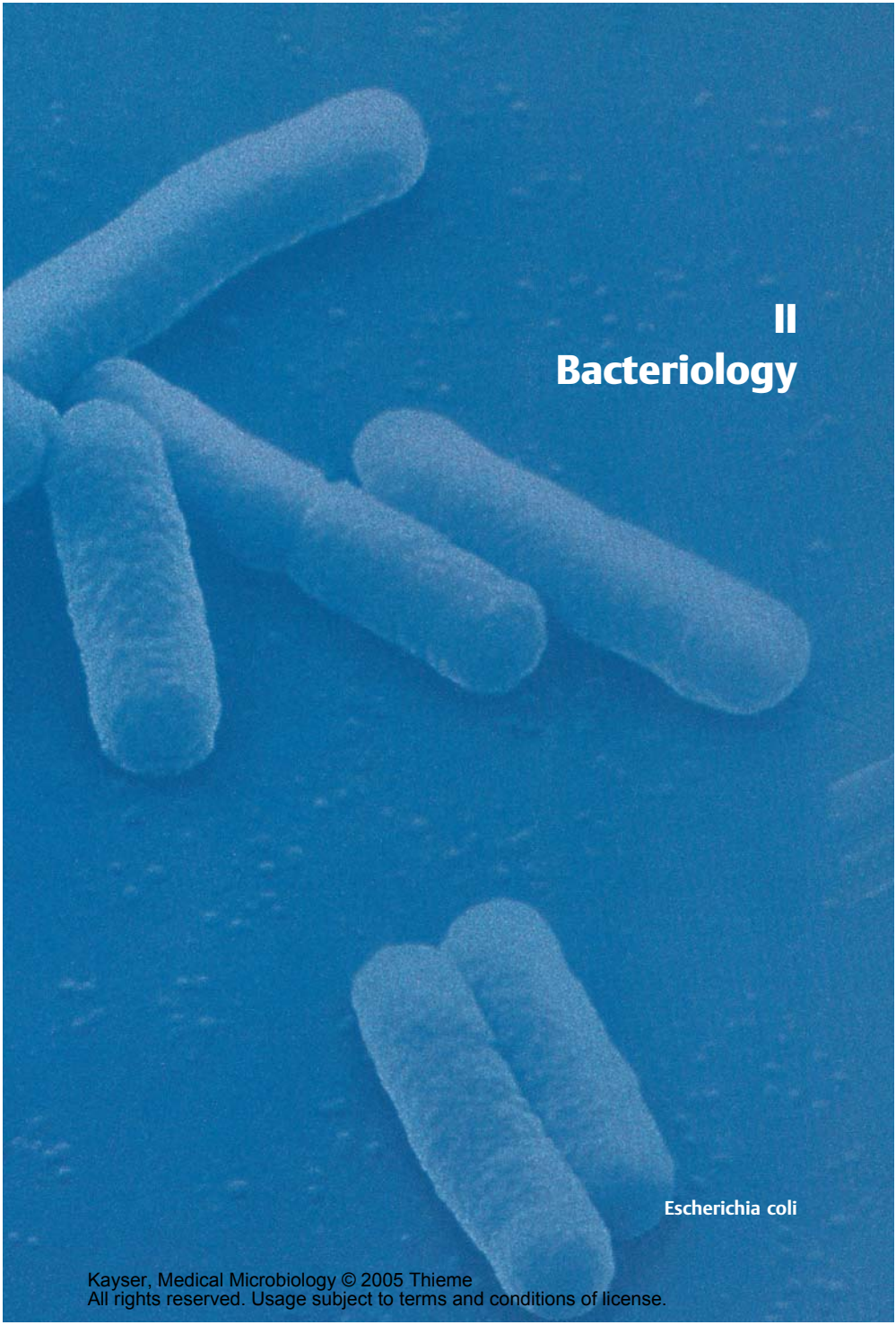
TNF: Tumor necrosis factor.

Tolerance: A state of specific immunological unresponsiveness.

Transformation (blastic): Morphological changes in a lymphocyte associated with the onset of cell division.

Transplantation antigens: See MHC.

Wiskott-Aldrich syndrome: A sex-linked, inheritable, recessive, combined immune deficiency in which IgM antibody production and cellular immune reactions are impaired.



II
Bacteriology

Escherichia coli

3 General Bacteriology

F. H. Kayser

The Morphology and Fine Structure of Bacteria

Bacterial cells are between 0.3 and 5 µm in size. They have three basic forms: cocci, straight rods, and curved or spiral rods. The **nucleoid** consists of a very thin, long, circular DNA molecular double strand that is not surrounded by a membrane. Among the nonessential genetic structures are the **plasmids**. The **cytoplasmic membrane** harbors numerous proteins such as permeases, cell wall synthesis enzymes, sensor proteins, secretion system proteins, and, in aerobic bacteria, respiratory chain enzymes. The membrane is surrounded by the **cell wall**, the most important element of which is the supporting murein skeleton. The cell wall of Gram-negative bacteria features a porous outer membrane into the outer surface of which the lipopolysaccharide responsible for the pathogenesis of Gram-negative infections is integrated. The cell wall of Gram-positive bacteria does not possess such an outer membrane. Its murein layer is thicker and contains teichoic acids and wall-associated proteins that contribute to the pathogenic process in Gram-positive infections. Many bacteria have **capsules** made of polysaccharides that protect them from phagocytosis. Attachment **pili** or **fimbriae** facilitate adhesion to host cells. Motile bacteria possess **flagella**. Foreign body infections are caused by bacteria that form a **biofilm** on inert surfaces. Some bacteria produce **spores**, dormant forms that are highly resistant to chemical and physical noxae.

Bacterial Forms

Bacteria differ from other single-cell microorganisms in both their cell structure and size, which varies from 0.3–5 µm. Magnifications of 500–1000×—close to the resolution limits of light microscopy—are required to obtain useful images of bacteria. Another problem is that the structures of objects the size of bacteria offer little visual contrast. Techniques like phase contrast and dark field microscopy, both of which allow for live cell observation, are used to overcome this difficulty. Chemical-staining techniques are also used, but the prepared specimens are dead.

Bacterial Morphology

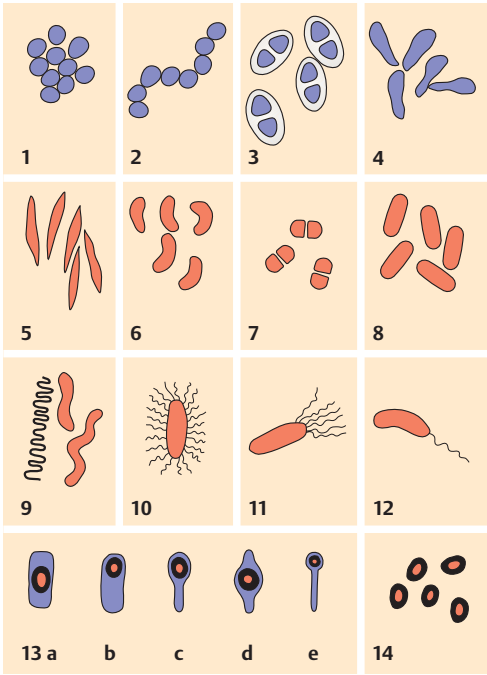


Fig. 3.1

1. Gram-positive cocci in grapelike clusters (staphylococci)
2. Gram-positive cocci in chains (streptococci)
3. Gram-positive cocci with capsules (pneumococci)
4. Gram-positive, clubshaped, pleomorphic rods (corynebacteria)
5. Gram-negative rods with pointed ends (fusobacteria)
6. Gram-negative curved rods (here commashaped vibrios)
7. Gram-negative diplococci, adjacent sides flattened (neisseria)
8. Gram-negative straight rods with rounded ends (coli bacteria)
9. Spiral rods (spirilla) and Gram-negative curved rods (*Helicobacter*)
10. Peritrichous flagellation
11. Lophotrichous flagellation
12. Monotrichous flagellation
13. Formation of endospores (sporulation) in cells of the genera *Bacillus* and *Clostridium* (spore stain)
    a) Central spore, vegetative cell shows no swelling
    b) Terminal spore, vegetative cell shows no swelling
    c) Terminal spore ("tennis racquet")
    d) Central spore, vegetative cell shows swelling
    e) Terminal spore ("drumstick")
14. Free spores (spore stain)

Table 3.**1** Morphological Characteristics of Bacteria (see Fig. 3.**1** for examples)

Bacterial form	Remarks
Cocci	Occur in clusters (Fig. 3.**2**), chains, pairs (diplococci), packets
Straight rods	Uniform thickness, rounded ends (Fig. 3.**3**), pointed ends, club form
Curved rods	Commashaped, spiral (Fig. 3.**4**), screwshaped
Mycoplasmas	Bacteria without a rigid cell wall; coccoid cells, long threads
Chlamydiae	Two forms: spherical/oval elementary bodies (300 nm); spherical/oval reticulate bodies (1000 nm)
Rickettsiae	Short coccoid rods (0.3–1 µm)

■ **Simple staining.** In this technique, a single staining substance, e.g., methylene blue, is used.

■ **Differential staining.** Two stains with differing affinities to different bacteria are used in differential staining techniques, the most important of which is gram staining. Gram-positive bacteria stain blue-violet, Gram-negative bacteria stain red (see p. 211 for method).

Three basic forms are observed in bacteria: spherical, straight rods, and curved rods (see Figs. 3.**1**–3.**4**).

Fine Structures of Bacteria

Nucleoid (Nucleus Equivalent) and Plasmids

The "cellular nucleus" in prokaryotes consists of a tangle of double-stranded DNA, not surrounded by a membrane and localized in the cytoplasm (Fig. 3.**5**). In *E. coli* (and probably in all bacteria), it takes the form of a single circular molecule of DNA. The genome of *E. coli* comprises 4.63×10^6 base pairs (bp) that code for 4288 different proteins. The genomic sequence of many bacteria is known.

The plasmids are nonessential genetic structures. These circular, twisted DNA molecules are 100–1000× smaller than the nucleoid genome structure and reproduce autonomously (Fig. 3.**6**). The plasmids of human pathogen bacteria often bear important genes determining the phenotype of their cells (resistance genes, virulence genes).

Cocci

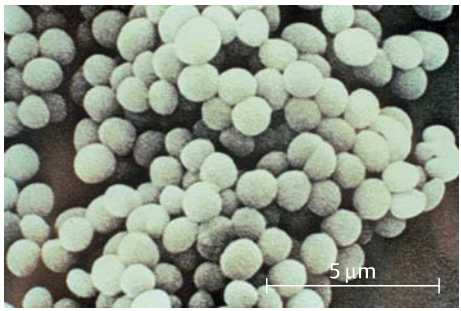


Fig. 3.**2** Cocci are spherical bacteria. Those found in grapelike clusters as in this picture are staphylococci (Scanning electron microscopy (SEM)).

Rod Bacteria

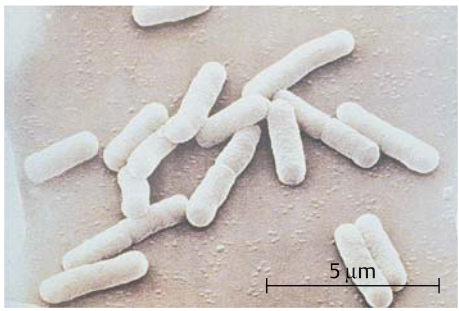


Fig. 3.**3** The straight rod bacteria with rounded ends shown here are coli bacteria (SEM).

Spirilla

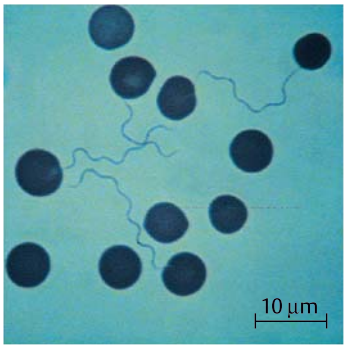


Fig. 3.**4** Spirilla, in this case borrelia are spiral bacteria (light microscopy (LM), Giemsa stain).

Bacteria During Cell Division

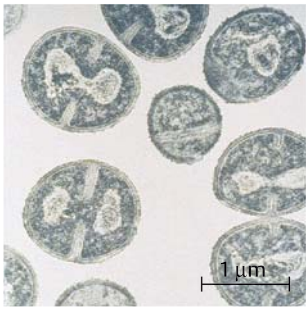


Fig. 3.**5** The nucleoid (nucleus equivalent) of bacteria consists of a tangled circular DNA molecule without a nuclear membrane. Transmission electron microscopy (TEM) image of staphylococci.

DNA Topology in Bacterial Cells

The DNA double helix (one winding/10 base pairs) is also wound counterclockwise about its helical axis (one winding/15 helical windings). This so-called supercoiling is necessary to save space and energy. Only supercoiled DNA can be replicated and transcribed. Topoisomerases steer the supercoiling process. DNA gyrase and topoisomerase IV are topoisomerases that occur only in bacteria. The 4-quinolones, an important group of anti-infection substances, inactivate these enzymes irreversibly.

Plasmids

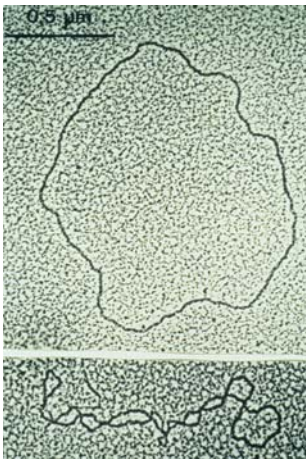


Fig. 3.**6** **a** Open circular form (OC). The result of a rupture in one of the two nucleic acid strands.
b Twisted (CCC = covalently closed circular), native form (TEM image).

a

b

Cytoplasm

The cytoplasm contains a large number of solute low- and high-molecular-weight substances, RNA and approximately 20 000 ribosomes per cell. Bacteria have 70S ribosomes comprising 30S and 50S subunits. Bacterial ribosomes function as the organelles for protein synthesis. The cytoplasm is also frequently used to store reserve substances (glycogen depots, polymerized metaphosphates, lipids).

The Most Important Bacterial Cytoplasmic Membrane Proteins

Permeases	Active transport of nutrients from outside to inside against a concentration gradient.
Biosynthesis enzymes	Required for biosynthesis of the cell wall, e.g., its murein (see under "Cell wall" p. 152). The enzymes that contribute to the final murein biosynthesis steps are for the most part identical with the "penicillin-binding proteins" (PBPs).
Secretion system proteins	Four secretion systems differing in structure and mode of action have been described to date. Proteins are moved out of the cell with the help of these systems. A common feature of all four is the formation of protein cylinders that traverse the cytoplasmic membrane and, in Gram-negative bacteria, the outer cell wall membrane as well. See p. 17 on the special relevance of the type III secretion system to virulence.
Sensor proteins (also known as signal proteins)	Transmit information from the cell's environment into its interior. The so-called receiver domain extends outward, the transmitter domain inward. The transmission activity is regulated by the binding of signal molecules to a receiver module. In two-component systems, the transmitter module transfers the information to a regulator protein, activating its functional module. This regulator segment can then bind to specific gene sequences and activate or deactivate one or more genes (see also Fig. 1.**4**, p. 19).
Respiratory chain enzymes	Occur in bacteria with aerobic metabolism. Aerobic respiration functions according to the same principles as cellular respiration in eurkaryotes.

The Cytoplasmic Membrane

This elementary membrane, also known as the plasma membrane, is typical of living cells. It is basically a double layer of phospholipids with numerous proteins integrated into its structure. The most important of these membrane

Basic Bacterial Cell Structure

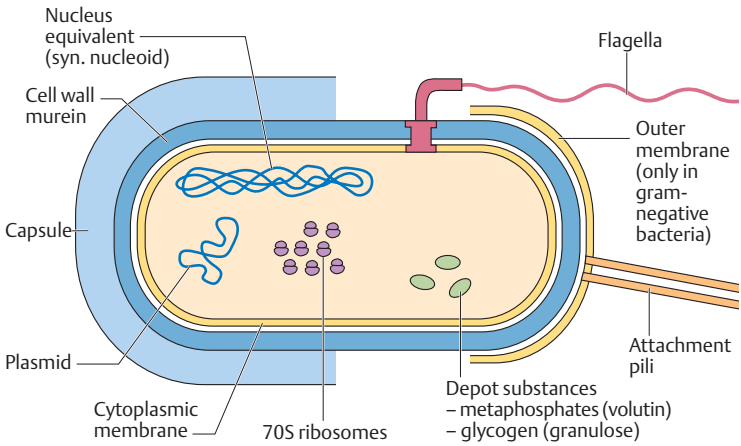


Fig. 3.**7** All bacteria have the same basic structure (not to scale).

proteins are permeases, enzymes for the biosynthesis of the cell wall, transfer proteins for secretion of extracellular proteins, sensor or signal proteins, and respiratory chain enzymes.

In electron microscopic images of Gram-positive bacteria, the mesosomes appear as structures bound to the membrane. How they function and what role they play remain to be clarified. They may be no more than artifacts.

Cell Wall

The tasks of the complex bacterial cell wall are to protect the protoplasts from external noxae, to withstand and maintain the osmotic pressure gradient between the cell interior and the extracellular environment (with internal pressures as high as 500–2000 kPa), to give the cell its outer form and to facilitate communication with its surroundings.

Murein (syn. peptidoglycan). The most important structural element of the wall is murein, a netlike polymer material surrounding the entire cell (sacculus). It is made up of polysaccharide chains crosslinked by peptides (Figs. 3.**8** and 3.**9**).

The cell wall of Gram-positive bacteria (Fig. 3.**10**). The murein sacculus may consist of as many as 40 layers (15–80 nm thick) and account for as much as

The Murein Building Block

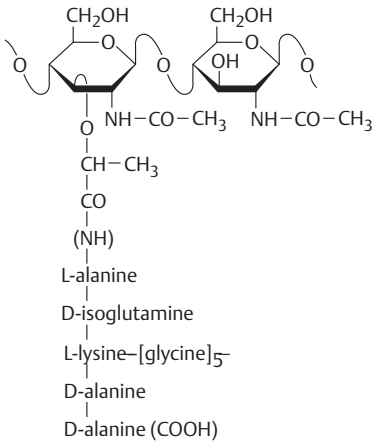


Fig. 3.**8** The murein (syn. peptidoglycan) of the cell wall is composed of a series of identical subunits. The terminal D-alanine is split off each time a new crosslink is synthesized. Only in staphylococci is a pentaglycine interpeptide bridge inserted between adjacent peptides.

30% of the dry mass of the cell wall. The membrane lipoteichoic acids are anchored in the cytoplasmic membrane, whereas the cell wall teichoic acids are covalently coupled to the murein. The physiological role of the teichoic

The Structure of Murein

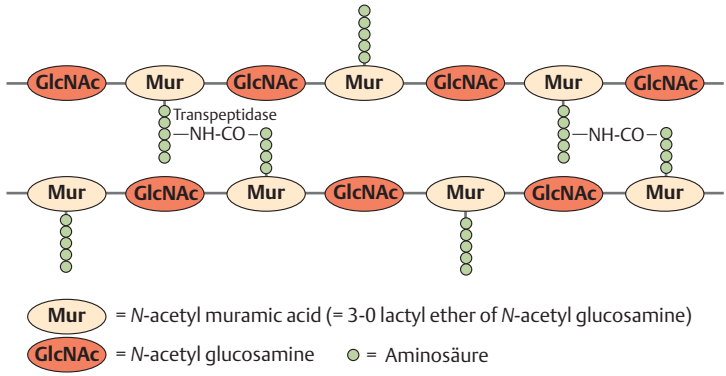


Fig. 3.**9** Soluble murein fragments of Gram-negative and Gram-positive bacteria can stimulate excessive cytokine secretion in macrophages by binding to toll-like receptors and CD14. Cytokines cause the clinical symptoms of sepsis or septic shock syndrome (see under Lipoid A, p. 156).

The Cell Wall of Gram-Positive Bacteria

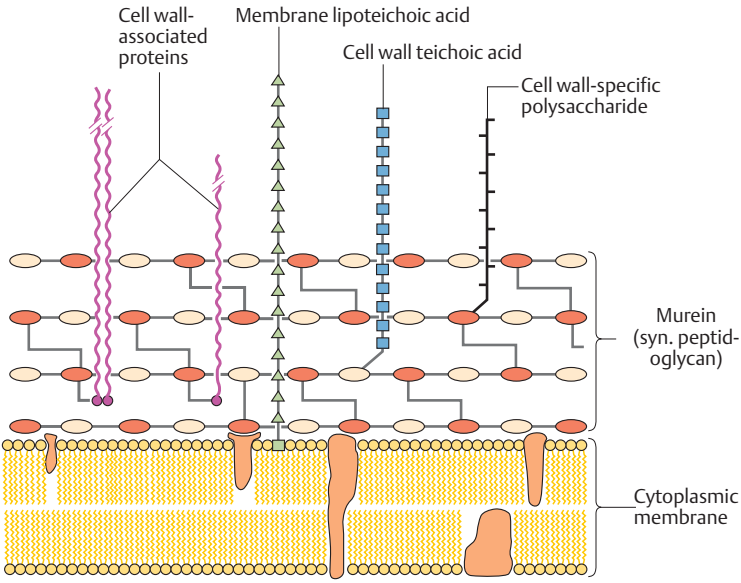


Fig. 3.**10** Note the characteristic thick murein layer, the proteins and teichoic acids anchored in the murein, and the lipoteichoic acid fixed to the membrane by a lipophilic anchor (not to scale).

acids is not known in detail; possibly they regulate the activity of the autolysins that steer growth and transverse fission processes in the cell. Within the macroorganism, teichoic acids can activate the alternative complement pathway and stimulate macrophages to secrete cytokines. Examples of cell wall-associated proteins are protein A, the clumping factor, and the fibronectin-binding protein of *Staphylococcus aureus* or the M protein of *Streptococcus pyogenes*. Cell wall anchor regions in these proteins extending far beyond the murein are bound covalently to its peptide components. Cell wall-associated proteins frequently function as pathogenicity determinants (specific adherence; phagocyte protection).

The cell wall of Gram-negative bacteria. Here, the murein is only about 2 nm thick and contributes up to 10% of the dry cell wall mass (Fig. 3.**11**). The outer membrane is the salient structural element. It contains numerous proteins (50% by mass) as well as the medically critical lipopolysaccharide.

The Cell Wall of Gram-Negative Bacteria

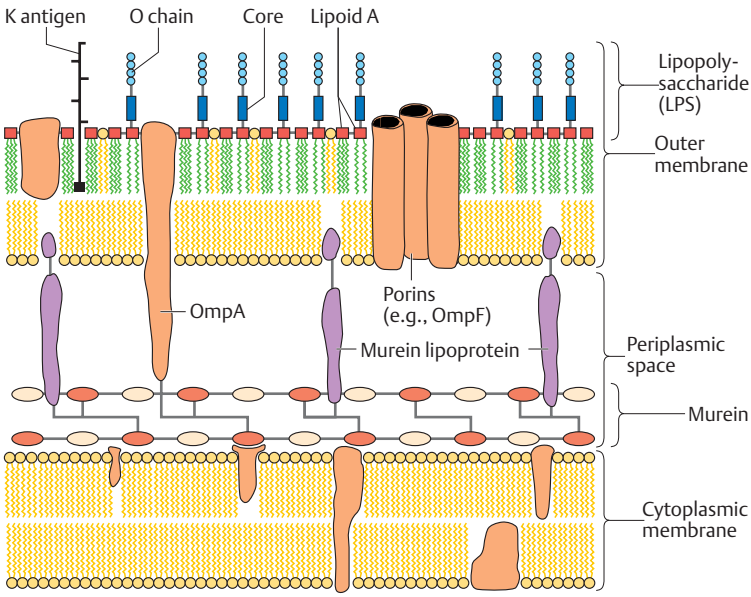


Fig. 3.**11** Note the characteristic thin murein layer and the outer membrane connected to it by proteins (OmpA, murein lipoprotein). Many different proteins are localized in the outer membrane. Its outer layer is made up of closely packed lipopolysaccharide complexes (see Fig. 3.12).

■ **Outer membrane proteins.**
- OmpA (outer membrane protein A) and the murein lipoprotein form a bond between outer membrane and murein.
- Porins, proteins that form pores in the outer membrane, allow passage of hydrophilic, low-molecular-weight substances into the periplasmic space.
- Outer membrane-associated proteins constitute specific structures that enable bacteria to attach to host cell receptors.
- A number of Omps are transport proteins. Examples include the LamB proteins for maltose transport and FepA for transport of the siderophore ferric (Fe^{3+}) enterochelin in *E. coli* (see also p. 13).

■ **Lipopolysaccharide (LPS).** This molecular complex, also known as endotoxin, is comprised of the lipoid A, the core polysaccharide, and the O-specific polysaccharide chain (Fig. 3.**12**).

The Lipopolysaccharide Complex

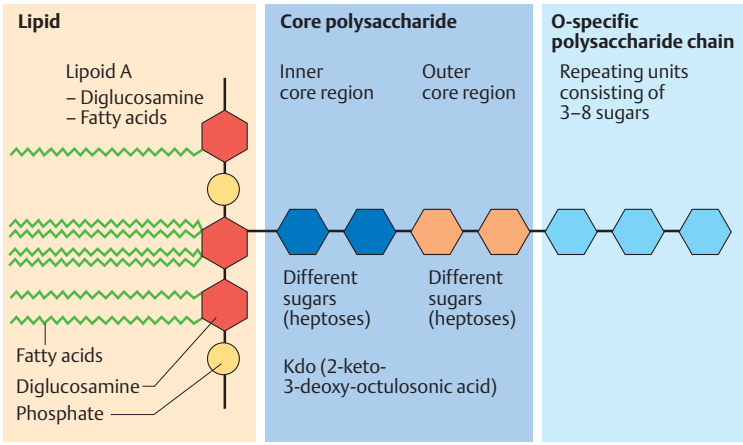


Fig. 3.**12** The three-part lipopolysaccharide complex (LPS) of Gram-negative bacteria is anchored in the outer membrane by means of its lipid moiety. LPS is also known as endotoxin.

– **Lipoid A** is responsible for the toxic effect. As a free substance, or bound up in the LPS complex, it stimulates—by binding together with the LPS binding protein (LBP) to the CD14 receptor of macrophages—the formation and secretion of cytokines that determine clinical endotoxin symptomatology. Interleukin 1 (IL-1) and tumor necrosis factor (TNF) induce an increased synthesis of prostaglandin E2 in the hypothalamus, thus setting the "thermostat" in the temperature control center higher, resulting in fever. Other direct and indirect endotoxin effects include granulopoiesis stimulation, aggregation and degeneration of thrombocytes, intravasal coagulation due to factor VII activation, a drop in blood pressure, and cachexia. LPS can also activate the alternative complement pathway. Release of large amounts of endotoxin can lead to septic (endotoxic) shock. Endotoxin is not inactivated by vapor sterilization. Therefore, the parent materials used in production of parenteral pharmaceuticals must be free of endotoxins (pyrogens).
– **The O-specific polysaccharide chain** is the so-called O antigen, the fine chemical structure of which results in a large number of antigenic variants useful in bacterial typing (e.g., detailed differentiation of salmonella types) (see p. 284f.).

L-forms (L = Lister Institute). The L-forms are bacteria with murein defects, e.g., resulting from the effects of betalactam antibiotics. L-forms are highly

unstable when subjected to osmotic influences. They are totally resistant to betalactams, which block the biosynthesis of murein. The clinical significance of the L-forms is not clear. They may revert to the normal bacterial form when betalactam therapy is discontinued, resulting in a relapse.

Capsule

Many pathogenic bacteria make use of extracellular enzymes to synthesize a polymer that forms a layer around the cell: the capsule. **The capsule protects bacterial cells from phagocytosis.** The capsule of most bacteria consists of a polysaccharide. The bacteria of a single species can be classified in different capsular serovars (or serotypes) based on the fine chemical structure of this polysaccharide.

Flagella

Flagella give bacteria the ability to move about actively. The flagella (singular flagellum) are made up of a class of linear proteins called flagellins. Flagel-

Bacterial flagella

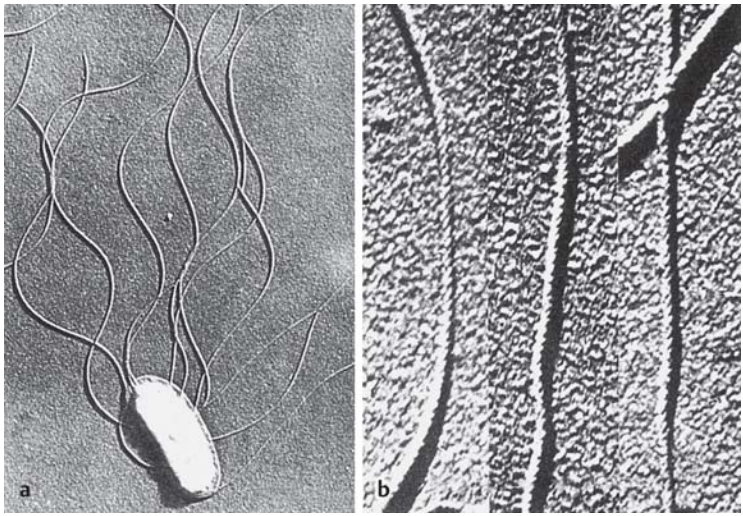


Fig. 3.**13** **a** Flagellated bacterial cell (SEM, 13 000×). **b** Helical structure of bacterial flagella (SEM, 77 000×).

lated bacteria are described as monotrichous, lophotrichous, or peritrichous, depending on how the flagella are arranged (see Fig. 3.**1**, p. 147). The basal body traverses the cell wall and cytoplasmic membrane to anchor the flagellum (see Figs. 3.**7** and 3.**13**) and enables it to whirl about its axis like a propeller. In *Enterobacteriaceae*, the flagellar antigens are called H antigens. Together with the O antigens, they are used to classify bacteria in serovars.

Attachment Pili (Fimbriae), Conjugation Pili

Many Gram-negative bacteria possess thin microfibrils made of proteins (0.1–1.5 nm thick, 4–8 nm long), the attachment pili. They are anchored in the outer membrane of the cell wall and extend radially from the surface. Using these structures, bacteria are capable of specific attachment to host cell receptors (ligand—receptor, key—keyhole).

The conjugation pili (syn. sex pili) in Gram-negative bacteria are required for the process of conjugation and thus for transfer of conjugative plasmids (see p. 175).

Examples of Attachment Pili in Gram-Negative Bacteria

PAP (syn. P pili)	Pyelonephritis-associated pili. Bind to receptors of the uroepithelium and to the P blood group antigen (hence "P" pili). The specific receptors for these pili are plentiful on the uroepithelial surface. PAP are characteristic of the uropathological variety of *Escherichia coli* that causes spontaneous urinary tract infections in patients showing no tract obstruction.
CFA1, CFA2	Colonization factors. Pili responsible for specific binding of enteropathogenic coli bacteria to enterocytes.
Gonococcal attachment pili	Used for specific attachment of gonococci mucosal cells of the urogenital epithelium.

Biofilm

A bacterial biofilm is a structured community of bacterial cells embedded in a self-produced polymer matrix and attached to either an inert surface or living tissue. Such films can develop considerable thickness (mm). The bacteria located deep within such a biofilm structure are effectively isolated from immune system cells, antibodies, and antibiotics. The polymers they secrete are frequently glycosides, from which the term glycocalyx (glycoside cup) for the matrix is derived.

Examples of Medically Important Biofilms

- Following implantation of endoprostheses, catheters, cardiac pacemakers, shunt valves, etc. these foreign bodies are covered by matrix proteins of the macroorganism such as fibrinogen, fibronectin, vitronectin, or laminin. Staphylococci have proteins on their surfaces with which they can bind specifically to the corresponding proteins, for example the clumping factor that binds to fibrinogen and the fibronectin-binding protein. The adhering bacteria then proliferate and secrete an exopolysaccharide glycocalyx: the biofilm matrix on the foreign body. Such biofilms represent **foreign body-associated infection foci**.
- Certain oral streptococci (*S. mutans*) bind to the proteins covering tooth enamel, then proceed to build a glucan matrix out of sucrose. Other bacteria then adhere to the matrix to form plaque (Fig. 3.**14**), the precondition for destruction of the enamel and formation of **caries** (see p. 243f.).
- Oral streptococci and other bacteria attach to the surface of the cardiac valves to form a biofilm. Professional phagocytes are attracted to the site and attempt, unsuccessfully, to phagocytize the bacteria. The frustrated phagocytes then release the tissue-damaging content of their lysosomes (see p. 23), resulting in an inflammatory reaction and the clinical picture of **endocarditis**.

Bacterial Spores

Bacterial spores (endospores) are purely dormant life forms. Their development from bacterial cells in a "vegetative" state does not involve assimilation of additional external nutrients. They are spherical to oval in shape and are characterized by a thick spore wall and a high level of resistance to chemical and physical noxae. Among human pathogen bacteria, only the genera *Clostridium* and *Bacillus* produce spores. The heat resistance of these spores is their most important quality from a medical point of view, since heat ster-

Dental Plaque

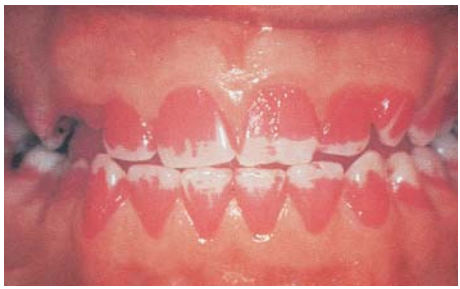


Fig. 3.**14** Dental plaque can be rendered visible with an erythrosin stain.

ilization procedures require very high temperatures to kill them effectively. Potential contributing factors to spore heat resistance include their thick wall structures, the dehydration of the spore, and crosslinking of the proteins by the calcium salt of pyridine-2,6-dicarboxylic acid, both of which render protein denaturing difficult. When a spore's milieu once again provides favorable conditions (nutrient medium, temperature, osmotic pressure, etc.) it returns to the vegetative state in which spore-forming bacteria can reproduce.

The Physiology of Metabolism and Growth in Bacteria

Human pathogenic bacteria are chemosynthetic and organotrophic (chemo-organotrophic). They derive energy from the breakdown of organic nutrients and use this chemical energy both for resynthesis and secondary activities. Bacteria oxidize nutrient substrates by means of either respiration or fermentation. In respiration, O_2 is the electron and proton acceptor, in fermentation an organic molecule performs this function. Human pathogenic bacteria are classified in terms of their O_2 requirements and tolerance as facultative anaerobes, obligate aerobes, obligate anaerobes, or aerotolerant anaerobes. Nutrient broth or agar is used to cultivate bacteria. Nutrient agar contains the inert substrate agarose, which liquefies at 100 °C and gels at 45 °C. Selective and indicator mediums are used frequently in diagnostic bacteriology.

Bacteria reproduce by means of simple transverse binary fission. The time required for complete cell division is called generation time. The in-vitro generation time of rapidly proliferating species is 15–30 minutes. This time is much longer in vivo. The growth curve for proliferation in nutrient broth is normally characterized by the phases lag, log (or exponential) growth, stationary growth, and death.

Bacterial Metabolism

Types of Metabolism

Metabolism is the totality of chemical reactions occurring in bacterial cells. They can be subdivided into anabolic (synthetic) reactions that consume energy and catabolic reactions that supply energy. In the anabolic, endergonic

reactions, the energy requirement is consumed in the form of light or chemical energy—by photosynthetic or chemosynthetic bacteria, respectively. Catabolic reactions supply both energy and the basic structural elements for synthesis of specific bacterial molecules. Bacteria that feed on inorganic nutrients are said to be lithotrophic, those that feed on organic nutrients are organotrophic.

Human pathogenic bacteria are always chemosynthetic, organotrophic bacteria (or chemo-organotrophs).

Catabolic Reactions

Organic nutrient substrates are catabolized in a wide variety of enzymatic processes that can be schematically divided into four phases:

Digestion. Bacterial exoenzymes split up the nutrient substrates into smaller molecules outside the cell. The exoenzymes represent important pathogenicity factors in some cases.

Uptake. Nutrients can be taken up by means of passive diffusion or, more frequently, specifically by active transport through the membrane(s). Cytoplasmic membrane permeases play an important role in these processes.

Preparation for oxidation. Splitting off of carboxyl and amino groups, phosphorylation, etc.

Oxidation. This process is defined as the removal of electrons and H^+ ions. The substance to which the H_2 atoms are transferred is called the hydrogen acceptor. The two basic forms of oxidation are defined by the final hydrogen acceptor (Fig. 3.**15**).

- **Respiration.** Here oxygen is the hydrogen acceptor. In anaerobic respiration, the O_2 that serves as the hydrogen acceptor is a component of an inorganic salt.
- **Fermentation.** Here an organic compound serves as the hydrogen acceptor.

The main difference between fermentation and respiration is the energy yield, which can be greater from respiration than from fermentation for a given nutrient substrate by as much as a factor of 10. Fermentation processes involving microorganisms are designated by the final product, e.g., alcoholic fermentation, butyric acid fermentation, etc.

The energy released by oxidation is stored as chemical energy in the form of a thioester (e.g., acetyl-CoA) or organic phosphates (e.g., ATP).

Bacterial Oxidation Pathways

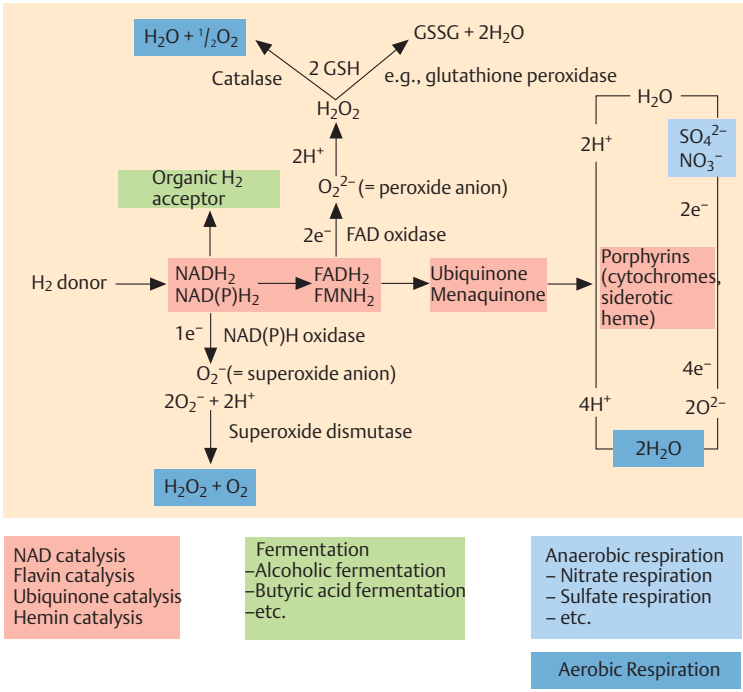


Fig. 3.**15** In oxidation of organic nutrient substrates, protons (H^+) and electrons (e^-) are transferred in more or less long chains. The respiration is aerobic when the final electron acceptor is free oxygen. Anaerobic respiration is when the electrons are transferred to inorganically bound oxygen. Fermentation is the transfer of H^+ and e^- to an organic acceptor.

The role of oxygen. Oxygen is activated in one of three ways:

- Transfer of $4e^-$ to O_2, resulting in two oxygen ions ($2\ O^{2-}$).
- Transfer of $2e^-$ to O_2, resulting in one peroxide anion ($1\ O_2^{2-}$).
- Transfer of $1e^-$ to O_2, resulting in one superoxide anion ($1\ O_2^-$).

Hydrogen peroxide and the highly reactive superoxide anion are toxic and therefore must undergo further conversion immediately (see Fig. 3.**15**).

Bacteria are categorized as the following according to their O_2-related behavior:

- **Facultative anaerobes.** These bacteria can oxidize nutrient substrates by means of both respiration and fermentation.
- **Obligate aerobes.** These bacteria can only reproduce in the presence of O_2.
- **Obligate anaerobes.** These bacteria die in the presence of O_2. Their metabolism is adapted to a low redox potential and vital enzymes are inhibited by O_2.
- **Aerotolerant anaerobes.** These bacteria oxidize nutrient substrates without using elemental oxygen although, unlike obligate anaerobes, they can tolerate it.

Basic mechanisms of catabolic metabolism. The principle of the biochemical unity of life asserts that all life on earth is, in essence, the same. Thus, the catabolic intermediary metabolism of bacteria is, for the most part, equivalent to what takes place in eukaryotic cells. The reader is referred to textbooks of general microbiology for exhaustive treatment of the pathways of intermediary bacterial metabolism.

Anabolic Reactions

It is not possible to go into all of the biosynthetic feats of bacteria here. Suffice it to say that they are, on the whole, quite astounding. Some bacteria (*E. coli*) are capable of synthesizing all of the complex organic molecules that they are comprised of, from the simplest nutrients in a very short time. These capacities are utilized in the field of microbiological engineering. Antibiotics, amino acids, and vitamins are produced with the help of bacteria. Some bacteria are even capable of using aliphatic hydrocarbon compounds as an energy source. Such bacteria can "feed" on paraffin or even raw petroleum. It is hoped that the metabolic capabilities of these bacteria will help control the effects of oil spills in surface water. Bacteria have also been enlisted in the fight against hunger: certain bacteria and fungi are cultivated on aliphatic hydrocarbon substrates, which supply carbon and energy, then harvested and processed into a protein powder (single cell protein). Culturing of bacteria in nutrient mediums based on methanol is another approach being used to produce biomass.

Metabolic Regulation

Bacteria are highly efficient metabolic regulators, coordinating each individual reaction with other cell activities and with the available nutrients as economically and rationally as possible. One form such control activity takes is regulation of the activities of existing enzymes. Many enzymes are allosteric proteins that can be inhibited or activated by the final products of metabolic pathways. One highly economical type of regulation controls the synthesis of enzymes at the genetic transcription or translation level (see the section on the molecular basis of bacterial genetics (p. 169ff.).

Growth and Culturing of Bacteria

Nutrients

The term bacterial culture refers to proliferation of bacteria with a suitable nutrient substrate. A nutrient medium (Table 3.**2**) in which chemoorganotrophs are to be cultivated must have organic energy sources (H_2 donors) and H_2 acceptors. Other necessities include sources of carbon and nitrogen for synthesis of specific bacterial compounds as well as minerals such as sulfur, phosphorus, calcium, magnesium, and trace elements as enzyme activators. Some bacteria also require "growth factors," i.e., organic compounds they are unable to synthesize themselves. Depending on the bacterial species involved, the nutrient medium must contain certain amounts of O_2 and CO_2 and have certain pH and osmotic pressure levels.

Table 3.**2** Nutrient Mediums for Culturing Bacteria

Nutrient medium	Description
Nutrient broth	Complex liquid nutrient medium.
Nutrient agar	Complex nutrient medium containing the polysaccharide agarose (1.5–2%). Nutrient agar liquefies when heated to 100 °C and does not return to the gel state until cooled to 45 °C. Agarose is not broken down by bacteria.
Selective mediums	Contain inhibitor substances that allow only certain bacteria to proliferate.
Indicator mediums	Indicate certain metabolic processes.
Synthetic mediums	Mediums that are precisely chemically defined.

Growth and Cell Death

Bacteria reproduce asexually by means of simple transverse binary fission. Their numbers (n) increase logarithmically ($n = 2^G$). The time required for a reproduction cycle (G) is called the generation time (g) and can vary greatly from species to species. Fast-growing bacteria cultivated in vitro have a generation time of 15–30 minutes. The same bacteria may take hours to reproduce in vivo. Obligate anaerobes grow much more slowly than aerobes; this is true in vitro as well. Tuberculosis bacteria have an in-vitro generation time of 12–24 hours. Of course the generation time also depends on the nutrient content of the medium.

The so-called **normal growth curve** for bacteria is obtained by inoculating a nutrient broth with bacteria the metabolism of which is initially quiescent, counting them at intervals and entering the results in a semilog coordinate system (Fig. 3.**16**). The lag phase (A) is characterized by an increase in bacterial mass per unit of volume, but no increase in cell count. During this phase, the metabolism of the bacteria adapts to the conditions of the nutrient medium. In the following log (or exponential) phase (C), the cell count increases logarithmically up to about 10^9/ml. This is followed by growth deceleration and transition to the stationary phase (E) due to exhaustion of the nutrients and the increasing concentration of toxic metabolites. Finally, death phase (F) processes begin. The generation time can only be determined during phase C, either graphically or by determining the cell count (n) at two different times and applying the formula:

$$g = \frac{t_2 - t_1}{\log_2 n_2 - \log_2 n_1} .$$

Normal Growth Curve of a Bacterial Culture

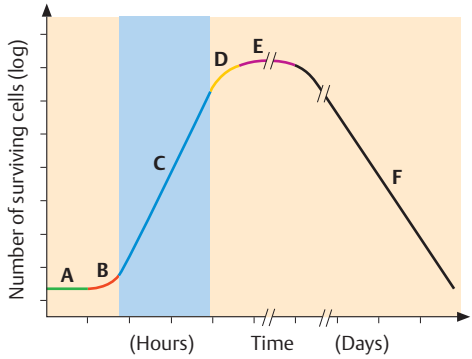


Fig. 3.**16** A = lag phase, B = acceleration phase, C = log (exponential) phase, D = deceleration phase, E = stationary phase, F = death phase.

Bacterial Cell Count and Bacterial Mass

The colony counting method. The number of living cells in a given culture or material can be determined by means of the colony counting method. The samples are diluted logarithmically by a dilution factor of 10. Using the pour plate technique, each dilution is mixed with 1 ml of liquid agar and poured out in a plate. In the surface inoculation method, 0.1 ml of each dilution is plated out on a nutrient agar surface. The plates are incubated, resulting in colony growth. The number of colonies counted, multiplied by the dilution factor, results in the original number of viable bacterial cells (CFU = colony forming units).

Bacterial mass. The bacterial mass can be established by weighing (dry or wet weight). The simplest way to determine the mass is by means of photometric adsorption measurement. The increases in mass and cell count run parallel during phase C on the growth curve.

The Molecular Basis of Bacterial Genetics

Bacteria possess two genetic structures: the **chromosome** and the **plasmid**. Both of these structures consist of a single circular DNA double helix twisted counterclockwise about its helical axis. **Replication** of this DNA molecule always starts at a certain point (the origin of replication) and is "semiconservative," that is, one strand in each of the two resulting double strands is conserved. Most **bacterial genes** code for proteins (polypeptides). Noncoding interposed sequences (introns), like those seen in eukaryotes, are the exception. Certain bacterial genes have a mosaic structure. The phases of **transcription** are promoter recognition, elongation, and termination. Many bacterial mRNAs are polycistronic, meaning they contain the genetic information for several polypeptides. **Translation** takes place on the 70S ribosomes. Special mRNA codons mark the start and stop of polypeptide synthesis. Many genes that code for functionally related polypeptides are grouped together in chromosome or plasmid segments known as operons. The most important regulatory mechanism is the positive or negative control of transcription initiation. This control function may be exercised by individual localized genes, the genes of an operon or genes in a regulon.

The Structure of Bacterial DNA

A bacterium's genetic information is stored in its chromosome and plasmids. Each of these structures is made of a single DNA double helix twisted to the right, then additionally twisted to the left about its helical axis (supercoiled, see p. 148ff. and Fig. 3.**17**). Plasmids consisting of linear DNA also occur, although this is rare. This DNA topology solves spatial problems and enables such functions as replication, transcription, and recombination. Some genes are composed of a mosaic of minicassettes interconnected by conserved DNA sequences between the cassettes (see Fig. 1.**2**, p. 14).

Chromosome. The chromosome corresponds to the nucleoid (p. 148ff.). The *E. coli* chromosome is composed of 4.63×10^6 base pairs (bp). It codes for 4288 proteins. The gene sequence is colinear with the expressed genetic products. The noncoding interposed sequences (introns) normally seen in eukaryotic genes are very rare. The chromosomes of E. coli and numerous other pathogenic bacteria have now been completely sequenced.

Plasmids. The plasmids are autonomous DNA molecules of varying size (3×10^3 to 4.5×10^5 bp) localized in the cytoplasm. Large plasmids are usually present in one to two copies per cell, whereas small ones may be present in 10, 40, or 100 copies. Plasmids are not essential to a cell's survival.

Resistance Plasmid in *Escherichia coli*

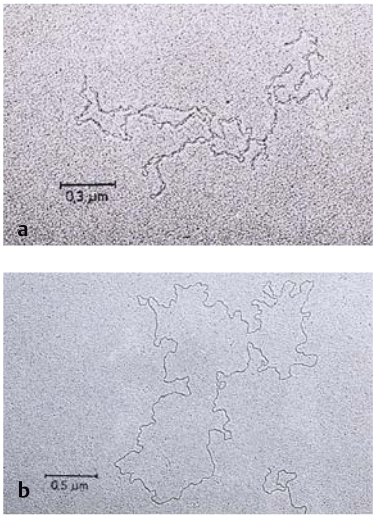


Fig. 3.**17** **a** Covalently closed circle (CCC), also known as a "supercoil" or "supertwist."
b Open circle. This open form is an artifact produced by a nick in one strand of the DNA double helix.

Many of them carry genes that code for certain phenotypic characteristics of the host cell. The following plasmid types are medically relevant:

- **Virulence plasmids.** Carry determinants of bacterial virulence, e.g., enterotoxin genes or hemolysin genes.
- **Resistance plasmids.** Carry genetic information bearing on resistance to anti-infective agents. R plasmids may carry several *R* genes at once (see also Fig. 3.**23**, p. 176). Plasmids have also been described that carry both virulence and resistance genes.

DNA Replication

The identical duplication process of DNA is termed semiconservative because the double strand of DNA is opened up during replication, whereupon each strand serves as the matrix for synthesis of a complementary strand. Thus each of the two new double strands "conserves" one old strand. The doubling of each DNA molecule (replicon) begins at a given starting point, the so-called origin of replication. This process continues throughout the entire fission cycle.

Transcription and Translation

- **Transcription.** Copying of the sense strand of the DNA into mRNA. The continuous genetic nucleotide sequence is transcribed "colinearly" into mRNA. This principle of colinearity applies with very few exceptions. The transcription process can be broken down into the three phases *promoter recognition*, *elongation*, and *termination*. The promoter region is the site where the RNA polymerase begins reading the DNA sequence. A sigma factor is required for binding to the promoter. Sigma factors are proteins that associate temporarily with the RNA polymerase (core enzyme) to form a holoenzyme, then dissociate themselves once the transcription process has begun, making them available to associate once again. Specific sigma factors recognize the standard promoters of most genes. Additional sigma factors, the expression of which depends on the physiological status of the cell, facilitate the transcription of special determinants. Genes that code for functionally related proteins, for example proteins that act together to catalyze a certain metabolic step, are often arranged sequentially at specific locations on the chromosome or plasmid. Such DNA sequences are known as operons (Fig. 3.**18**). The mRNA synthesized by the transcription of an operon is polycistronic, i.e., it contains the information sequences of several genes. The information sequences are

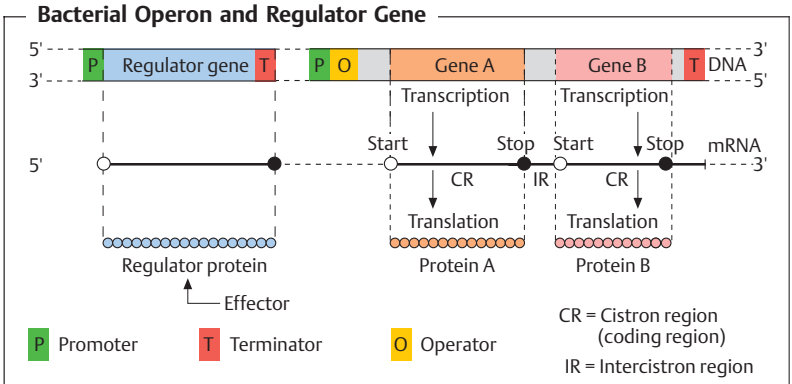


Fig. 3.**18** An operon is a DNA sequence that usually includes several structural genes. These genes code for proteins that are functionally related. Transcription of an operon is often activated or repressed by the product of a regulator gene located elsewhere on the chromosome.

separated by intercistronic regions. Each cistron has its own start and stop codon in the mRNA.

Translation. Transformation of the nucleotide sequence carried by the mRNA into the polypeptide amino acid sequence at the 70S ribosomes. In principle, bacterial and eukaryotic translation is the same. The enzymes and other factors involved do, however, differ structurally and can therefore be selectively blocked by antibiotics (p. 198ff.).

Regulation of Gene Expression

Bacteria demonstrate a truly impressive capacity for adapting to their environment. A number of regulatory bacterial mechanisms are known, for example posttranslational regulation, translational regulation, transcription termination, and quorum sensing (see Fig. 1.**5**, p. 20). The details of all these mechanisms would exceed the scope of this book. The most important is regulation of the initiation of transcription by means of activation or repression, a process not observed in this form in eukaryotes: a single gene, or several genes in an operon at one DNA location, may be affected (see Fig. 3.**18**). The mechanism that has been investigated most thoroughly is transcriptional regulation of catabolic and anabolic operons by a repressor or activator.

Transcriptional Regulation of an Operon:

- **Catabolic operons** have genes that code for enzymes of catabolic metabolism. Anabolic operons code for enzymes of anabolic metabolism.
- **Regulators.** Code for proteins that can repress or activate transcription by binding to the operator or promoter of an operon.
- **Effectors.** Low-molecular-weight signal molecules from the immediate environment of the bacterial cell. Can activate (= corepressor) or inactivate (= inducer) the repressor by means of an allosteric effect.
- **Induction of a catabolic operon.** The effector molecule is a nutrient substrate that is broken down by the products of the operon genes (e.g., lactose). Lactose inactivates the repressor, initializing transcription of the genes for *β-galactosidase* and *β-galactoside permease* in the lactose operon. These genes are normally not read off because the repressor is bound to the operator. The cell is not induced to produce the necessary catabolic enzymes until the nutrient substrate is present.
- **Repression of an anabolic operon.** The signal molecule is the final product of an anabolic process, for instance an amino acid. If this acid is present in the medium, it can be obtained from there and the cell need not synthesize the anabolic enzymes it would require to produce it. In such a case, binding to the effector is what turns the regulator protein into an active repressor.

A single regulator protein can also activate or repress several genes not integrated in an operon, i.e., at various locations on the DNA. Such functional gene groups are called **regulons**. Alternative **sigma factors** (see p. 168) may be involved in the transcriptional activation of special genes with special promoters. Physiological cell status determines whether or not these alternative factors are produced.

The Genetic Variability of Bacteria

Changes in bacterial DNA are the result of spontaneous **mutations** in individual genes as well as recombination processes resulting in new genes or genetic combinations. Based on the molecular mechanisms involved, bacterial **recombinations** are classified as homologous, site-specific, and transpositional. The latter two in particular reflect the high level of mobility of many genes and have made essential contributions to the evolution of bacteria.

Although sexual heredity is unknown in bacteria, they do make use of the mechanisms of intercellular transfer of genomic material known as parasexual processes. **Transformation** designates transfer of DNA that is essentially

chemically pure from a donor into a receptor cell. In **transduction**, bacteriophages serve as the vehicles for DNA transport. **Conjugation** is the transfer of DNA by means of cell-to-cell contact. This process, made possible by conjugative plasmids and transposons, can be a high-frequency one and may even occur between partners of different species, genera, or families. The transfer primarily involves the conjugative elements themselves. Conjugative structures carrying resistance or virulence genes are of considerable medical significance.

The processes of **restriction** and **modification** are important factors limiting genetic exchange among different taxa. Restriction is based on the effects of restriction endonucleases capable of specific excision of foreign DNA sequences. These enzymes have become invaluable tools in the field of genetic engineering.

Molecular Mechanisms of Genetic Variability

Spontaneous Mutation

In the year 1943, Luria and Delbrück used the so-called fluctuation test to demonstrate that changes in the characteristics of bacterial populations were the results of rare, random mutations in the genes of individual cells, which then were selected. Such mutations may involve substitution of a single nucleotide, frame-shifts, deletions, inversions, or insertions. The frequency of mutations is expressed as the **mutation rate**, which is defined as the probability of mutation per gene per cell division. The rate varies depending on the gene involved and is approximately 10^{-6} to 10^{-10}. Mutation rates may increase drastically due to mutagenic factors such as radioactivity, UV radiation, alkylating chemicals, etc.

Recombination

The term recombination designates processes that lead to the restructuring of DNA, formation of new genes or genetic combinations.

Homologous (generalized) recombination. A precise exchange of DNA between corresponding sequences. Several enzymes contribute to the complex breakage and reunion process involved, the most important being the RecA enzyme and another the RecBC nuclease. Fig. 1.**2** (p. 14) shows an example of homologous recombination resulting in the exchange of minicassettes between two genes.

Site-specific recombination. Integration or excision of a sequence in or from target DNA. Only a single sequence of a few nucleotides of the integrated DNA needs to be homologous with the recombination site on the target DNA. The integration of bacteriophage genomes is an example of what this process facilitates (p. 184f.) Integration of several determinants of antibiotic resistance in one integron can also utilize this process (Fig. 3.**19**). Resistance integrons may be integrated in transposable DNA.

Transposition. The transposition process does not require the donor and target DNA to be homologous. DNA sequences can either be transposed to a different locus on the same molecule or to a different replicon. Just as in site-specific recombination, transposition has always played a major role in the evolution of multi-resistance plasmids (see Fig. 3.**23**, p. 176).

Site-Specific Recombination (Integron)

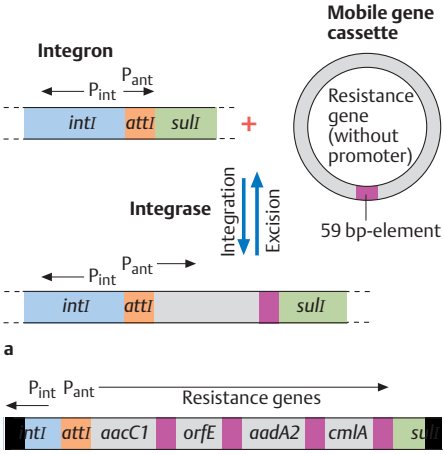


Fig. 3.**19** **a** Integration or excision of a circular gene cassette into or out of an integron. An integron is a genetic structure containing the determinants of a site-specific recombination system. This structure is capable of capturing or mobilizing mobile gene cassettes. It also provides the promoter for transcription of the cassette genes, which themselves have no promoter. The cassettes occur in both free circularized and integrated forms.

b

intI	*Integrase* gene. Codes for site-specific integrase (int = integrase).
attI	Attachment sequence for the integrase. Site of recombination (att = attachment).
P_{ant}	Promoter for the integrated gene cassette (P = promoter).
P_{int}	Promoter for the *integrase* gene.
sulI	Gene for sulfonamide resistance (sul = sulfonamide).
59bp	Integrase-specific recombination site in cassette.

b Model of integron 4 (In*4*), which contains three resistance genes and an open reading frame (*orfE*), the function of which is unknown. In*4* is the result of successive integration of several resistance genes at the *att1* site.

Transposable DNA Elements

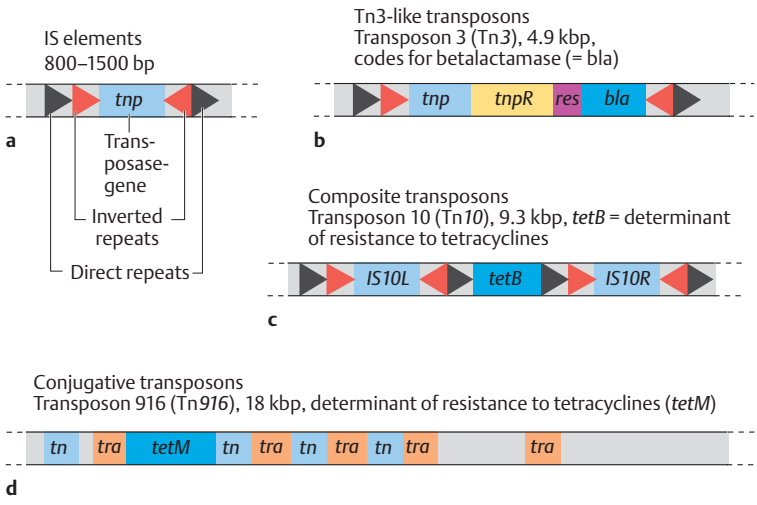


Fig. 3.**20** **a–d** Explanation of the structures and abbreviations used in the text box "Details of Transposable DNA elements" below.

Details of Transposable DNA Elements

- **Insertion sequences (IS elements**, Fig. 3.**20**). These are the simplest transposable DNA sequences. They are terminated by identical, but reversed, sequences of 10–40 nucleotides known as inverted repeats (IR). They frame the segment that codes for the enzyme transposase. The target structures for this enzyme are the so-called *direct repeats*, nucleotide sequences comprising 5–9 bp that are duplicated in the integration process.

- **Tn3 transposons** (Fig. 3.**20b**). In addition to the transposase gene *tnpA*, they contain the regulator sequence *tnpR* and the *res* site to which resolvase must bind. Tn3-like transposons are duplicated in the transposition process, so that one copy remains at the original location and the other is integrated at the new location.

- **Composite transposons** (Fig. 3.**20c**). They consist of two IS elements framing a sequence of variable size that is not required for transposition, e.g., a resistance gene.

- **Conjugative transposons** (Fig. 3.**20d**). These genetic elements code in certain regions for factors that control the transfer (Tra) and transposition (Tn) processes. Conjugative transposons have been discovered mainly in Gram-positive cocci and Gram-negative anaerobes (*Bacteroides*).

Intercellular Mechanisms of Genetic Variability

Although bacteria have no sexual heredity in the strict sense, they do have mechanisms that allow for intercellular DNA transfer. These mechanisms, which involve a unilateral transfer of genetic information from a donor cell to a receptor cell, are subsumed under the term **parasexuality**.

Transformation

Transfer of "naked" DNA. In 1928, Griffith demonstrated that the ability to produce a certain type of capsule could be transferred between different pneumococci. Then Avery showed in 1944 that the transforming principle at work was DNA. This transformation process has been observed mainly in the genera *Streptococcus*, *Neisseria*, *Helicobacter* and *Haemophilus*.

Transduction

Transfer of DNA from a donor to a receptor with the help of transport bacteriophages (Fig. 3.**21**).

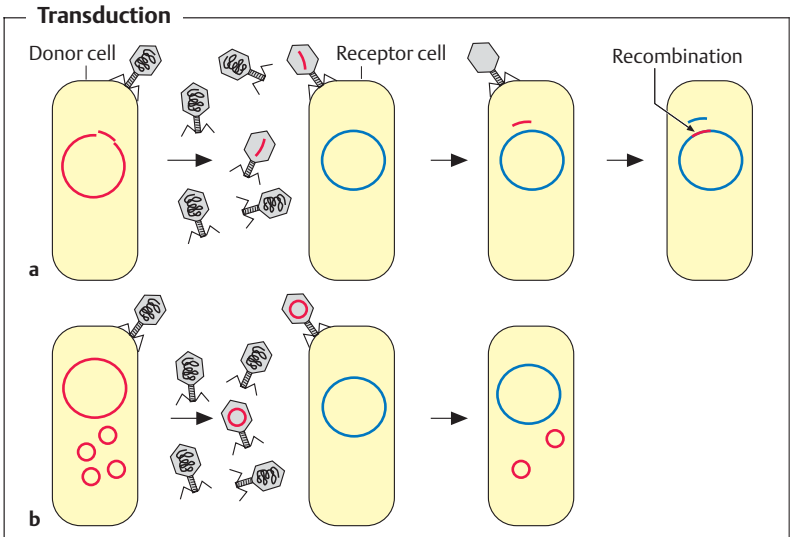


Fig. 3.**21** Transduction of a chromosomal DNA sequence (**a**) and a plasmid (**b**).

Bacteriophages are viruses that infect bacteria (p. 182ff.). During their replication process, DNA sequences from the host bacterial cell may replace all or part of the genome in the phage head. Such phage particles are then defective. They can still dock on receptor cells and inject their DNA, but the infected bacterial cell will then neither produce new phages nor be destroyed.

Conjugation

Conjugation is the transfer of DNA from a donor to a receptor in a conjugal process involving cell-to-cell contact. Conjugation is made possible by two genetic elements: the conjugative plasmids and the conjugative transposons.

Conjugation

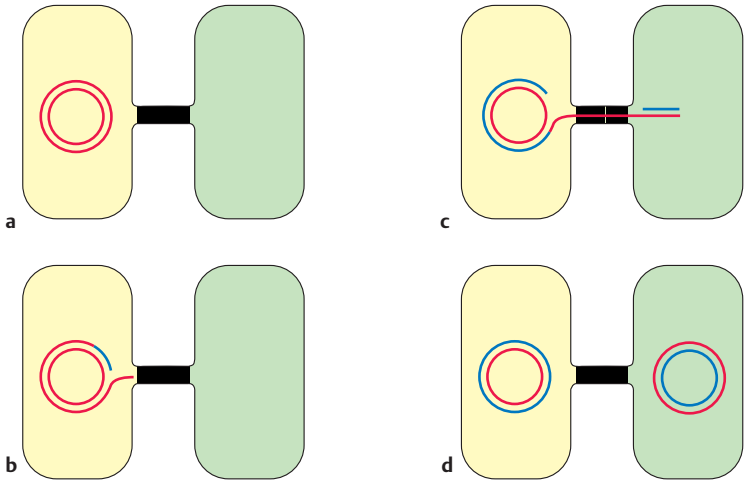


Fig. 3.**22** Transfer/replication process of a conjugative plasmid.
a Conjugation: connection between two bacterial cells by means of sex pili. This initial step alone does not necessarily always lead to effective conjugation.
b Effective conjugation: formation of a specific conjugal bridge between donor cell and receptor cell.
c Plasmid mobilization and transfer: an endonuclease cleaves one strand of the circular DNA double helix at a specific point (**b**). The single strand with the "leader region" enters the receptor cell.
d Synthesis: the double-stranded structure of both the transferred single strand and the remaining DNA strand is restored by means of complementary DNA synthesis. The receptor cell, now plasmid-positive, is called a transconjugant.

In the conjugation process, the conjugative elements themselves are what are primarily transferred. However, these elements can also mobilize chromosomal genes or otherwise nontransferable plasmids. Conjugation is seen frequently in Gram-negative rods (*Enterobacteriaceae*), in which the phenomenon has been most thoroughly researched, and enterococci.

The F-factor in *Escherichia coli*. This is the prototype of a conjugative plasmid. This factor contains the so-called *tra* (transfer) genes responsible both for

Model of a Hypothetical Conjugative Multiple-Resistance Plasmid

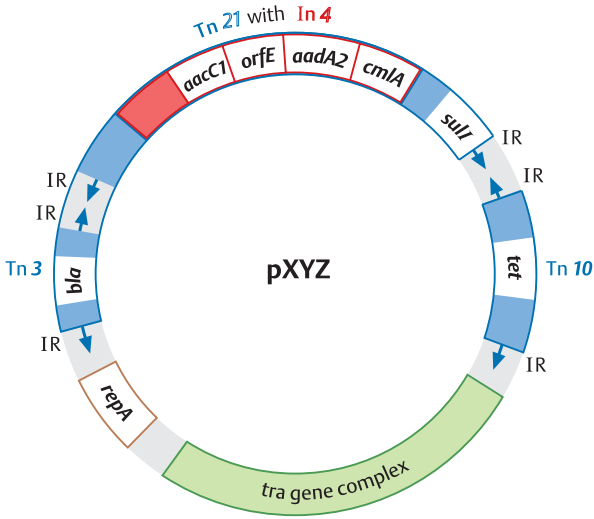


Fig. 3.**23** Multiple resistance plasmids can result from successive integration of transposable resistance DNA or integration of resistance integrons (see Figs. 3.**19** and 3.**20**, p. 173 and 174)

Tn*21* Transposon of the Tn*21* family, codes for resistance to sulfonamides (*sulI*) and contains an R integron (In*4*).

In*4* Codes for chloramphenicol acetyltransferase (= *cmlA*), an aminoglycoside acetyltransferase (= *aacC1*) and an aminoglycoside adenylyltransferase (= *aadA2*); also contains an open reading frame (*orfE*) of unknown function.

Tn*3* Transposon 3; codes for a betalactamase (= *bla*).

Tn*10* Transposon 10; codes for resistance to tetracyclines (= *tet*).

repA Codes for the replication enzyme of the plasmid.

tra Plasmid DNA region containing 25 *tra* genes; *tra* genes are responsible for the transfer and replication process (see Fig. 3.**22**).

the formation of conjugal pili on the surface of F cells and for the transfer process. The transfer of the conjugative plasmid takes place as shown here in schematic steps (Fig. 3.**22**).

Occasional integration of the F factor into the chromosome gives it the conjugative properties of the F factor. Such an integration produces a sort of giant conjugative element, so that chromosomal genes can also be transferred by the same mechanism. Cells with an integrated F factor are therefore called Hfr ("high frequency of recombination") cells.

Conjugative resistance and virulence plasmids. Conjugative plasmids that carry determinants coding for antibiotic resistance and/or virulence in addition to the *tra* genes and *repA* are of considerable medical importance. Three characteristics of conjugative plasmids promote a highly efficient horizontal spread of these determinant factors among different bacteria:

- **High frequency of transfer.** Due to the "transfer replication" mechanism, each receptor cell that has received a conjugative plasmid automatically becomes a donor cell. Each plasmid-positive cell is also capable of multiple plasmid transfers to receptor cells.

- **Wide range of hosts.** Many conjugative plasmids can be transferred between different taxonomic species, genera, or even families.

- **Multiple determinants.** Many conjugative plasmids carry several genes determining the phenotype of the carrier cell. The evolution of a hypothetical conjugative plasmid carrying several resistance determinants is shown schematically in Fig. 3.**23**.

Conjugative transposons. These are DNA elements (p. 173) that are usually integrated into the bacterial chromosome. They occur mainly in Gram-positive cocci, but have also been found in Gram-negative bacteria (*Bacteroides*). Conjugative transposons may carry determinants for antibiotic resistance and thus contribute to horizontal resistance transfer. In the transfer process, the transposon is first excised from the chromosome and circularized. Then a single strand of the double helix is cut and the linearized single strand—analogous to the F factor—is transferred into the receptor cell. Conjugative transposons are also capable of mobilizing nonconjugative plasmids.

Restriction, Modification, and Gene Cloning

The above descriptions of the mechanisms of genetic variability might make the impression that genes pass freely back and forth among the different bacterial species, rendering the species definitions irrelevant. This is not the case. A number of control mechanisms limit these genetic exchange processes. Among the most important are **restriction** and **modification**. Re-

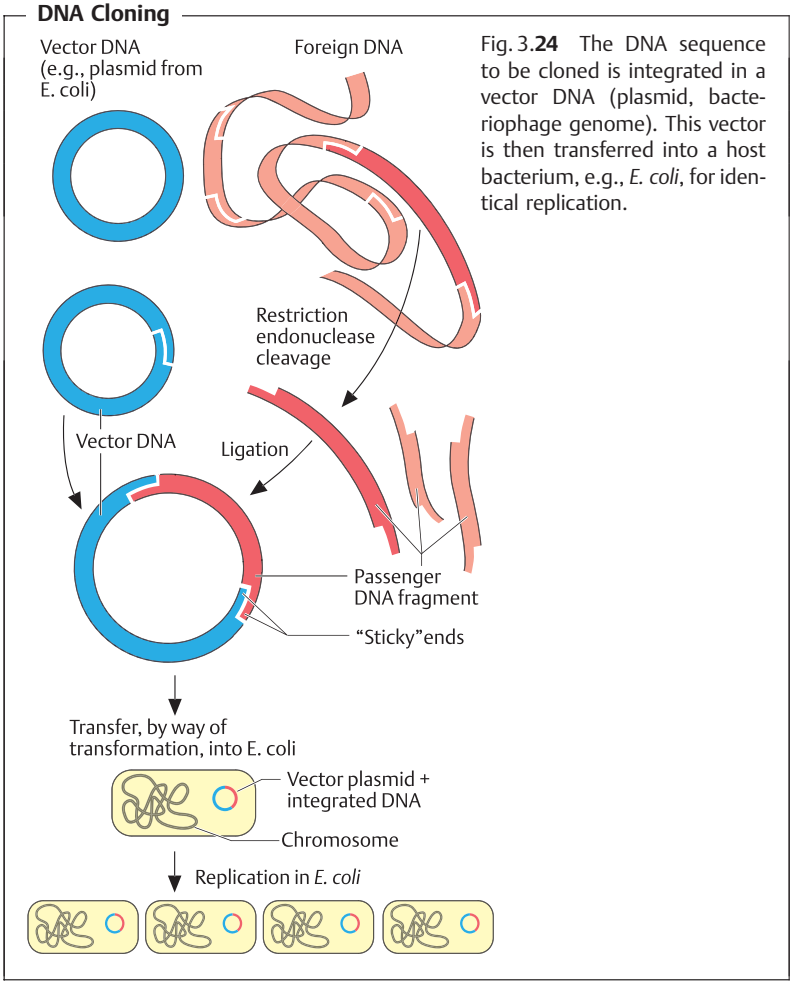


Fig. 3.**24** The DNA sequence to be cloned is integrated in a vector DNA (plasmid, bacteriophage genome). This vector is then transferred into a host bacterium, e.g., *E. coli*, for identical replication.

striction endonucleases can destroy foreign DNA that bears no "fingerprint" (modification) signifying "self." These modifications take the form of methylation of the DNA bases by modification enzymes.

Bacterial restriction endonucleases are invaluable tools in modern **gene cloning** techniques. The process is termed gene "cloning" because it involves replication of DNA that has been manipulated in vitro in a suitable host cell so as to produce identical copies of this DNA: molecular clones or gene clones.

The technique simplifies the replication of DNA, making experimental manipulations easier. On the other hand, the bacteria can also be used to synthesize gene products of the foreign genes. Such foreign proteins are called recombinant proteins. Bacterial plasmids often function in the role of vectors into which the sequences to be cloned are inserted. Fig. 3.**24** illustrates the principle of gene cloning in simplified form.

Table 3.**3** lists the most important terms used in the field of bacterial genetics.

Table 3.**3** Glossary of Important Terminology in Bacterial Genetics

Anticodon	Triplet sequences of transfer RNA complementary to the codons of mRNA
Chromosome	See nucleoid
Cistron	Genetic unit, identical to "gene"
Code	Key relating the DNA nucleotide (n = 3) sequence to the polypeptide amino acid sequence
Codon	Sequence of three nucleotides, triplet
Corepressor	See effector molecules
Deletion	Loss of a DNA sequence in a replicon
Effector molecules	Small molecules that inactivate (= inductor) or activate (= corepressor) a regulator protein by means of an allosteric effect
Episome	Historical term; characterizes a replicon (e.g., F plasmid) occurring either in the cytoplasm or integrated in the bacterial chromosome
F factor	Prototype of a conjugative plasmid (fertility factor)
Gene	DNA segment containing the information used in synthesis of a polypeptide or RNA
Genome	All of the genetic information contained in a cell
Genotype	The totality of genetically determined characteristics
Hfr cells	Coli bacteria with F factor integrated into their chromosomes, therefore capable of transferring chromosomal genes at a high frequency by means of conjugation (Hfr = high frequency of recombination)
Inductor	See effector molecules
Integron	Genetic structure containing the determinants for a site-specific recombination system; responsible for integration or excision of mobile gene cassettes

Table 3.**3** *Continued: Glossary of Important Terminology. . .*

Inverted repeats	Nucleotide sequences repeated in reverse order at the ends of transposable DNA
IS	Insertion sequences; transposable DNA elements
Cassette	Sequence in a gene that can be transferred to other genes by homologous recombination
Clone	Population of identical cells or DNA molecules
Conjugation	Transfer of hereditary material in a pairing process
Lysogenic bacteria	Cells with a phage genome (prophage) integrated into their chromosomes
Lysogenic conversion	Change in cell phenotype brought about by prophage genes
Messenger RNA	Synthesized at the DNA by transcription; carries genetic information to the ribosomes
Modification enzymes	Methylases that label DNA as "self" by methylation
Mutation	A permanent alteration of the genome
Nucleoid	Nuclear region, nucleus equivalent
Operator	DNA sequence of an operon; regulator binding site
Operon	Regulatory unit comprising the promoter, operator, structural genes, and terminator
Parasexuality	Unilateral gene transfer from a donor to a receptor
Phenotype	The totality of characteristics expressed in a bacterial cell
Plasmid	Extrachromosomal, autonomous, in most cases circular DNA molecule
Promoter	Recognition and binding site for RNA polymerase
Prophage	Phage genome integrated into the chromosome
Regulator	Regulatory protein that controls gene transcription; repressor or activator
Regulon	Functional unit of genes at different loci controlled by the same regulator
Recombination, Legitimate or homologous recombination	Replacement of a DNA sequence by a homologous sequence from a different genome; breakage and reunion model

Table 3.**3** *Continued: Glossary of Important Terminology. . .*

Recombination, Illegitimate recombination	Insertion of transposable DNA
Site-specific recombination	Integration or excision of a DNA sequence by means of homologous recombination in a specific DNA segment comprising only a small number of nucleotides
Replication	Reproduction, duplication of DNA
Replicon	DNA molecule that replicates autonomously
Restriction endonucleases	Enzymes that recognize and cleave specific DNA nucleotide sequences
Semiconservative replication	DNA duplication mechanism in which one old strand is conserved in each of the two new double strands
Conjugal (or sex) pili	Surface structures essential to conjugation in Gram-negative rod bacteria
Sigma factors	Proteins that temporarily associate with prokaryotic RNA polymerase for specific promoter binding
Supercoil	Circular DNA molecule additionally twisted about the helical axis in the opposite direction
Terminator	Sequence marking the end of a transcription process
Transduction	Gene transfer using bacteriophages as vehicles
Transfer RNA	Specifically binds an amino acid (aminoacyl tRNA) and transfers it to the ribosome
Transformation	Transfer of genes from a donor in the form of "naked" DNA
Transcription	RNA synthesis at DNA
Translation	Ribosomal synthesis of polypeptides
Transposase	Transposition enzyme; facilitates illegitimate recombination
Transposition	Translocation of a mobile DNA element within a replicon or between different replicons
Transposon	Transposable DNA; frequently contains—in addition to the genes for transposition—determinants that change the phenotype of a bacterial cell
Triplet code	Three nucleotides coding for one amino acid
Vector	Vehicle for foreign (passenger) DNA; usually a plasmid or phage genome

Bacteriophages

■ Bacteriophages, or simply phages, are viruses that infect bacteria. They possess a protein shell surrounding the phage genome, which with few exceptions is composed of DNA. A bacteriophage attaches to specific receptors on its host bacteria and injects its genome through the cell wall. This forces the host cells to synthesize more bacteriophages. The host cell lyses at the end of this reproductive phase. So-called temperate bacteriophages lysogenize the host cells, whereby their genomes are integrated into the host cell chromosomes as the so-called prophage. The phage genes are inactive in this stage, although the prophage is duplicated synchronously with host cell proliferation. The transition from prophage status to the lytic cycle is termed spontaneous or artificial induction. Some genomes of temperate phages may carry genes which have the capacity to change the phenotype of the host cell. Integration of such a prophage into the chromosome is known as lysogenic conversion. ■

Definition

Bacteriophages are viruses the host cells of which are bacteria. Bacteriophages are therefore obligate cell parasites. They possess only one type of nucleic acid, either DNA or RNA, have no enzymatic systems for energy supply and are unable to synthesize proteins on their own.

Morphology

Similarly to the viruses that infect animals, bacteriophages vary widely in appearance. Fig. 3.**25a** shows a schematic view of a T series coli phage. Research on these phages has been particularly thorough. Fig. 3.**25b** shows an intact T phage next to a phage that has injected its genome.

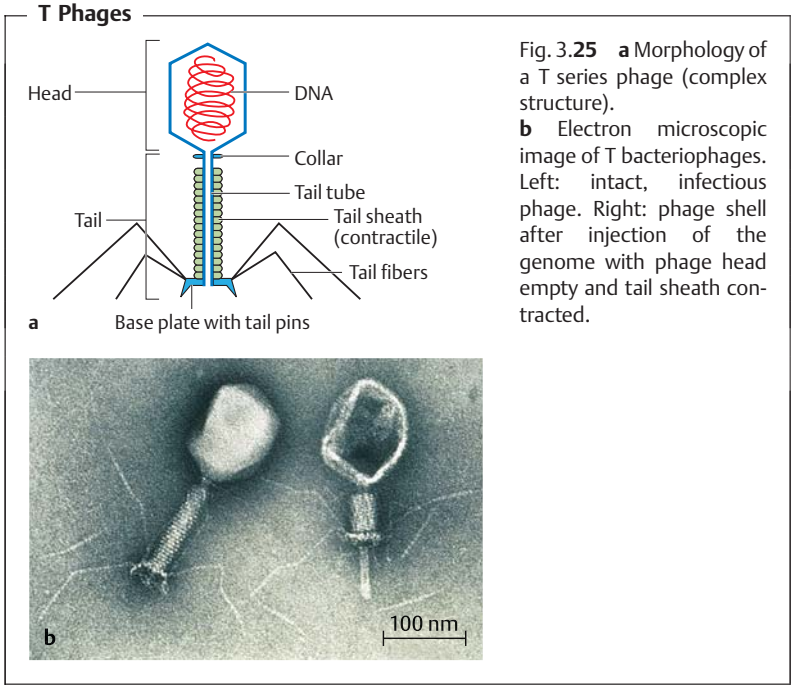


Fig. 3.**25** **a** Morphology of a T series phage (complex structure). **b** Electron microscopic image of T bacteriophages. Left: intact, infectious phage. Right: phage shell after injection of the genome with phage head empty and tail sheath contracted.

Composition

Phages are made up of protein and nucleic acid. The proteins form the head, tail, and other morphological elements, the function of which is to protect the phage genome. This element bears the genetic information, the structural genes for the structural proteins as well as for other proteins (enzymes) required to produce new phage particles. The nucleic acid in most phages is DNA, which occurs as a single DNA double strand in, for example, T series phages. These phages are quite complex and have up to 100 different genes. In spherical and filamentous phages, the genome consists of single-stranded DNA (example: ΦX174). RNA phages are less common.

Reproduction

The phage reproduction process involves several steps (Fig. 3.**26**).

- **Adsorption.** Attachment to cell surface involving specific interactions between a phage protein at the end of the tail and a bacterial receptor.

Lytic Phage Reproduction and Lysogenization

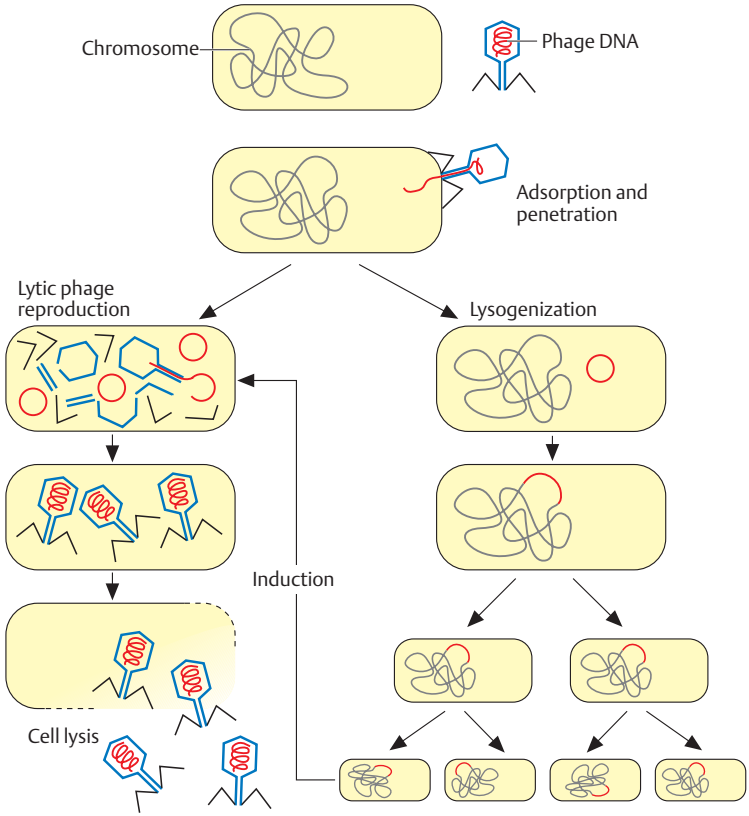


Fig. 3.**26** Injection of the phage genome is followed either by direct intracellular (lytic) phage reproduction or lysogenization of the host cell. In the lysogenization process, the phage DNA is integrated into the host cell chromosome and replicated together with it in the process of cell fission.

Release of Phages from the Host Cell

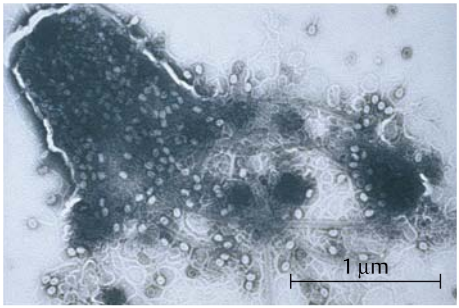


Fig. 3.**27** At the end of the phage maturation process, the host cell is lysed to release the new phages. Lysis occurs by a phage-encoded murein hydrolase, which gains access to the murein through membrane channels formed by the phage-encoded protein holin.

- **Penetration.** Injection of the phage genome. Enzymatic penetration of the wall by the tail tube tip and injection of the nucleic acid through the tail tube.

- **Reproduction**. Beginning with synthesis of early proteins (zero to two minutes after injection), e.g., the phage-specific replicase that initiates replication of the phage genome. Then follows transcription of the late genes that code for the structural proteins of the head and tail. The new phage particles are assembled in a maturation process toward the end of the reproduction cycle.

- **Release.** This step usually follows the lysis of the host cell with the help of murein hydrolase coded by a phage gene that destroys the cell wall (Fig. 3.**27**).

Depending on the phage species and milieu conditions, a phage reproduction cycle takes from 20 to 60 minutes. This is called the **latency period**, and can be considered as analogous to the generation time of bacteria. Depending on the phage species, an infected cell releases from 20 to several hundred new phages, which number defines the **burst size**. Thus phages reproduce more rapidly than bacteria. In view of this fact, one might wonder how any bacteria have survived in nature at all. It is important not to forget that cell population density is a major factor determining the probability of finding a host cell in the first place and that such densities are relatively small in nature. Another aspect is that only a small proportion of phages reproduce solely by means of these lytic or vegetative processes. Most are temperate phages that lysogenize the infected host cells.

Lysogeny

Fig. 3.**26** illustrates the **lysogeny** of a host cell. Following injection of the phage genome, it is integrated into the chromosome by means of region-specific recombination employing an integrase. The phage genome thus integrated is called a **prophage**. The prophage is capable of changing to the vegetative state, either spontaneously or in response to induction by physical or chemical noxae (UV light, mitomycin). The process begins with excision of the phage genome out of the DNA of the host cell, continues with replication of the phage DNA and synthesis of phage structure proteins, and finally ends with host cell lysis. Cells carrying a prophage are called **lysogenic** because they contain the genetic information for lysis. Lysogeny has advantages for both sides. It prevents immediate host cell lysis, but also ensures that the phage genome replicates concurrently with host cell reproduction.

Lysogenic conversion is when the phage genome lysogenizing a cell bears a gene (or several genes) that codes for bacterial rather than viral processes. Genes localized on phage genomes include the gene for diphtheria toxin, the gene for the pyrogenic toxins of group A streptococci and the *cholera toxin* gene.

The Importance of the Bacteriophages

Biological research	Bacteriophages are often used as models in studies of fundamental biological processes: DNA replication, gene expression, gene regulation, viral morphogenesis, studies of the details, and function of supramolecular structures
Genetic engineering	Vectors for gene cloning, adjuvants in sequencing
Therapy and prevention	An older concept now receiving increased attention. Administration of suitable phage mixtures in therapy and prevention of gastrointestinal infections. In animal husbandry, a number of phages that attack only EHEC (enterohemorrhagic *E. coli*) are used against EHEC infections
Epidemiology	Bacterial typing. Strains of a bacterial species are classified in phagovars (syn. lysotypes) based on their sensitivity to typing bacteriophages. Recognition of the bacterial strain responsible for an epidemic, making it possible to follow up the chain of infection and identify the infection sources. This typing method has been established for *Salmonella typhi*, *Salmonella paratyphi B*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and other bacteria, although it is now increasingly being replaced by new molecular methods, in particular DNA typing

The Principles of Antibiotic Therapy

Specific antibacterial therapy refers to treatment of infections with anti-infective agents directed against the infecting pathogen. The most important group of anti-infective agents are the antibiotics, which are products of fungi and bacteria (*Streptomycetes*). Anti-infective agents are categorized as having a broad, narrow, or medium spectrum of action. The efficacy, or effectiveness, of a substance refers to its bactericidal or bacteriostatic effect. Anti-infective agents have many different mechanisms of action. Under the influence of sulfonamides and trimethoprim, bacteria do not synthesize sufficient amounts of tetrahydrofolic acid. All betalactam antibiotics irreversibly block the biosynthesis of murein. Rifamycin inhibits the DNA-dependent RNA polymerase (transcription). Aminoglycosides, tetracyclines, and macrolides block translation. All 4-quinolones damage cellular DNA topology by inhibiting bacterial topoisomerases. Due to their genetic variability, bacteria may develop resistance to specific anti-infective agents. The most important resistance mechanisms are: inactivating enzymes, resistant target molecules, reduced influx, increased efflux. Resistant strains (problematic bacteria) occur frequently among hospital flora, mainly *Enterobacteriaceae*, pseudomonads, staphylococci, and enterococci. Laboratory resistance testing is required for specific antibiotic therapy. Dilutions series tests are quantitative resistance tests used to determine the minimum inhibitory concentration (MIC). The disk test is a semiquantitative test used to classify the test bacteria as resistant or susceptible. In combination therapies it must be remembered that the interactions of two or more antibiotics can give rise to an antagonistic effect. Surgical chemoprophylaxis must be administered as a short-term antimicrobial treatment only.

Definitions

Specific **antibacterial therapy** designates treatment of infections with **anti-infective agents** directed against the infecting pathogen (syn. **antibacterial chemotherapeutics, antibiotics**). One feature of these pharmaceuticals is "selective toxicity," that is, they act upon bacteria at very low concentration levels without causing damage to the macroorganism. The most important group of anti-infective agents is the **antibiotics**. These natural substances are produced by fungi or bacteria (usually *Streptomycetes*). *The term "antibiotic" is often used in medical contexts to refer to all antibacterial pharmaceuticals, not just to antibiotics in this narrower sense.* Fig. 3.**28** illustrates

Interactions: Anti-Infective Agent/Bacterium/Host Organism

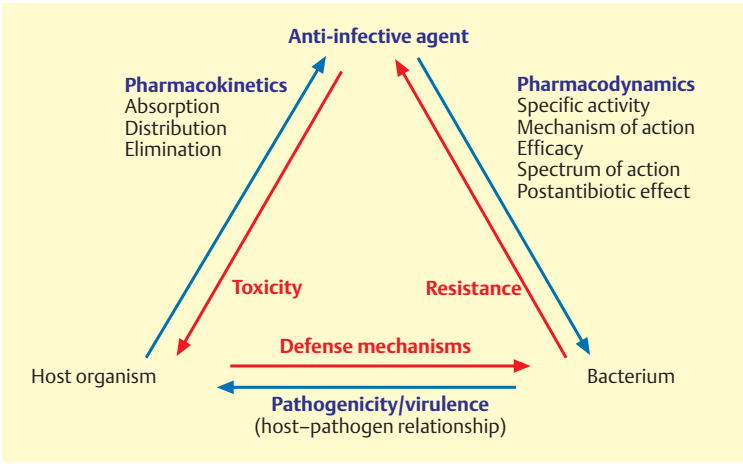


Fig. 3.**28** Interactions between the anti-infective agent and host organism are characterized by the terms pharmacokinetics and toxicity; interactions between the anti-infective agent and the bacterial pathogen are characterized in terms of pharmacodynamics and resistance.

the relations between an anti-infective agent, the host organism, and a bacterial pathogen. Table 3.**4** lists frequently used anti-infective agents. The most important groups (cephalosporins, penicillins, 4-quinolones, macrolides, tetracyclines) are in bold print. Fig. 3.**29** presents the basic chemical structures of the most important anti-infective agents.

Table 3.**4** Frequently Used Anti-Infective Agents

Class/active substance	Remarks
Aminoglycoside/aminocyclitol antibiotics	
(dihydro)streptomycin	For treatment of tuberculosis
neomycin, paramomycin	Only for oral or topical application
kanamycin	Parenteral administration; resistance frequent
gentamicin, tobramycin, amikacin, netilmicin, sisomicin	Newer aminoglycosides; broad spectrum; no effect on streptococci and enterococci; ototoxicity and nephrotoxicity; control of serum levels during therapy
spectinomycin	Against penicillinase-positive gonococci, in urogenital gonorrhea
Carbacephems	Betalactams structured like cephalosporins, but with a C atom instead of sulfur in the second ring system (see fig. 3.**29**, p. 195)
loracarbef	Oral carbacephem; stable in the presence of penicillinases from *Haemophilus* and *Moraxella*
4-Quinolones	
norfloxacin, pefloxacin	Oral quinolones; only in urinary tract infections
ciprofloxacin, ofloxacin, fleroxacin, enoxacin	Oral and systemic quinolones with broad spectrum of indications
levofloxacin, sparfloxacin	Quinolones with enhanced activity against Gram-positive and "atypical" pathogens (chlamydias, mycoplasmas); caution—sparfloxacin is phototoxic
gatifloxacin, moxifloxacin	Quinolones with enhanced activity against Gram-positive and "atypical" pathogens (chlamydias, mycoplasmas) and Gram-negative anaerobes
Cephalosporins	
Group 1	
cefazolin, cephalothin	Effective against Gram-positive and some Gram-negative bacteria; stable in the presence of staphylococci penicillinases; unstable in the presence of betalactamases of Gram-negative bacteria

Table 3.4 *Continued: Frequently Used Anti-Infective Agents*

Class/active substance	Remarks
Group 2 cefuroxime, cefotiam, cefamandole	Effective against Gram-positive bacteria; more effective against Gram-negative bacteria than Group 1; stable in the presence of staphylococci penicillinases; stable in the presence of some betalactamases of Gram-negative bacteria
Group 3a cefotaxime, ceftriaxone, ceftizoxime, cefmenoxime, cefodizime	Much more effective than Group 1 against Gram-negative bacteria; stable in the presence of numerous betalactamases of Gram-negative bacteria; show weak activity against staphylococci
Group 3b Ceftazidime, cefepime, cefpirome, cefoperazone	Spectrum of action as in Group 3a; also effective against *Pseudomonas aeruginosa*
Further cephalosporins cefsulodin	Narrow spectrum of action; the only therapeutically relevant activity is that against *Pseudomonas aeruginosa*
cefoxitin	Effective against the anaerobic *Bacteroidaceae*; activity against Gram-negative bacteria as in Group 2; insufficient activity against staphylococci
Oral cephalosporins ceflaclor, cefadroxil, cephalexin, cefradine	Spectrum of action similar as cephalothin
cefpodoxime, cefuroxime (axetil), cefixime, cefprozil, cefdinir, cefetamet, ceftibuten	Newer oral cephalosporins with broad spectra of action
Chloramphenicol	Broad spectrum, mainly bacteriostatic effect; risk of aplastic anemia
Diaminobenzyl pyrimidine trimethoprim	Broad spectrum; inhibition of dihydrofolic acid reductase; frequent bactericidal synergism with sulfonamides (e.g., cotrimoxazole)

Table 3.4 *Continued: Frequently Used Anti-Infective Agents*

Class/active substance	Remarks
Ethambutol	Only against tuberculosis bacteria
Fosfomycin	Broad spectrum, bactericidal effect in bacterial cell division phase; blocks murein biosynthesis; rapid development of resistance; use in combination therapy
Fusidic acid	Steroid antibiotic; only against Gram-positive bacteria; bacteriostatic; blocks protein biosynthesis (translation); development of resistance is frequent
Glycopeptides vancomycin teicoplanin	Narrow spectrum including only Gram-positive bacteria; moderate bactericidal efficacy during bacterial cell division phase; blocks murein biosynthesis; nephrotoxicity, allergy, thrombophlebitis
Isonicotinamides isoniazid (INH)	Only against tuberculosis bacteria, inhibition of enzymes requiring pyridoxal or pyridoxamine as a coenzyme
Lincosamides lincomycin, clindamycin	Effective against Gram-positive bacteria and Gram-negative anaerobes; good penetration into bone tissue
Macrolides/ketolides	
erythromycin, roxithromycin, clarithromycin, azithromycin	Against Gram-positive and Gram-negative cocci, chlamydias, and mycoplasmas
telithromycin	Ketolide; effective against many macrolide-resistant strains

Table 3.**4** *Continued: Frequently Used Anti-Infective Agents*

Class/active substance	Remarks
Monobactams	Betalactam antibiotics with only the betalactam ring (see fig. 3.**29**, p. 195)
aztreonam, carumonam	Good activity against *Enterobacteriaceae*; moderate efficacy against *Pseudomonas*; very high level of betalactamase stability; no effect against Gram-positive bacteria
Nitrofurans	
nitrofurantoin, furazolidone, nitrofural, etc.	Against Gram-positive and Gram-negative bacteria; use only in urinary tract infections
Nitroimidazoles	
metronidazole, tinidazole, omidazole	Active against various protozoans and obligate anaerobic bacteria; bactericidal effect
Oxalactams	Betalactam antibiotics with oxygen instead of sulfur in the second ring system (see Fig. 3.**29**, p. 195)
lamoxactam	Broad spectrum; moderate efficacy against *Pseudomonas*; poor efficacy against Gram-positive cocci; highly stable in the presence of beta-lactamases; also effective against Gram-negative anaerobes
flomoxef	No activity against *Pseudomonas*; good activity against staphylococci; otherwise like lamoxactam
clavulanic acid	Only minimum antibacterial activity; inhibits beta-lactamases; used in combination with amoxicillin (Augmentin)
Oxazolidinones	
linezolid	Only against Gram-positive bacteria; inhibits bacterial translation; no crossresistance with other translation inhibitors
Para-aminosalicylic acid (PAS)	Only against tuberculosis bacteria; affects folic acid biosynthesis

Table 3.4 *Continued: Frequently Used Anti-Infective Agents*

Class/active substance	Remarks
Penicillins	
Classic penicillins	
penicillin G (benzyl penicillin), penicillin V (oral penicillin), pheneticillin, propicillin	Effective against Gram-positive bacteria and Gram-negative cocci; bactericidal effect during bacterial cell division phase; inactivated by penicillinase of staphylococci, gonococci, *Haemophilus influenzae*, *Moraxella catarrhalis*
Penicillinase-resistant penicillins	
methicillin, oxacillin, cloxacillin, flucloxacillin	Stable in the presence of penicillinase of staphylococci; agent of choice in staphylococci infections (flucloxacillin)
Aminopenicillins	
ampicillin, amoxicillin, epicillin, hetacillin, etc.	Also effective against *Enterobacteriaceae*; labile against Gram-positive and Gram-negative penicillinases
Carboxyl penicillins	
carbenicillin, ticarcillin, carfecillin, etc.	Effective against *Enterobacteriaceae* and *Pseudomonas*; labile against Gram-positive and Gram-negative penicillinases
temocillin (6-α-methoxy ticarcillin)	No effect against *Pseudomonas*; highly stable in the presence of betalactamases
Acylureidopenicillins	
azlocillin, mezlocillin, piperacillin, apalcillin	Effective against *Enterobacteriaceae* and *Pseudomonas*; despite lability against beta-lactamases active against many enzyme-producing strains due to good penetration and high levels of sensitivity of the target molecules
Penems	Penicillins with a double bond in the second ring system
N-formimidoyl thienamycin (imipenem = N-F-thienamycin + cilastatin)	A carbapenem (C atom instead of sulfur in second ring); very broad spectrum and high level of activity against Gram-positive and Gram-negative bacteria, including anaerobes; frequently effective against *Enterobacteriaceae* and *Pseudomonas* with resistance to the cephalosporins of Group 3b; inactivated by renal enzymes; is therefore administered in combination with the enzyme inhibitor cilastatin

Table 3.4 *Continued: Frequently Used Anti-Infective Agents*

Class/active substance	Remarks
meropenem	Like imipenem, but stable against renal dehydropeptidase
Polypeptides	
bacitracin	Only against Gram-positive bacteria; is only used topically
Polymyxin B, colistin	Only against Gram-negative rod bacteria; neurotoxicity, nephrotoxicity
Rifamycins	
rifampicin	Against Gram-positive bacteria and tuberculosis bacteria; mainly bacteriostatic; rapid development of resistance, for which reason combination therapy is recommended
Streptogramins	
quinopristin/dalfopristin	Fixed combination preparation of two streptogramins; effective mainly against Gram-positive bacteria
Sulfamethoxazole/trimethoprim (cotrimoxazole)	Fixed combination; five parts sulfamethoxazole and one part trimethoprim
Sulfonamides	
sulfanilamide, sulfamethoxazole, sulfafurazole, etc.	Broad spectrum; bacteriostatic effect only; resistance frequent
Sulfones	
dapsone	diaminodiphenylsulfone; for therapy of leprosy
Tetracyclines	
doxycycline tetracycline, oxytetracycline, rolitetracycline, minocycline	Broad spectrum including all bacteria, chlamydias, and rickettsias; resistance frequent; dental deposits in small children

Basic Chemical Structures of the Most Important Anti-infectives

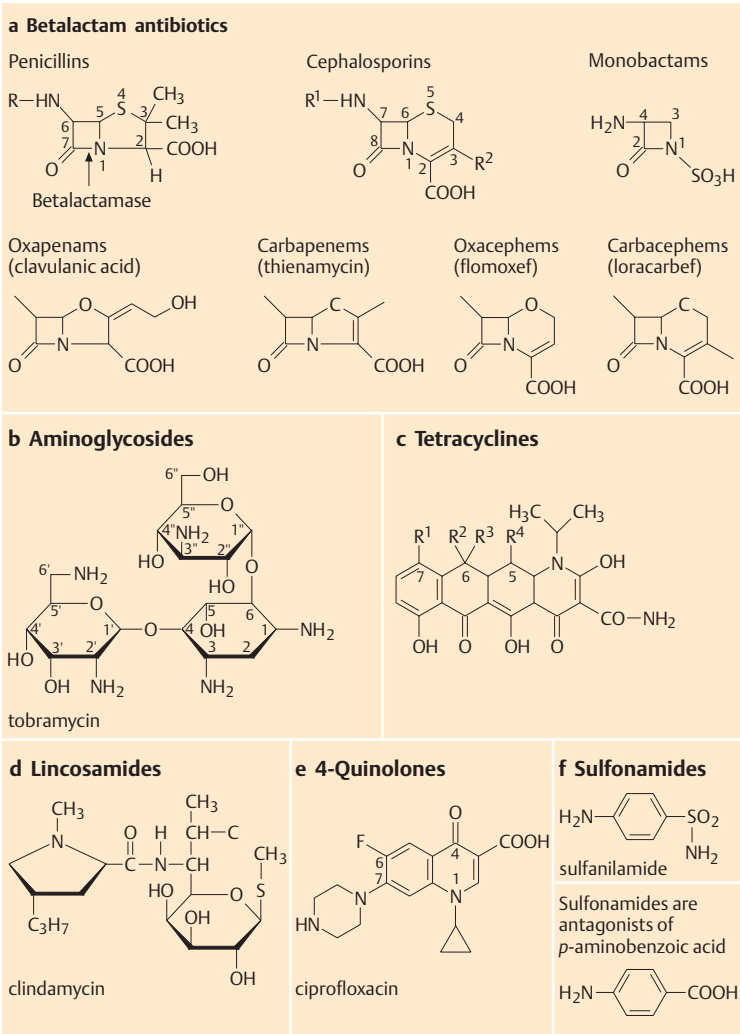


Fig. 3.**29** Antibiotic groups often include many drug substances with different substituents.

Spectrum of Action

Each anti-infective agent has a certain **spectrum of action**, which is a range of bacterial species showing natural sensitivity to the substance. Some anti-infective agents have a narrow spectrum of action (e.g., vancomycin). Most, however, have broad spectra like tetracyclines, which affect all eubacteria.

Efficacy

The efficacy of an anti-infective agent (syn. kinetics of action) defines the way it affects a bacterial population. Two basic effects are differentiated: **bacteriostasis**, i.e., reversible inhibition of growth, and irreversible **bactericidal activity** (Fig. 3.**30**). Many substances can develop both forms of efficacy depending on their concentration, the type of organism, and the growth phase. Many of these drugs also have a **postantibiotic effect (PAE)** reflecting the damage inflicted on a bacterial population. After the anti-infective agent is no longer present, the bacterial cells not killed require a recovery phase before they can reproduce again. The PAE may last several hours.

A bacteriostatic agent alone can never completely eliminate pathogenic bacteria from the body's tissues. "Healing" results from the combined effects of the anti-infective agent and the specific and nonspecific immune defenses of the host organism. In tissues in which this defense system is inefficient (endocardium), in the middle of a purulent lesion where no functional phagocytes are present, or in immunocompromised patients, bactericidal substances must be required. The clinical value of knowing whether an antibacterial drug is bacteriostatic or bactericidal is readily apparent.

All of the bacteria from an infection focus cannot be eliminated without support from the body's immune defense system. A bacterial population always includes several cells with phenotypic resistance that is not genotypically founded. These are the so-called **persisters**, which occur in in-vitro cultures at frequencies ranging from $1{:}10^6$ to $1{:}10^8$ (Fig. 3.**30**). The cause of such persistence is usually a specific metabolic property of these bacteria that prevents bactericidal substances from killing them. Following discontinuation of therapy, such persisters can lead to relapses. Infections with **L-forms** show a special type of persistence when treated with antibiotics that block murein synthesis (p. 156).

Efficacy of Selected Anti-Infective Agents

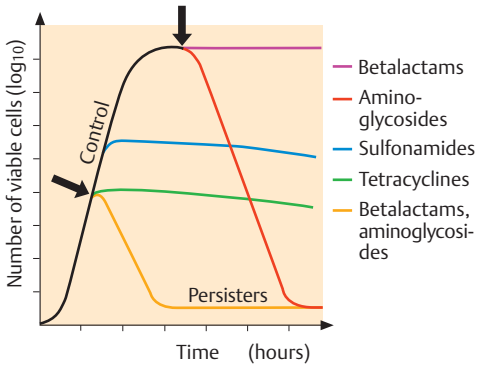


Fig. 3.**30** The arrows indicate addition of substances in the different phases of the normal growth curve (see Fig. 3.**16**). Betalactams are bactericidal only during the bacterial cell division phase, whereas aminoglycosides show this activity in all growth phases. Sulfonamides are always bacteriostatic, tetracyclines are mainly bacteriostatic. Some cells in every culture (so-called persisters) are phenotypically (but not genotypically) resistant to the bactericidal effects of anti-infective agents.

Mechanisms of Action

Table 3.**5** provides a concise summary of the molecular mechanisms of action of the most important groups of anti-infective agents.

Table 3.**5** Mechanisms of Action of the Most Important Anti-Infective Agents

Substance group	Mechanism, activity site
Sulfonamides	Competition with *p*-aminobenzoic acid as a substrate for dihydropteric acid synthetase, thus too little tetrahydrofolic acid
Trimethoprim	Inhibition of dihydrofolic acid reductase, thus too little tetrahydrofolic acid
Betalactam antibiotics	Disturbance of murein biosynthesis: — Irreversible inhibition of DD-transpeptidase, which catalyzes the peptide crosslinkage in murein — Release of an inhibitor of autolytic murein enzymes — Enzymatic destruction of murein architecture with autolysins: "wrong place at the wrong time" — Lysis due to high internal osmotic pressure
Vancomycin Teicoplanin Fosfomycin Bacitracin	Disturbance of murein biosynthesis at various different molecular stages
Rifamycin	Transcription: Blockage of DNA-dependent RNA polymerase
Aminoglycosides	Translation: — Genetic code is not read correctly (miscoding) — Blockage of e-type (elongation ribosome) A-position occupancy by AA-tRNA
Tetracyclines	Translation: — Blockage of e-type (elongation ribosome) and i-type (initiation ribosome) A-position occupancy by AA-tRNA
Chloramphenicol	Translation: Inhibition of peptidyl transferase activity
Macrolides, ketolides	Translation: Inhibition of elongation of the polypeptide chain
4-Quinolones	Inhibition of the DNA gyrase and topoisomerase IV resulting in the inhibition of DNA replication
Polymyxins	Cytoplasmic membrane: Structural disruption

Details of the Mechanisms of Action of Anti-Infective Agents

Sulfonamides and trimethoprim	Tetrahydrofolic acid (THFA) acts as a coenzyme to regulate the C1 metabolism for transfer of the hydroxymethyl and formyl groups. Too little THFA results in the cessation of growth. The combination of sulfamethoxazole and trimethoprim (cotrimoxazole) results in a potentiated efficacy.
Betalactam antibiotics	The mechanisms described in Table 3.**5** refer to penicillin and pneumococci. They probably hold in similar form for other betalactams and other bacteria as well. All bacteria with cell walls containing murein possess autolysins. These enzymes create gaps in the murein sacculus while the bacterium is growing, these gaps are then filled in with new murein material. Bacteria the growth of which is inhibited, but which are not lysed, show betalactam **tolerance** (bacteriostatic, but not bactericidal effects).
Protein synthesis inhibitors	The biosynthesis of bacterial proteins differs in detail from that observed in eukaryotes, permitting a selective inhibition by antibiotics. The principle of selective toxicity still applies.
4-Quinolones	DNA gyrase, which only occurs in bacteria, catalyzes the counterclockwise supercoiling of the double helix, which is, in itself, wound to the right, about its helical axis (see Fig. 3.**17**, p. 167). Only in supercoiled form can the DNA fit economically into the cell. DNA replication depends on this supercoiled topology. 4-Quinolones also inhibit bacterial topoisomerase IV of Gram-positive bacteria.

Pharmacokinetics

Pharmacokinetics covers the principles of absorption, distribution, and elimination of pharmacons by the macroorganism. The reader is referred to standard textbooks of pharmacology for details. The dosage and dosage interval recommendations for antibacterial therapy take into account the widely differing pharmacokinetic parameters of the different anti-infective agents, among them:

- Absorption rate and specific absorption time
- Volume of distribution
- Protein binding
- Serum (blood) concentration
- Tissue concentration
- Metabolization
- Elimination

Side Effects

Treatment with anti-infective agents can cause side effects, resulting either from noncompliance with important therapeutic principles or specific patient reactivity. On the whole, such side effects are of minor significance.

- **Toxic effects.** These effects arise from direct cell and tissue damage in the macroorganism. Blood concentrations of some substances must therefore be monitored during therapy if there is a risk of cumulation due to inefficient elimination (examples: aminoglycosides, vancomycin).

- **Allergic reactions.** See p. 108 for possible mechanisms (example: penicillin allergy).

- **Biological side effects.** Example: change in or elimination of normal flora, interfering with its function as a beneficial colonizer (see p. 25).

The Problem of Resistance

Definitions

Clinical resistance. Resistance of bacteria to the concentration of anti-infective agents maintained at the infection site in the macroorganism.

Natural resistance. Resistance characteristic of a bacterial species, genus, or family.

Acquired resistance. Strains of sensitive taxa can acquire resistance by way of changes in their genetic material.

Biochemical resistance. A biochemically detectable resistance observed in strains of sensitive taxa. The biochemical resistance often corresponds to the clinically relevant resistance. Biochemically resistant strains sometimes show low levels of resistance below the clinically defined boundary separating resistant and sensitive strains. Such strains may be medically susceptible.

Incidence, Significance

Problematic bacteria. Strains with acquired resistance are encountered frequently among *Enterobacteriaceae*, pseudomonads, staphylococci, and enterococci. Specific infection therapy directed at these pathogens is often fraught with difficulties, which explains the label problematic bacteria. They are responsible for most nosocomial infections (p. 342f.). Usually harmless in otherwise healthy persons, they may cause life-threatening infections in highly susceptible, so-called **problematic patients**. Problematic bacteria are often characterized by **multiple resistance**. Resistance to anti-infective agents is observed less frequently in nonhospital bacteria.

Genetic variability. The basic cause of the high incidence of antibiotic resistance experienced with problematic bacteria is the pronounced genetic variability of these organisms, the mechanisms of which are described in the section “Genetic variability” (p. 171 and p. 174). Most important are the mechanisms of horizontal transfer of resistance determinants responsible for the efficient distribution of resistance markers among these bacteria.

Selection. The origin and distribution of resistant strains is based to a significant extent on selection of resistance variants. The more often anti-infective substances are administered therapeutically, the greater the number of strains that will develop acquired resistance. Each hospital has a characteristic flora reflecting its prescription practice. A physician must be familiar with the resistance characteristics of this hospital flora so that the right

anti-infective agents for a **"calculated antibiotic therapy"** can be selected even before the resistance test results are in. Such therapies take into account the frequency of infections by certain bacterial species (pathogen epidemiology) as well as current resistance levels among these bacteria (resistance epidemiology).

Resistance Mechanisms

Inactivating enzymes. Hydrolysis or modification of anti-infective agents.

- **Betalactamases.** Hydrolyze the betalactam ring of betalactam antibiotics (see Fig. 3.**29**). Over 200 different betalactamases are known. A course classification system is based on the substrate profile in penicillinases and cephalosporinases. Production of some betalactamases is induced by betalactams (see p. 169), others are produced constitutively (unregulated).

- **Aminoglycosidases.** Modify aminoglycosides by means of phosphorylation and nucleotidylation of free hydroxyl groups (phosphotransferases and nucleotidyl transferases) or acetylation of free amino groups (acetyltransferases).

- **Chloramphenicol acetyltransferases.** Modification, by acetylation, of chloramphenicol.

Resistant target molecules.

- Gene products with a low affinity to anti-infective agents are produced based on mutations in natural genes. Example: DNA gyrase subunit A, resistant to 4-quinolones.

- Acquisition of a gene that codes for a target molecule with low affinity to anti-infective agents. The resistance protein assumes the function of the sensitive target molecule. Example: methicillin resistance in staphylococci; acquisition of the penicillin-binding protein 2a, which is resistant to betalactam antibiotics and assumes the function of the naturally sensitive penicillin-binding proteins.

- Acquisition of the gene for an enzyme that alters the target structure of an anti-infective agent to render it resistant. Example: 23S rRNA methylases; modification of ribosomal RNA to prevent binding of macrolide antibiotics to the ribosome.

Permeability mechanisms.

- **Reduced influx.** Reduction of transport of anti-infective agents from outside to inside through membranes; rare.

Increased efflux. Active transport of anti-infective agents from inside to outside by means of efflux pumps in the cytoplasmic membrane, making efflux greater than influx; frequent.

Evolution of Resistance to Anti-Infective Agents

Resistance to anti-infective agents is genetically determined by resistance genes. Many resistance determinants are not new developments in response to the use of medical antibiotics, but developed millions of years ago in bacteria with no human associations. The evolutionary process is therefore a **"nonanthropogenic"** one. The determinants that code for resistance to anti-infective agents that are not antibiotics did develop after the substances began to be used in therapy, hence this is **"anthropogenic"** evolution. Factors contributing to the resistance problem have included the molecular mechanisms of genetic variability (mutation, homologous recombination, site-specific integration, transposition) and the mechanisms of intercellular gene transfer in bacteria (transformation, transduction, conjugation).

Nonanthropogenic and Anthropogenic Evolution

Nonanthropogenic evolution. The need for resistance developed parallel to the ability to produce antibiotics. The producing organisms protect themselves from their own products by means of such R mechanisms. Resistance genes also evolved in bacteria that shared the natural habitat of the antibiotic producers. They secured their own ecological niche in the presence of the producers by means of the characteristic of resistance. The genetic sequences from which the resistance genes evolved were those that coded for the anabolic or catabolic metabolism genes. At a later point in evolutionary history, such "nonanthropogenic" genes have accidentally, and rarely, found their way into the genetic material of human pathogen bacteria. Therefore, when new antibiotic substances come to be used for therapeutic purposes, there are always a small number of bacteria that already show resistance to them.

Anthropogenic evolution. This term refers to the evolution of resistance genes in bacteria associated with humans based on mutations in native genes. An example is the mutation that brings about resistance to 4-quinolones in gene *gyrA*, which codes for subunit A of the DNA gyrase. A special case of anthropogenic evolution is the development of new resistance genes resulting from mutations in "nonanthropogenic" resistance genes already established in human pathogen bacteria.

The best-known example of this is provided by mutations in TEM and SHV *betalactamase* genes that code for betalactamases with a very broad substrate profile (ESBL = extended spectrum betalactamases).

Resistance Tests

Two standard test systems are used to determine the in-vitro resistance levels of bacteria.

In **dilution series tests**, the minimum inhibitory concentration (MIC) of an anti-infective agent required to inhibit proliferation of a bacterial population is determined. A factor 2 geometrical dilution series of the agent is prepared in a nutrient medium, inoculated with the test organism and incubated, whereupon the lowest growth-inhibiting concentration level (mg/l) is determined. Three standardized dilution methods are available. In the agar dilution test, nutrient agar plates containing antibiotic are inoculated ("spotted") with the test organisms. In the microbroth dilution test, the final volume is usually 100 µl per microplate well. This test type can also be automated. The final volume in a macrobroth dilution test is 2 ml per tube.

Due to the complexity and time-consuming nature of the above test types, routine laboratories often use the **agar diffusion test**. This involves diffuse inoculation of the nutrient agar plate with the test strain. Then disks of filter paper containing the anti-infective agents are placed on the agar. After the plates thus prepared are incubated, the inhibition zones around the disks (i.e., whether or not they develop and their size) provide information on the resistance of the microorganisms tested (Fig. 3.**31**). This is possible because of the linear relation between the $\log_2$ MIC and the diameter of the inhibition zones (Fig. 3.**32**).

To **interpret the results**, the MICs or inhibition zones are brought into relation with the substance concentrations present at a site of infection at standard dosage levels. This calculation is based on known averages for various pharmacokinetic parameters (serum concentration, half-life) and pharmacodynamic parameters (bactericidal activity or not, postantibiotic effect,

Agar Diffusion Test

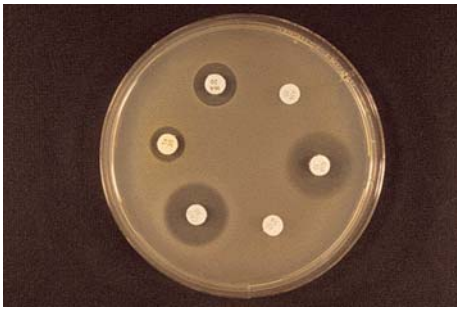


Fig. 3.**31** This method, also known as the "disk test," is used to test the resistance of a bacterial culture to various anti-infective agents. The method provides a basis for classification of a bacterial strain as "susceptible," "resistant," or "intermediate" according to the dimension of the inhibition zone.

Inhibition Zone and Minimum Inhibitory Concentration (MIC)

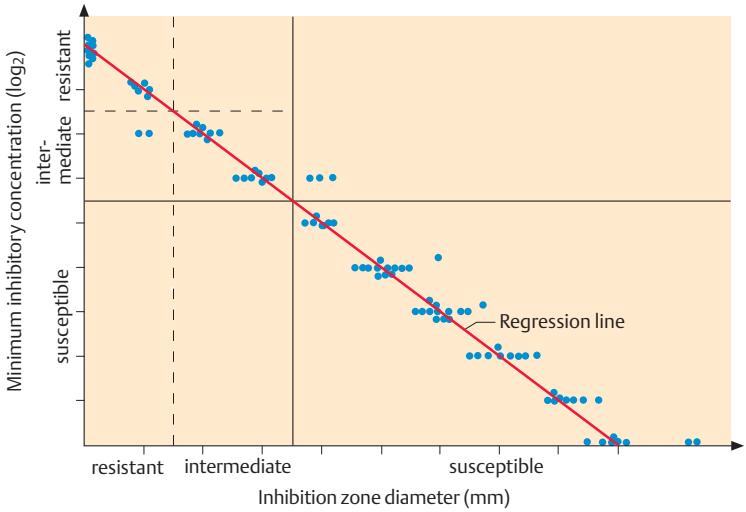


Fig. 3.**32** Each point represents a bacterial strain. The size of the inhibition zone is determined in the agar diffusion test (disk test) and the minimum inhibitory concentration (MIC) in the dilution test. The MIC corresponds logarithmically (log_2) with the diameter of the inhibition zone.

etc.). The interpretation also takes into account clinical experience gained from therapy of infections with pathogens of given suceptibility. Such data are used to establish general guideline values defining the boundary between susceptible and resistant bacteria.

The **minimum bactericidal concentration** (MBC) is the smallest concentration of a substance required to kill 99.9% of the cells in an inoculum.

The MBC is determined using quantitative subcultures from the macroscopically unclouded tubes or (microplate) wells of an MIC dilution series.

Combination Therapy

Combination therapy is the term for concurrent administration of two or more anti-infective agents. Some galenic preparations combine two components in a fixed ratio (example: cotrimoxazole). Normally, however, the in-

dividual substances in a combination therapy are administered separately. Several different objectives can be pursued with combination therapy:

- **Broadening of the spectrum of action.** In mixed infections with pathogens of varying resistance; in calculated therapy of infections with unknown, or not yet known, pathogenic flora and resistance characteristics.
- **Delay of resistance development.** In therapy of tuberculosis; when using anti-infective agents against which bacteria quickly develop resistance.
- **Potentiation of efficacy.** In severe infections requiring bactericidal activity at the site of infection. Best-known example: penicillin plus gentamicin in treatment of endocarditis caused by enterococci or streptococci.

Combining the effects of anti-infective drugs can have several different effects:

- **No difference.** The combination is no more efficacious than the more active of the two components alone.
- **Addition.** Summation of the effects.
- **Synergism.** Potentiation of the effects.
- **Antagonism.** The combination is less efficacious than one of the two components alone.

Rule of thumb: combinations of bacteriostatics with substances that are bactericidal in the cell division phase only often result in antagonism, e.g., penicillin plus tetracycline in therapy of pneumococcal pneumonia.

In-vitro investigations of the mechanism of action of a combination when used against a pathogen usually employ the so-called "checkerboard titration" technique, in which the combinatory effects of substances A and B are compared using a checkerboard-like pattern.

Chemoprophylaxis

One of the most controversial antibiotic uses is prophylactic antibiosis. There are no clear-cut solutions here. There are certain situations in which chemoprophylaxis is clearly indicated and others in which it is clearly contraindicated. The matter must be decided on a case-by-case basis by weighing potential benefits against potential harm (side effects, superinfections with highly virulent and resistant pathogens, selection of resistant bacteria).

Chemoprophylaxis is considered useful in malaria, rheumatic fever, pulmonary cystic fibrosis, recurring pyelonephritis, following intensive contact

with meningococci carriers, before surgery involving massive bacterial contamination, in heavily immunocompromised patients, in cardiac surgery or in femoral amputations due to circulatory problems. Chemoprophylaxis aimed at preventing a postsurgical infection should begin a few hours before the operation and never be continued for longer than 24–72 hours.

Immunomodulators

Despite the generally good efficacy of anti-infective agents, therapeutic success cannot be guaranteed. Complete elimination of bacterial pathogens also requires a functioning immune defense system. In view of the fact that the number of patients with severe immunodeficiencies is on the rise, immunomodulators are used as a supportive adjunct to specific antibiotic therapy in such patients. Many of these "cytokines" (see p. 77ff.) produced by the cells of the immune system can now be produced as "recombinant proteins." Myelopoietic growth factors have now been successfully used in patients suffering from neutropenia. Additional immunomodulators are also available, e.g., interferon gamma (IFNγ) and interleukin 2 (IL-2).

Laboratory Diagnosis

Infections can be diagnosed either directly by detection of the pathogen or components thereof or indirectly by antibody detection methods. The reliability of laboratory results is characterized by the terms sensitivity and specificity, their value is measured in terms of positive to negative predictive value. These predictive values depend to a great extent on prevalence. In direct laboratory diagnosis, correct material sampling and adequate transport precautions are an absolute necessity. The classic methods of direct laboratory diagnosis include microscopy and culturing. Identification of pathogens is based on morphological, physiological, and chemical characteristics. Among the latter, **the importance of detection of pathogen-specific nucleotide sequences is constantly increasing**. Development of sensitive test systems has made direct detection of pathogen components in test materials possible in some cases. The molecular biological methods used are applied with or without amplification of the sequence sought as the case warrants. Direct detection can also employ polyclonal or monoclonal antibodies to detect and identify antigens.

Preconditions, General Methods, Evaluation

Preconditions

The field of medical microbiology dealing with laboratory diagnosis of infectious diseases is known as diagnostic or clinical microbiology. Modern medical practice, and in particular hospital-based practice, is inconceivable without the cooperation of a special microbiological laboratory.

To ensure optimum patient benefit, the physician in charge of treatment and the laboratory staff must cooperate closely and efficiently. The preconditions include a basic knowledge of pathophysiology and clinical infectiology on the part of the laboratory staff and familiarity with the laboratory work on the part of the treating physician. The following sections provide a brief rundown on what physicians need to know about laboratory procedures.

General Methods and Evaluation

An infectious disease can be diagnosed **directly** by finding the causal pathogen or its components or products. It can also be diagnosed **indirectly** by means of antibody detection (Chapter 2, p. 121ff.). The accuracy and value of each of the available diagnostic methods are characterized in terms of sensitivity, specificity, and positive or negative predictive value. These parameters are best understood by reference to a 2 × 2 table (Table 3.**6**).

By inserting fictitious numbers into the 2 × 2 table, it readily becomes apparent that a positive predictive value will fall rapidly, despite high levels of specificity and sensitivity, if the prevalence level is low (Baye's theorem).

Sampling and Transport of Test Material

It is very important that the material to be tested be correctly obtained (sampled) and transported. In general, material from which the pathogen is to be isolated should be sampled as early as possible before chemotherapy is begun. Transport to the laboratory must be carried out in special containers provided by the institutes involved, usually containing transport mediums—either enrichment mediums (e.g., blood culture bottle), selective growth mediums or simple transport mediums without nutrients. An invoice must be attached to the material containing the information required for processing (using the form provided).

Table 3.6 2 × 2 Table: Explanation and Calculation of Sensitivity, Specificity, and Predictive Value (Positive–Negative)

Collective	Test positive	Test negative
Infected	Correct positive *cp*	False negative *fn*
Noninfected	False positive *fp*	Correct negative *cn*

- Sensitivity (%) measures the frequency of correct positive results in the **infected** collective (horizontal addition).
- Specificity (%) measures the frequency of correct negative results in the **noninfected** collective (horizontal addition).

$$\text{Sensitivity (\%)} = \frac{cp}{cp + fn} \times 100; \quad \text{Specificity (\%)} = \frac{cn}{fp + cn} \times 100$$

- The predictive value of the positive result expresses the probability that a positive result indicates an infection. It analyzes the positive test results both in the infected collective and in the noninfected collective (vertical addition).
- The predictive value of the negative result expresses the probability that a negative result indicates noninfection.

$$\text{Pos. pred. value (\%)} = \frac{cp}{cp + fp} \times 100; \quad \text{Neg. pred. value (\%)} = \frac{cn}{cn + fn} \times 100$$

Material from the respiratory tract:

- Swab smear from tonsils.
- Sinus flushing fluid.
- Pulmonary secretion. Expectorated sputum is usually contaminated with saliva and the flora of the oropharynx. Since these contaminations include pathogens that may cause infections of the lower respiratory tract organs, the value of positive findings would be limited. The material can be considered unsuitable for diagnostic testing if more than 25 oral epithelia are present per viewing frame at 100× magnification. Morning sputum from flushing the mouth or after induction will result in suitable samples. Sputum is not analyzed for anaerobes.
- Useful alternatives to expectorated sputum include bronchoscopically sampled bronchial secretion, flushing fluid from bronchoalveolar lavage (BAL), transtracheal aspirate or a pulmonary puncture biopsy. These types of material are required if an anaerobe infection is suspected. The material must then be transported in special anaerobe transport containers.

Material from the urogenital tract:

- Urine. Midstream urine is in most cases contaminated with the flora of the anterior urethra, which often corresponds to the pathogen spectrum of urinary tract infections. Bacterial counts must be determined if "contamination" is to be effectively differentiated from "infection." At counts in morning urine of $\geq 10^5$/ml an infection is highly probable, at counts of $\leq 10^3$ rather improbable. At counts of around 10^4/ml the test should be repeated. Lower counts may also be diagnostically significant in urethrocystitis. The dipstick method, which can be used in any medical practice, is a simple way of estimating the bacterial count: a stick coated with nutrient medium is immersed in the midstream urine, then incubated. The colony count is then estimated by comparing the result with standardized images.
- Catheterizing the urinary bladder solely for diagnostic purposes is inadvisable due to the potential for iatrogenic infection. Uncontaminated bladder urine is obtainable only by means of a suprapubic bladder puncture.
- Genital secretions are sampled with smear swabs and must be transported in special transport mediums.

Blood:

- For a blood culture, at least 10–20 ml of venous blood should be drawn sterilely into one aerobic and one anaerobic blood culture bottle. Sample three times a day at intervals of several hours (minimum interval one hour).
- For serology, (2–)5 ml of native blood will usually suffice. Take the initial sample as early as possible and a second one 1–3 weeks later to register any change in the antibody titer.

Pus and wound secretions:

- For surface wounds sample material with smear swabs and transport in preservative transport mediums. Such material is only analyzed for aerobic bacteria.
- For deep and closed wounds, liquid material (e.g., pus) should be sampled, if possible, with a syringe. Use special transport mediums for anaerobes.

Material from the gastrointestinal tract:

- Use a small spatula to place a portion of stool about the size of a cherry in liquid transport medium for shipment.
- Transport duodenal juice and bile in sterile tubes. Use special containers if anaerobes are suspected.

Cerebrospinal fluid, puncture biopsies, exudates, transudates:

- Ensure sampling sterility. Use special containers if anaerobes are suspected.

Microscopy

Bacteria are so small that a magnification of 1000× is required to view them properly, which is at the limit of light microscope capability. At this magnification, bacteria can only be discerned in a preparation in which their density is at least 10^4–10^5 bacteria per ml.

Microscopic examination of such material requires a slide preparation:

- **Native preparations**, with or without vital staining, are used to observe living bacteria. The poor contrast of such preparations makes it necessary to amplify this aspect (dark field and phase contrast microscopy). Native preparations include the coverslip and suspended drop types.

- **Stained preparations** are richer in contrast so that bacteria are readily recognized in an illuminated field at 1000×. The staining procedure kills the bacteria. The material is first applied to a slide in a thin layer, dried in the air, and fixed with heat or methyl alcohol. **Simple** and **differential staining techniques** are used. The best-known simple staining technique employs methylene blue. **Gram staining** is the most important differential technique (Table 3.**7**): Gram-positive bacteria stain blue-violet, Gram-negative bacteria stain red. The Gram-positive cell wall prevents alcohol elution of the stain-

Table 3.**7** Procedure for the Three Most Important Types of Staining

Methylene blue	Gram staining	Ziehl–Neelsen staining
Methylene blue 1–5 minutes	Gentian violet or crystal violet, 1 minute	Concentrated carbolfuchsin; heat three times until vapor is observed
Rinse off with water	Pour off stain, rinse off with Lugol's solution, then cover with Lugol's solution for 2–3 minutes	Rinse off with water Destain with HCl (3%)/alcohol mixture
	Pour off Lugol's solution	Counterstain with methylene blue, 1–5 minutes
	Destain with acetone/ethyl alcohol (1:4)	Rinse off with water
	Rinse off with water	
	Counterstain with dilute carbolfuchsin, 1 minute	
	Rinse off with water	

iodine complex. In old cultures in which autolytic enzymes have begun to break down the cell walls, Gram-positive cells may test Gram-negative ("Gram-labile" bacteria).

Another differential stain is the **Ziehl-Neelsen** technique. It is used to stain mycobacteria, which do not "take" gram or methylene blue stains due to the amounts of lipids in their cell walls. Since mycobacteria cannot be destained with HCl-alcohol, they are called acid-resistant rods. The mycobacteria are stained red and everything else blue.

Fluorescence microscopy is another special technique. A fluorochrome absorbs shortwave light and emits light with a longer wavelength. Preparations stained with fluorochromes are exposed to light at the required wavelength. The stained particles appear clearly against a dark background in the color of the emitted light. This technique requires special equipment. Its practical application is in the observation of mycobacteria. In **immunofluorescence** detection, a fluorochrome (e.g., fluorescein isothiocyanate) is coupled to an antibody to reveal the presence of antigens on particle surfaces.

Culturing Methods

Types of nutrient mediums. Culturing is required in most cases to detect and identify bacteria. Almost all human pathogen bacteria can be cultivated on nutrient mediums. Nutrient mediums are either liquid (nutrient broth) or gelatinous (nutrient agar, containing 1.5–2% of the polysaccharide agarose). Enrichment mediums are complex mediums that encourage the proliferation of many different bacterial species. The most frequently used enrichment medium is the blood agar plate containing 5% whole blood. Selective mediums allow only certain bacteria to grow and suppress the reproduction of others. Indicator mediums are used to register metabolic processes.

Proliferation forms. Most bacteria show diffuse proliferation in **liquid mediums**. Some proliferate in "crumbs," other form a grainy bottom sediment, yet others a biofilm skin at the surface (pseudomonads). Isolated colonies are observed to form on, or in, **nutrient agar** if the cell density is not too high. These are pure cultures, since each colony arises from a single bacterium or colony-forming unit (CFU). The pure culture technique is the basis of bacteriological culturing methods. The procedure most frequently used to **obtain isolated colonies** is fractionated inoculation of a nutrient agar plate (Figs. 3.**33**–3.**35**).

Use of this technique ensures that isolated colonies will be present in one of the three sectors. Besides obtaining pure cultures, the isolated colony technique has the further advantage of showing the form, appearance, and

Fractionated Inoculation of Nutrient Agar

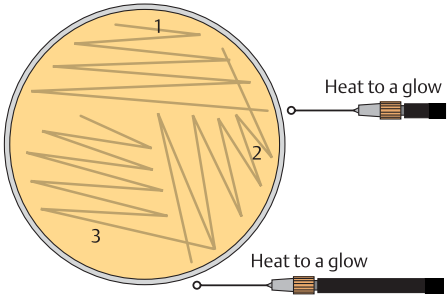


Fig. 3.**33** Isolated colonies are obtained by means of fractionated inoculation of nutrient agar. The wire loop must be sterilized between inoculations.

Blood Agar Plate Following Fractionated Inoculation and Incubation

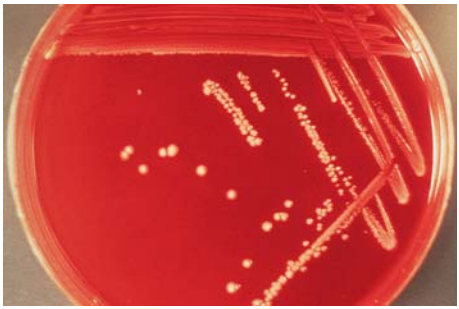


Fig. 3.**34** Blood agar is frequently used as a universal enrichment medium. Most human bacterial pathogens grow on it. Here is a pure culture of *Staphylococcus aureus*.

Endo Agar Following Fractionated Inoculation with a Mixed Culture

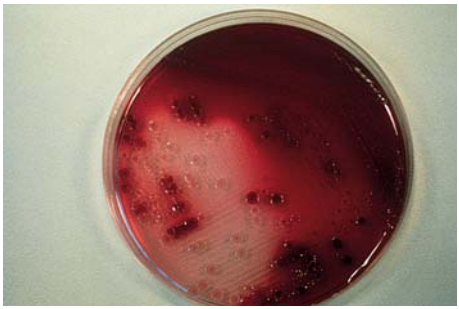


Fig. 3.**35** Endo agar is a combined selective/indicator medium. It allows growth of *Enterobacteriaceae*, *Pseudomonadaceae*, and other Gram-negative rod bacteria but inhibits the growth of Gram-positive bacteria and Gram-negative cocci. The red color of the colonies and agar is characteristic of lactose breakdown (= *Escherichia coli*); the light-colored colonies are lactose-negative (= *Salmonella enterica*).

color of single colonies. The special proliferation forms observed in nutrient broth and nutrient agar give an experienced bacteriologist sufficient information for an initial classification of the pathogen so that identifying reactions can then be tested with some degree of specificity.

Conditions required for growth. The optimum proliferation temperature for most human pathogen bacteria is 37 °C.

Bacteria are generally cultured under atmospheric conditions. It often proves necessary to incubate the cultures in 5% CO_2. Obligate anaerobes must be cultured in a milieu with a low redox potential. This can be achieved by adding suitable reduction agents to the nutrient broth or by proliferating the cultures under a gas atmosphere from which most of the oxygen has been removed by physical, chemical, or biological means.

Identification of Bacteria

The essential principle of bacterial identification is to assign an unknown culture to its place within the taxonomic classification system based on as few characteristics as possible and as many as necessary (Table 3.**8**).

- **Morphological characteristics**, including staining, are determined under the microscope.
- **Physiological characteristics** are determined with indicator mediums. Commercially available miniaturized systems are now frequently used for this purpose (Fig. 3.**36**).

Determination of Metabolic Characteristics with Indicator Mediums

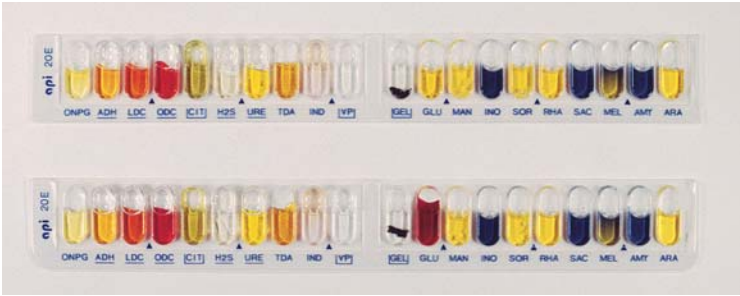


Fig. 3.**36** Identification of *Enterobacteriaceae* using API 20E, a standardized microplate method. Positive and negative reactions are shown by color reactions.

Table 3.**8** Characteristics Useful in Identification of Bacteria

Morphological characteristics
Form (sphere, rod, spiral)
Size; pseudogroupings (clusters, chains, diplococci)
Staining (Gram-positive, Gram-negative); flagella (presence, arrangement); capsule (yes, no); spores (form, within cell formation)
Physiological characteristics
Respiratory chain enzymes (oxidases, catalases)
Enzymes that break down carbohydrates, alcohols, glycosides (e.g., betagalactosidase)
Protein metabolism enzymes (e.g., gelatinase, collagenase)
Amino acid metabolism enzymes (e.g., decarboxylases, deaminases, urease)
Other enzymes: hemolysins, lipases, lecithinases, DNases, etc.
End products of metabolism (e.g., organic acids detected by gas chromatography)
Resistance/sensitivity to chemical noxae
Characteristics of anabolic metabolism (e.g., citrate as sole source of C)
Chemical characteristics
DNA structure (base sequences)
Structure of cell wall murein
Antigen structure: fine structures detectable with antibodies (e.g., flagellar protein or polysaccharides of the cell wall or capsule)
Fatty acids in membranes and cell wall; analysis using different chromatographic methods

■ **Chemical characteristics** have long been in use to identify bacteria, e.g., in detection of antigen structures. Molecular genetic methods (see below) will play an increasing role in the future.

Molecular Methods

The main objective of the molecular methods of bacterial identification is direct recognition of pathogen-specific nucleotide sequences in the test material. These methods are used in particular in the search for bacteria that are not culturable, are very difficult to culture, or proliferate very slowly. Of course, they can also be used to identify pure bacterial cultures (see above). In principle, any species-specific sequence can be used for identification, but the specific regions of genes coding for 16S rRNA and 23S rRNA are particularly useful in this respect. The following methods are used:

DNA probes. Since DNA is made up of two complementary strands of nucleic acids, it is possible to detect single-strand sequences with the hybridization technique using complementary marking of single strands. The probes can be marked with radioactivity (^{32}P, ^{35}S) or nonradioactive reporter molecules (biotin, dioxigenin):

- *Solid phase hybridization*. The reporter molecule or probe is fixed to a nylon or nitrocellulose membrane (colony blot technique, dot blot technique).
- *Liquid phase hybridization*. The reporter molecule and probe are in a solute state.
- *In-situ hybridization*. Detection of bacterial DNA in infected tissue.

Amplification. The main objective here is to increase the sensitivity level so as to find the "needle in a haystack." A number of techniques have been developed to date, which can be classified in three groups:

- *Amplification of the target sequence*. The oldest and most important among the techniques in this group is the polymerase chain reaction (PCR), which is described on p. 409f.). With "real time PCR," a variant of PCR, the analysis can be completed in 10 minutes.
- *Probe amplification*.
- *Signal amplification*.

Identification by Means of Amplification and Sequencing

The target sequence for identification of bacteria that have not yet been cultured (e.g., *Tropheryma whipplei*, the causal pathogen in Whipple's disease) or of pathogens very difficult to identify with classic methods, is often a certain region of the 16S rRNA, some sections of which are identical in all bacteria. Between these highly conservative segments are other sections that are specific for a species or genus. Using primers that can recognize the conserved regions of 16S rDNA to the right and left of the specific regions, the specific sequence is amplified, then sequenced. The base sequence thus obtained is then identified by comparison with a reference data library.

Identification by Means of Amplification and Gene Chips

In this technique, thousands of oligonucleotides specific for human pathogen bacteria are deposited on the surface of a chip about 2 cm^2 in size. This chip is then charged with amplified and marked single-strand DNA from the test material (containing, for example, species-specific sequences of the 16S rDNA or other species-specific sequences). Then the level of binding to complementary nucleotide sequences is measured as fluorescence using confocal laser scanning microscopy. The occurrence of antibiotic resistance genes can also be measured by this method.

Direct Detection of Bacterial Antigens

Antigens specific for particular species or genera can be detected directly by means of polyclonal or (better yet) monoclonal antibodies present in the test material. This allows for rapid diagnosis. Examples include the detection of bacterial antigens in cerebrospinal fluid in cases of acute purulent meningitis, detection of gonococcal antigens in secretion from the urogenital tract, and detection of group A streptococcal antigen in throat smear material. These direct methods are not, however, as sensitive as the classic culturing methods. Adsorbance, coagglutination, and latex agglutination tests are frequently used in direct detection. In the agglutination methods, the antibodies with the Fc components are fixed either to killed staphylococcal protein A or to latex particles.

Diagnostic Animal Tests

Animal testing is practically a thing of the past in diagnostic bacteriology. Until a few years ago, bacterial toxins (e.g., diphtheria toxin, tetanus toxin, botulinus toxin) were confirmed in animal tests. Today, molecular genetic methods are used to detect the presence of the toxin gene, which process usually involves an amplification step.

Bacteriological Laboratory Safety

Microbiologists doing diagnostic work will of course have to handle potentially pathogenic microorganisms and must observe stringent regulations to avoid risks to themselves and others. Laboratory safety begins with suitable room designs and equipment (negative-pressure lab rooms, safety hoods)

and goes on to include compliance with the basic rules of work in a microbiological laboratory: protective clothing, no eating, drinking, or smoking, mechanical pipetting aids, hand and working surface disinfection (immediately in case of contamination, otherwise following each procedure), proper disposal of contaminated materials, staff health checks, and proper staff training.

3

Taxonomy and Overview of Human Pathogenic Bacteria

Taxonomy includes the two disciplines of classification and nomenclature. The bacteria are classified in a hierarchic system based on phenotypic characteristics (morphological, physiological, and chemical characteristics). The basic unit is the species. Similar and related species are classified in a single genus and related genera are placed in a single family. Classification in yet higher taxa often takes practical considerations into account, e.g., division into "descriptive sections." A species is designated by two Latin names, the first of which denotes the genus, both together characterizing the species. Family names end in *-aceae*. Table 3.**9** provides an overview of human pathogenic bacteria.

Classification

Bacteria are grouped in the domain bacteria to separate them from the domains archaea and eucarya (see p. 5). Within their domain, bacteria are further broken down into taxonomic groups (taxa) based on relationships best elucidated by knowledge of the evolutionary facts. However, little is known about the phylogenetic relationships of bacteria, so their classification is often based on similarities among phenotypic characteristics (phenetic relationships). These characteristics are **morphological**, **physiological** (metabolic), or **chemical** (see Table 3.**8,** p. 215) in nature. The role of chemical characteristics in classification is growing in importance, for instance, murein composition or the presence of certain fatty acids in the cell wall. DNA and RNA structure is highly important in classification. DNA composition can be roughly estimated by determining the proportions of the bases: mol/l of guanine + cytosine (GC). The GC content (in mol%) of human pathogenic bacteria ranges from 25% to 70%. Measurement of how much heterologous duplex DNA is formed, or of RNA-DNA hybrids, provides information

on the similarity of different bacteria and thus about their degree of relationship. Another highly useful factor in determining phylogenetic relationship is the sequence analysis of the (16S/23S) rRNA or (16S/23S) rDNA. This genetic material contains highly conserved sequences found in all bacteria alongside sequences characteristic of the different taxa.

In formal terms, the prokaryotes are classified in phyla, classes, orders, families, genera, and species, plus subtaxa if any:

Family (familia)	*Enterobacteriaceae*
Genus	*Escherichia*
Species	*E. coli*
Var(iety) or type	Serovar O157:H7
Strain	xyz

Taxonomic classification is based on the concept of the species. Especially in an epidemiological setting, we often need to subclassify a species in **vars** or (syn.) **types**, in which cultures of a species that share certain characteristics are grouped together. Examples: biovar, phagovar, pathovar, morphovar, serovar (also biotype, phagotype, etc.). Use of the term **strain** varies somewhat: in clinical bacteriology it often designates the first culture of a species isolated from an infected patient. In an epidemiological context, isolates of the same species obtained from different patients are considered to belong to the same epidemic strain.

There is no official, internationally recognized classification of bacteria. The higher taxa therefore often reflect practical considerations.

Table 3.**9** Overview of the Medically Most Important Bacteria[1]

Family Genus, species	Characteristics	Clinical manifestations
Section 1. Gram-positive cocci		
Staphylococcaceae	Cluster-forming cocci, nonmotile; catalase-positive	
Staphylococcus aureus	Coagulase-positive, yellow-pigmented colonies	Pyogenic infections, toxicoses
S. epidermidis	Coagulase-negative, whitish colonies, normal flora	Foreign body infections
S. saprophyticus	Coagulase-negative	Urinary tract infections in young women
Streptococcaceae	Chain-forming cocci and diplococci, nonmotile, catalase-negative	
Streptococcus pyogenes	Chain-forming cocci, Lancefield group A, β-hemolysis	Tonsillitis, scarlet fever, skin infections
S. pneumoniae	Diplococci, no group antigen present, α-hemolysis	Pneumonia, otitis media, sinusitis
S. agalactiae	Chain-forming cocci, group antigen B, β-hemolysis	Meningitis/sepsis in neonates
"***Enterococcaceae***"	Chain-forming cocci and diplococci, α, β, or γ-hemolysis, group antigen D, catalase-negative	Part of the flora of intestines of humans and animals
Enterococcus faecalis *Enterococcus faecium*	Aesculin-positive, growth in 6.5% NaCl, pH 9.6	Opportunistic infections
Section 2. Endospore-forming Gram-positive rods		
Bacillaceae	Aerobic soil bacteria	
Bacillus anthracis	Nonmotile, ubiquitous	Anthrax
Clostridiaceae	Anaerobic soil bacteria	
Clostridium tetani	Motile, anaerobic, tetanus toxin (tetanospasmin)	Tetanus

[1] (Nomenclature according to Bergey's *Manual of Systematic Bacteriology*, 2001, Vol. 1, pp. 155–166. Names in quotation marks not yet validated).

Table 3.9 *Continued: Overview of the Medically Most Important Bacteria*

Family Genus, species	Characteristics	Clinical manifestations
Continued: Section 2.		
Clostridium botulinum	Motile, neurotoxins A, B, and G	Botulism, usually ingestion of toxin with food
Clostridium perfringens and further clostridiae	Nonmotile, exotoxins, and exoenzymes	1. Anaerobic cellulitis 2. Gas gangrene (myonecrosis)
Clostridium difficile	Motile, enterotoxin (toxin A), cytotoxin (toxin B)	Pseudomembranous colitis (often antibotic associated)
Section 3. Regular, nonsporing, Gram-positive rods		
Listeria monocytogenes	Slender rods, weak β-hemolysis on blood agar, motile at 20 °C, ubiquitous (soil)	Meningitis, sepsis (neonates, immunocompromised persons), epidemic gastroenteritis
Erysipelothrix rhusiopathiae	Transmitted from diseased pigs	Erysipeloid (today rare)
Gardnerella vaginalis	Flora of the normal genital mucosa	Contributes to vaginosis
Section 4. Irregular, nonsporing, Gram-positive rods		
Corynebacteriaceae	Mostly normal bacterial flora of the skin and mucosa, aerobic	Only few species cause disease
Corynebacterium diphtheriae	Club shape, pleomorphic, diphtheria exotoxin (A + B)	Diphtheria (throat, nose, wounds)
Actinomycetaceae	Normal bacterial flora of the mucosa, anaerobic or microaerophilic	
Actinomyces israelii and further *Actinomyces* spp.	Filaments (also branched)	Actinomycosis (cervicofacial, thoracic, abdominal, pelvic)
Nocardiaceae	Nonmotile, obligately aerobic, filaments, partially acid-fast	Habitat: soil and aquatic biotopes

Table 3.**9** *Continued: Overview of the Medically Most Important Bacteria*

Family Genus, species	Characteristics	Clinical manifestations
Continued: Section 4.		
Nocardia asteroides *Nocardia brasiliensis* and further species	Infections in patients with impaired cell-mediated immunity	Pulmonary, systemic, and dermal nocardioses
Section 5. Mycobacteria (acid-fast rods)		
Mycobacteriaceae	Slender rods, Ziehl-Neelsen staining (Gram-positive cell wall), aerobic, nonmotile	
Mycobacterium tuberculosis	Slow proliferation (culturing 3–6–8 weeks)	Tuberculosis (pulmonary and extrapulmonary)
Mycobacterium leprae	In-vitro culture not possible	Leprosy (lepromatous, tuberculoid)
Nontuberculous mycobacteria (NTM) (e.g., *Mycobacterium avium/intracellulare* complex, and numerous other species)	Ubiquitous. Low level of pathogenicity, opportunists	Pulmonary disease, lymphadenitis, infections of skin, soft tissue, bones, joints, tendons. Disseminated disease in immunosuppressed patients (AIDS)
Section 6. Gram-negative aerobic cocci and coccobacilli		
Neisseriaceae	Coffee bean-shaped diplococci, nonmotile, oxidase (+), catalase (+)	
Neisseria gonorrheae	Cocci often in phagocytes, acid from fermentation of glucose	Gonorrhea
Neisseria meningitidis	Acid from fermentation of glucose and maltose	Meningitis/sepsis
Eikenella corrodens	HAC**E**K-group. Low pathogenicity	Nosocomial infections
Kingella kingae	HACE**K**-group. Low pathogenicity	Nosocomial infections

Table 3.**9** *Continued: Overview of the Medically Most Important Bacteria*

Family Genus, species	Characteristics	Clinical manifestations
Continued: Section 6.		
Moraxellaceae	Cocci and short rods	
Moraxella catarrhalis	Normal respiratory tract flora	Sinusitis, otitis media in children
Acinetobacter baumannii *Acinetobacter calcoaceticus*	Ubiquitous, coccobacillary rods	Nosocomial infections, often multiple resistance against anti-infective agents
Section 7. Gram-negative facultatively anaerobic rods		
Enterobacteriaceae	Inhabitat intestine of man and animals. Genera (41) and species (hundreds) identified biochemically	
Escherichia coli	Lactose-positive, most frequent human pathogen, various pathovars.	Nosocomial infections, Gut disease caused by pathovars EPEC, ETEC, EIEC, EHEC, and EAggEC
Salmonella enterica	Lactose-negative, motile, over 2000 serovars	Typhoid/paratypoid fever, gastroenteritis
Shigella dysenteriae, S. flexneri, S. boydii, S. sonnei	Lactose-negative (in most cases), nonmotile, O-serovars	Bacterial dysentery
Klebsiella, Enterobacter, Citrobacter, Proteus, Serratia, Morganella, Providencia, and other genera	Opportunists, frequently resistant to antibiotics	Nosocomial infections
Yersinia pestis	Bipolar staining, motile, no acid from lactose. Rodent pathogen	Bubonic plague, pulmonary plague
Yersinia enterocolitica	Reservoir: wild animals, domestic animals, pets	Enteritis, lymphadenitis
Calymmatobacterium granulomatis	Encapsulated, nonmotile	Granuloma inguinale (venereal disease)

Table 3.**9** *Continued: Overview of the Medically Most Important Bacteria*

Family Genus, species	Characteristics	Clinical manifestations
Continued: Section 7.		
Vibrionaceae	Comma-shaped, polar flagella, oxidase-positive	
Vibrio cholerae	Alkaline tolerance, exotoxin, no invasion of the small intestine's mucosa	Cholera, massive watery diarrhea
Aeromonadaceae		
Aeromonas spp.	Aquatic biotopes, fish infections	Occasionally the cause of enteritis in man
Pasteurellaceae	Small straight rods, nonmotile	
Pasteurella multocida	Pathogen of various animals (sepsis)	Infections via dermal injuries (rare)
Haemophilus influenzae	X and V factors for culturing, capsule serovar "b" (Hib)	Meningitis, respiratory tract infections
Cardiobacteriaceae		
Cardiobacterium hominis	HA**C**EK group. Normal mucosal flora of humans, nonmotile	Endocarditis (rare). Opportunistic infections
Section 8. Gram-negative aerobic rods		
Pseudomonadaceae	Straight or curved rods, motile, oxidase-positive. Ubiquitous bacteria	Nosocomial infections
Pseudomonas aeruginosa and many further species	Fluorescent pigments produced. Other properties as above	Nosocomial infections, frequent multiple antibiotic resistance
"Burkholderiaceae"		
Burkholderia cepacia	Ubiquitous	Nosocomial infections. Often resistance to multiple antibiotics
B. mallei	Malleus of horses	Skin abscesses. Very rare
B. pseudomallei	Habitat: soil	Melioidosis (Asia)

Table 3.**9** *Continued: Overview of the Medically Most Important Bacteria*

Family Genus, species	Characteristics	Clinical manifestations
Continued: Section 8.		
"Xanthomonadaceae" *Stenotrophomonas maltophilia*	Low pathogenicity	Nosocomial infections. Often resistance to multiple antibiotics
Legionellaceae	Motile, difficult to stain, requires special culturing mediums	
Legionella pneumophila	Most frequent species, aquatic biotopes	Legionnaire's pneumonia, Pontiac fever
Brucellaceae	Short rods, nonmotile, facultative intracellular parasite, fastidious growth	Zoonoses
Brucella abortus *Brucella melitensis* *Brucella suis* *Brucella canis*	Transmission via direct contact or foods (milk and milk products)	Brucellosis (Bang disease, Malta fever)
Alcaligenaceae *Bordetella pertussis*	Short rods, nonmotile, only in humans	Pertussis (whooping cough)
"Francisellaceae" *Francisella tularensis*	Minute pleomorphic rods. Requires enriched media for culturing	Tularemia, zoonosis (rodents)
Section 9. Gram-negative rods, straight, curved, and helical, strictly anaerobic		
Bacteroidaceae *"Fusobacteriaceae"* *"Porphyromonadaceae"* *"Prevotellaceae"*	Pleomorphic rods, major component of normal mucosal flora	Subacute necrotic infections, mostly together with other bacteria
Bacteroides spp. *Porphyromonas* spp. *Prevotella* spp. *Fusobacterium spp.*		Necrotic abscesses in CNS, head region, lungs, abdomen, female genital tract

Table 3.**9** *Continued: Overview of the Medically Most Important Bacteria*

Family Genus, species	Characteristics	Clinical manifestations
Continued: Section 9.		
Streptobacillus moniliformis (belongs new to *Fusobacteriaceae)*	Normal flora in rats, mice, and cats	Rat-bite fever (also caused by *Spirillum minus* (= Sodoku)
Section 10. Aerobic/microaerophilic, motile, helical/vibrioid Gram-negative rod bacteria		
Campylobacteriaceae	Thin, helical, and vibrioid, culturable	
Campylobacter jejuni	Animal pathogen	Enteritis
Campylobacter fetus		Opportunistic infections: sepsis, endocarditis
"Helicobacteriaceae" *Helicobacter pylori*	Helical, culturing difficult, produces large amounts of urease	Type B gastritis, peptic ulcers of stomach and duodenum
Section 11. The Spirochetes. Gram-negative, helical bacteria		
Spirochaetaceae	Helical, motile, thin	
Treponema pallidum	Only in humans, not culturable	Syphilis, three stages
Borrelia burgdorferi *B. afzelii* *B. garinii*	Tickborne, culturable	Lyme disease, three stages
Borrelia duttonii *Borrelia hermsii* and further species	Tickborne, antigen variability	Endemic relapsing fever
Borrelia recurrentis	Transmitted by body lice	Epidemic relapsing fever
Leptospiraceae	Helical, motile, culturable	
Leptospira interrogans	Serogroups and serovars (e.g., icterohemorrhagiae, pomona, grippotyphosa, etc.)	Leptospirosis, morbus Weil

Table 3.**9** *Continued: Overview of the Medically Most Important Bacteria*

Family Genus, species	Characteristics	Clinical manifestations
Section 12. Rickettsiae, Coxiellae, Ehrlichiae, Bartonellae, and Chlamydiae		
Rickettsiaceae	Small short rods, usually intracellular bacteria transmitted by arthropods	Rickettsioses
Rickettsia prowazekii	Transmitted by body lice	Typhus
Rickettsia rickettsii	Transmitted by ticks	Rocky Mountains Spotted Fever (RMSF)
"Coxelliaceae" *Coxiella burnetii*	Reservoir: sheep, cattle, rodents; infection by inhalation	Q fever (pneumonia)
Ehrlichiaceae	Coccobacillary. Culture possible	Zoonoses
Ehrlichia chaffeensis	Transmission by ticks	Human monocytrophic ehrlichiosis (HME)
Ehrlichia ewingii and Anaplasma (formerly Ehrlichia) phagocytophilum	Transmission by ticks	Human granulocytotrophic ehrlichiosis (HEG)
Bartonellaceae	Short pleomorphic rods	
Bartonella bacilliformis	Tropism for erythrocytes/endothelia. Transmitted by sand flea	Oroya fever and verruga peruana
Bartonella henselae and *Bartonella claridgeia*	Animal reservoir: cats	Sepsis, bacillary angiomatosis in immunosuppressed patients (AIDS). Cat scratch disease in immunocompetent persons
Bartonella quintana	Transmission by body lice	Five-day fever
Chlamydiaceae	Obligate intracellular pathogen, reproductive cycle	
Chlamydia trachomatis	Biovar trachoma	Trachoma, inclusion conjunctivitis, urethritis (nonspecific)
	Biovar lymphogranuloma venerum	Lymphogranuloma venereum

Table 3.**9** *Continued: Overview of the Medically Most Important Bacteria*

Family Genus, species	Characteristics	Clinical manifestations
Continued: Section 12.		
Chlamydia psittaci	Reservoir: infected birds. Infection by inhalation of pathogen-containing dust	Ornithosis (pneumonia)
Chlamydia pneumoniae	Only in humans, aerogenic transmission	Infections of the respiratory tract, often subacute. Role in atherosclerosis of coronary arteries still unclear
Section 13. Mycoplasmas (bacteria without cell walls)		
Mycoplasmataceae	Pleomorphic; no murein, therefore resistant to antibiotics that attack the cell wall	
Mycoplasma pneumoniae	Reservoir human, aerogenic infection	Pneumonia (frequently atypical)
Ureaplasma urealyticum	Component of the normal flora of the urogenital tract	Urethritis (nonspecific)

Nomenclature

The rules of bacterial nomenclature are set out in the *International Code for the Nomenclature of Bacteria*. A species is designated with two latinized names, the first of which characterizes the genus and the second the species. Family names always end in *-aceae*. Taxonomic names approved by the "International Committee of Systematic Bacteriology" are considered official and binding. In medical practice, short handles have become popular in many cases, for instance gonococci instead of *Neisseria gonorrheae* or pneumococci (or even "strep pneumos") instead of *Streptococcus pneumoniae*.

4 Bacteria as Human Pathogens

F. H. Kayser

Staphylococcus

■ Staphylococci are Gram-positive cocci occurring in clusters. They can be cultured on normal nutrient mediums both aerobically and anaerobically. The most important species from the viewpoint of human medicine is *S. aureus*. A number of extracellular enzymes and exotoxins such as coagulase, alphatoxin, leukocidin, exfoliatins, enterotoxins, and toxic shock toxin are responsible for the clinical symptoms of infections by this pathogen, which are observed in the three types invasive infections, pure toxicoses, and mixed forms. The antibiotics of choice for therapy of these infections are penicillinase-resistant penicillins. Laboratory diagnosis involves identification of the pathogen by means of microscopy and culturing. *S. aureus* is a frequent pathogen in nosocomial infections and limited outbreaks in hospitals. Hand washing by medical staff is the most important prophylactic measure in hospitals.

Coagulase-negative staphylococci are classic opportunists. *S. epidermidis* and other species are frequent agents in foreign body infections due to their ability to form biofilms on the surfaces of inert objects. *S. saprophyticus* is responsible for between 10 and 20 % of acute urinary tract infections in young women. ■

Staphylococci are small spherical cells (1 µm) found in grapelike clusters. Staphylococci are nonmotile, catalase-producing bacteria. The genus *Staphylococcus* includes over 30 species and subspecies. Table 4.**1** briefly summarizes the characteristics of those most important in the medical context. *S. aureus* (and *E. coli*) are among the most frequent causal organisms in human bacterial infections.

Table 4.1 Overview of the *Staphylococcus* Species That Affect Humans Most Frequently

Species	Parameter
S. aureus	Coagulase-positive; colonies golden yellow. Local purulent infections: furuncles, carbuncles, bullous impetigo, wound infections, sinusitis, otitis media, mastitis puerperalis, ostitis, postinfluenza pneumonia, sepsis. Toxin-caused illnesses: food poisoning, dermatitis exfoliativa, toxic shock syndrome
S. epidermidis	Coagulase-negative; sensitive to novobiocin; most frequent CNS* pathogen; opportunist; infection requires host predisposition; foreign body infections with discrete clinical symptoms
S. saprophyticus	Coagulase-negative; resistant to novobiocin. Urinary tract infections in young women (10–20 %); occasional nonspecific urethritis in men

* CNS: coagulase-negative staphylococci

Staphylococcus Aureus

Morphology and culturing. Fig. 4.**1a** shows the appearance of Gram-stained *S. aureus*. This is a facultative anaerobe that is readily cultured on normal nutrient mediums at 37 °C. Colonies as in Fig. 4.**1b** develop after 24 hours of incubation. Hemolytic zones are frequently observed around the colonies.

Fine structure. The cell wall consists of a thick layer of murein. Linear teichoic acids and polysaccharides are covalently coupled to the murein polysaccharide (Fig. 3.**10**, p. 154). The lipoteichoic acids permeating the entire murein layer are anchored in the cell membrane. Teichoic and lipoteichoic acids can trigger activation of complement by the alternative pathway and stimulate macrophages to secrete cytokines. Cell wall-associated proteins are bound to the peptide components of the murein. Clumping factor, fibronectin-binding protein, and collagen-binding protein bind specifically to fibrinogen, fibronectin, and collagen, respectively, and are instrumental in adhesion to tissues and foreign bodies covered with the appropriate matrix protein. Protein A binds to the Fc portion of immunoglobulins (IgG). It is assumed that "false" binding of immunoglobulins by protein A prevents "correct" binding of opsonizing antibodies, thus hindering phagocytosis.

Staphylococcus aureus

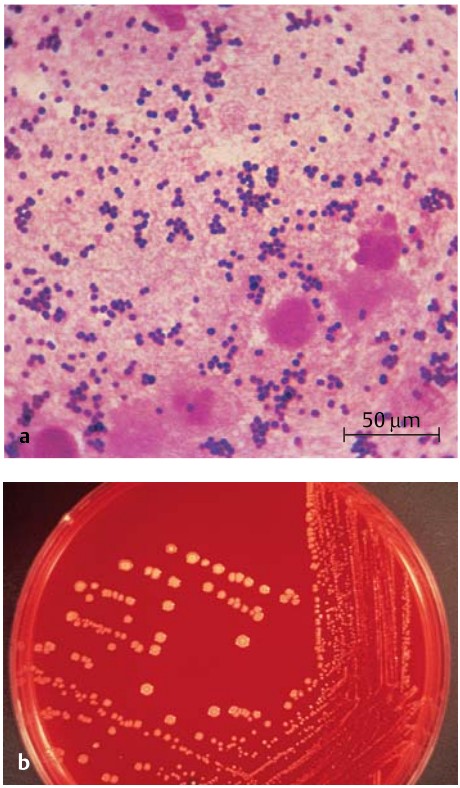


Fig. 4.1 **a** Gram staining of a pus preparation: Gram-positive cocci, some in grapelike clusters. Clinical diagnosis: furunculosis. **b** Culture on blood agar: convex colonies with yellowish pigment and porcelainlike surface.

Extracellular toxins and enzymes. *S. aureus* secretes numerous enzymes and toxins that determine, together with the fine structures described above, the pathogenesis of the attendant infections. The most important are:

- **Plasma coagulase** is an enzyme that functions like thrombin to convert fibrinogen into fibrin. Tissue microcolonies surrounded by fibrin walls are difficult to phagocytose.
- **α-toxin** can have lethal CNS effects, damages membranes (resulting in, among other things, hemolysis), and is responsible for a form of dermonecrosis.

- **Leukocidin** damages microphages and macrophages by degranulation.
- **Exfoliatins** are responsible for a form of epidermolysis.
- Food poisoning symptoms can be caused by eight serologically differentiated **enterotoxins** (A-E, H, G, and I). These proteins (MW: 35 kDa) are not inactivated by heating to 100 °C for 15–30 minutes. *Staphylococcus* enterotoxins are superantigens (see p. 72).
- **Toxic shock syndrome toxin-1** (TSST-1) is produced by about 1% of *Staphylococcus* strains. TSST-1 is a superantigen that induces clonal expansion of many T lymphocyte types (about 10%), leading to massive production of cytokines, which then give rise to the clinical symptoms of toxic shock.

Pathogenesis and clinical pictures. The pathogenesis and symptoms of *S. aureus* infections take one of three distinct courses:

- **Invasive infections.** In this type of infection, the pathogens tend to remain in situ after penetrating through the derma or mucosa and to cause local infections characterized by purulence. Examples include furuncles (Fig. 4.**2**), carbuncles, wound infections, sinusitis, otitis media, and mastitis puerperalis.

Other kinds of invasive infection include postoperative or posttraumatic ostitis/osteomyelitis, endocarditis following heart surgery (especially valve replacement), postinfluenza pneumonia, and sepsis in immunocompromised patients. *S. aureus* and *E. coli* are responsible for approximately equal shares of nearly half of all cases of inpatient sepsis.

Inert foreign bodies (see p. 158 for examples) can be colonized by *S. aureus*. Colonization begins with specific binding of the staphylococci, by means of cell wall-associated adhesion proteins, to fibrinogen or fibronectin covering the foreign body, resulting in a biofilm that may function as a focus of infection.

Multiple Furuncles

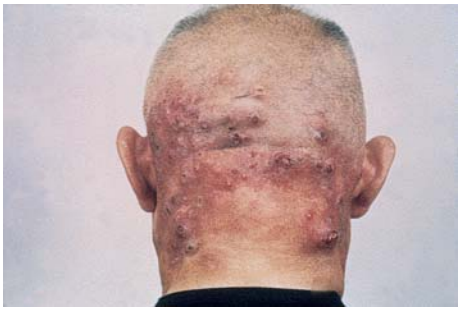


Fig. 4.**2** Furuncles in a patient with type 2 diabetes mellitus.

■ **Toxicoses.** Food poisoning results from ingestion of food contaminated with enterotoxins. The onset a few hours after ingestion takes the form of nausea, vomiting, and massive diarrhea.

■ **Mixed forms.** Dermatitis exfoliativa (staphylococcal scalded skin syndrome, Ritter disease), pemphigus neonatorum, and bullous impetigo are caused by exfoliatin-producing strains that infect the skin surface. Toxic shock syndrome (TSS) is caused by strains that produce TSST-1. These strains can cause invasive infections, but may also only colonize mucosa. The main symptoms are hypotension, fever, and a scarlatiniform rash.

Diagnosis. This requires microscopic and culture-based pathogen identification. Differentiating *S. aureus* from the coagulase-negative species is achieved by detection of the plasma coagulase and/or the clumping factor. The enterotoxins and TSST-1 can be detected by means of immunological and molecular biological methods (special laboratories).

Plasma Coagulase and Clumping Factor Test

■ To detect plasma coagulase, suspend several colonies in 0.5 ml of rabbit plasma, incubate the inoculated plasma for one, four, and 24 hours and record the levels of coagulation.

■ For the clumping factor test, suspend colony material in a drop of rabbit plasma on a slide. Macroscopically visible clumping confirms the presence of the factor.

Therapy. Aside from surgical measures, therapy is based on administration of antibiotics. The agents of choice for severe infections are penicillinase-resistant penicillins, since 70–80% of all strains produce penicillinase. These penicillins are, however, ineffective against methicillin-resistant strains, and this resistance applies to all betalactams.

Epidemiology and prevention. *S. aureus* is a frequent colonizer of skin and mucosa. High carrier rates (up to 80%) are the rules among hospital patients and staff. The principle localization of colonization in these persons is the anterior nasal mucosa area, from where the bacteria can spread to hands or with dust into the air and be transmitted to susceptible persons.

S. aureus is frequently the causal pathogen in nosocomial infections (see p. 343f.). Certain strains are known to cause hospital epidemics. Identification of the epidemic strain requires differentiation of relevant infection isolates from other ubiquitous strains. Lysotyping (see p. 186) can be used for this purpose, although use of molecular methods to identify genomic DNA "fingerprints" is now becoming more common.

The most important preventive measure in hospitals is washing the hands thoroughly before medical and nursing procedures. Intranasal application of antibiotics (mupirocin) is a method of reducing bacterial counts in carriers.

Coagulase-Negative Staphylococci (CNS)

CNS are an element in the normal flora of human skin and mucosa. They are classic opportunists that only cause infections given a certain host disposition.

■ ***S. epidermidis.*** This is the pathogen most frequently encountered in CNS infections (70–80% of cases). CNS cause mainly foreign body infections. Examples of the foreign bodies involved are intravasal catheters, continuous ambulant peritoneal dialysis (CAPD) catheters, endoprostheses, metal plates and screws in osteosynthesis, cardiac pacemakers, artificial heart valves, and shunt valves. These infections frequently develop when foreign bodies in the macroorganism are covered by matrix proteins (e.g., fibrinogen, fibronectin) to which the staphylococci can bind using specific cell wall proteins. They then proliferate on the surface and produce a polymeric substance—the basis of the developing biofilm. The staphylococci within the biofilm are protected from antibiotics and the immune system to a great extent. Such biofilms can become infection foci from which the CNS enter the bloodstream and cause sepsislike illnesses. Removal of the foreign body is often necessary.

■ ***S. saprophyticus*** is responsible for 10–20% of acute urinary tract infections, in particular dysuria in young women, and for a small proportion of cases of nonspecific urethritis in sexually active men.

Antibiotic treatment of CNS infections is often problematic due to the multiple resistance often encountered in these staphylococci, especially *S. hemolyticus*.

Streptococcus and Enterococcus

■ **Streptococci** are Gram-positive, **nonmotile**, catalase-negative, facultatively anaerobic cocci that occur in chains or pairs. They are classified based on their hemolytic capacity (α-, β-, γ-hemolysis) and the antigenicity of a carbohydrate occurring in their cell walls (Lancefield antigen).

β-hemolytic group A streptococci (*S. pyogenes*) cause infections of the upper respiratory tract and invasive infections of the skin and subcutaneous connective tissue. Depending on the status of the immune defenses and the genetic disposition, this may lead to scarlet fever and severe infections such as necrotizing fasciitis, sepsis, or septic shock. Sequelae such as acute rheumatic fever and glomerulonephritis have an autoimmune pathogenesis. The α-hemolytic pneumococci (*S. pneumoniae*) cause infections of the respiratory tract. Penicillins are the antibiotics of choice. Resistance to penicillins is

known among pneumococci, and is increasing. Laboratory diagnosis involves pathogen detection in the appropriate material. Persons at high risk can be protected from pneumococcal infections with an active prophylactic vaccine containing purified capsular polysaccharides. Certain oral streptococci are responsible for dental caries. Oral streptococci also cause half of all cases of endocarditis.

Although **enterococci** show only low levels of pathogenicity, they frequently cause nosocomial infections in immunocompromised patients (usually as elements of a mixed flora).

Streptococci are round to oval, Gram-positive, nonmotile, nonsporing bacteria that form winding chains (streptos [greek] = twisted) or diplococci. They do not produce catalase. Most are components of the normal flora of the mucosa. Some can cause infections in humans and animals.

Classification. The genera *Streptococcus* and *Enterococcus* comprise a large number of species. Table 4.**2** lists the most important.

α-, β-, γ-hemolysis.

α-hemolysis. Colonies on blood agar are surrounded by a green zone. This "greening" is caused by H_2O_2, which converts hemoglobin into methemoglobin.

β-hemolysis. Colonies on blood agar are surrounded by a large, yellowish hemolytic zone in which no more intact erythrocytes are present and the hemoglobin is decomposed.

γ-hemolysis. This (illogical) term indicates the absence of macroscopically visible hemolytic zones.

Lancefield groups. Many streptococci and enterococci have a polymeric carbohydrate (C substance) in their cell walls called the Lancefield antigen. They are classified in Lancefield groups A-V based on variations in the antigenicity of this antigen.

Specific characteristics of enterococci that differentiate them from streptococci include their ability to proliferate in the presence of 6.5% NaCl, at 45 °C and at a pH level of 9.6.

Table 4.2 The Most Important Human Pathogen Streptococci and Enterococci

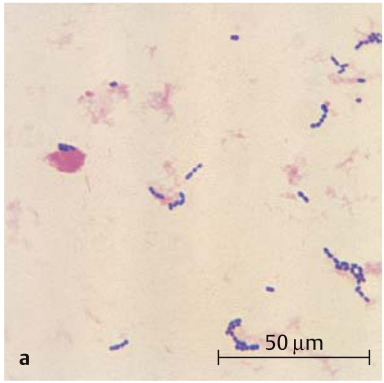
Species	Hemolysis	Group antigen	Remarks
Pyogenic, hemolytic streptococci			
Streptococcus pyogenes (A streptococci)	β	A	Frequent pathogen in humans; invasive infections, sequelae
S. agalactiae (B streptococci)	β	B	Meningitis/sepsis in neonates; invasive infections in predisposed persons
C streptococci	β(α; γ)	C	Rare; purulent infections (similar to *S. pyogenes* infections)
G streptococci	β	G	Rare; purulent infections (similar to *S. pyogenes* infections)
S. pneumoniae	α	–	Pneumococci; respiratory tract infections; sepsis; meningitis
S. bovis	α; γ	D	Not enterococci, although in group D; rare sepsis pathogen; if isolated from blood work up for pathological colon processes
Oral streptococci (selection)			
S. salivarius *S. sanguis* *S. mutans* *S. mitis* *S. anginosus* *S. constellatus* *S. intermedius* etc.	α; γ *S. milleri* group	A, C, E, F, G, H, K occasionally detectable	Greening (viridans) streptococci; occur in oral cavity; endocarditis; caries (*S. mutans*, *S. sanguis*, *S. mitis*) Purulent abscesses
Enterococci (Enterococcus)			
E. faecalis	α; γ; β	D	Occur in human and animal intestines; low-level pathogenicity; endocarditis; nosocomial infections. Often component of mixed florae.
E. faecium	α	D	

Streptococcus pyogenes (A Streptococci)

Morphology and culturing. Gram-positive cocci with a diameter of 1 µm that form chains (Fig. 4.**3a**). Colonies on blood agar (Fig. 4.**3b**) show β-hemolysis caused by streptolysins (see below).

Fine structure. The murein layer of the cell wall is followed by the serogroup A carbohydrate layer, which consists of C substance and is covalently bound to the murein. Long, twisted protein threads that extend outward are anchored in the cell wall murein: the M protein. A streptococci are classified in serovars with characteristic M protein chemistry. Like the hyaluronic acid capsules seen in some strains, the M protein has an antiphagocytic effect.

Streptococcus Pyogenes



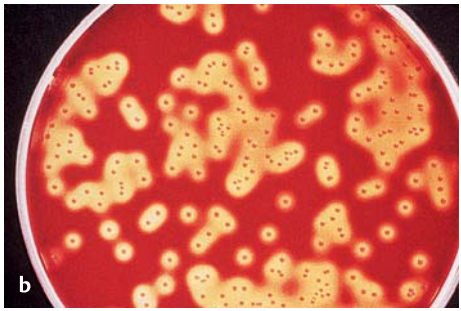


Fig. 4.**3** **a** Gram staining of pleural puncture biopsy material: gram-positive cocci in twisted chains. **b** Culture on blood agar: small, whitish-gray colonies surrounded by large β-hemolysis zones; a 5% CO_2 atmosphere provides optimum conditions for β-hemolysis.

Extracellular toxins and enzymes. The most important in the context of pathogenicity are:

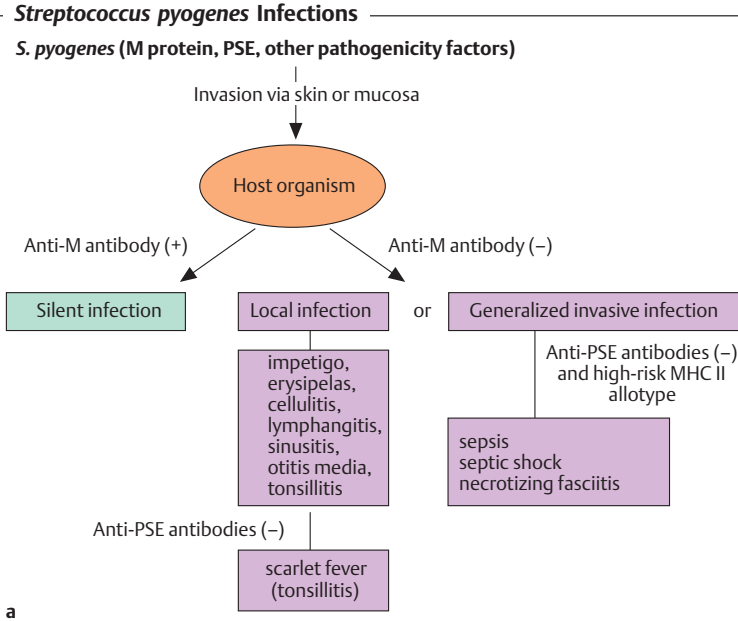
- **Streptolysin O, streptolysin S.** Destroy the membranes of erythrocytes and other cells. Streptolysin O acts as an antigen. Past infections can be detected by measuring the antibodies to this toxin (antistreptolysin titer).
- **Pyrogenic streptococcal exotoxins** (PSE) A, B, C. Responsible for fever, scarlet fever exanthem and enanthem, sepsis, and septic shock. The pyrogenic exotoxins are superantigens and therefore induce production of large amounts of cytokines (p. 77).
- **Streptokinase.** Dissolves fibrin; facilitates spread of streptococci in tissues.
- **Hyaluronidase.** Breaks down a substance that cements tissues together.
- **DNases.** Breakdown of DNA, producing runny pus.

Pathogenesis and clinical pictures. Streptococcal diseases can be classified as either acute, invasive infections or sequelae to them.

- **Invasive infections.** The pathogens enter through traumas or microtraumas in the skin or mucosa and cause invasive local or generalized infections (Fig. 4.**4**). The rare cases of severe septic infection and necrotizing fasciitis occur in persons with a high-risk MHC II allotype. In these patients, the PSE superantigens (especially PSEA) induce large amounts of cytokine by binding at the same time to the MHC II complex and the β chain of the T cell receptor. The excess cytokines thus produced are the cause of the symptoms.
- **Sequelae.** Glomerulonephritis is an immune complex disease (p. 113) and acute rheumatic fever may be a type II immune disease (p. 109).

Diagnosis. What is involved in diagnosis is detection of the pathogen by means of microscopy and culturing. Group A antigen can be detected using particles coated with antibodies that precipitate agglutination (latex agglutination, coagglutination). Using these methods, direct detection of A streptococci in tonsillitis is feasible in the medical practice. However, this direct detection method is not as sensitive as the culture. Differentiation of A streptococci from other β-hemolytic streptococci can be realized in the laboratory with the bacitracin disk test, because A streptococci are more sensitive to bacitracin than the other types.

Therapy. The agents of choice are penicillin G or V. Resistance is unknown. Alternatives are oral cephalosporins or macrolide antibiotics, although resistance to the latter can be expected. In treatment of septic shock, a polyvalent immunoglobulin is used to inactivate the PSE.



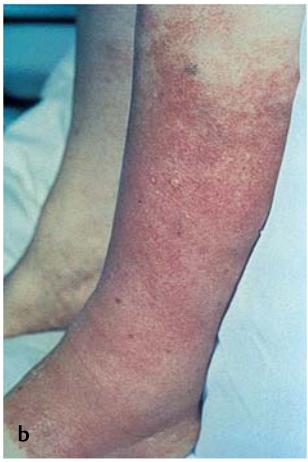


Fig. 4.4 **a** Pathogenesis and clinical pictures of *S. pyogenes* infections (simplified scheme). **b** Erysipelas caused by *S. pyogenes*.

Epidemiology and prophylaxis. Infection frequency varies according to geographical area, season, and age. Humans are the only pathogen reservoir for *S. pyogenes*. Transmission is by direct contact (smear infection) or droplets. The incubation period is one to three days. The incidence of carriers among children is 10–20%, but can be much higher depending on the epidemiological situation. Carriers and infected persons are no longer contagious 24 hours after the start of antibiotic therapy. Microbiological follow-up checks of patients and first-degree contacts are not necessary (exception: rheumatic history).

In persons with recurring infections or with acute rheumatic fever in their medical histories, continuous penicillin prophylaxis with a long-term penicillin is appropriate (e.g., 1.2 million IU benzathine penicillin per month).

Streptococcus pneumoniae (Pneumococci)

Morphology and culturing. Pneumococci are Gram-positive, oval to lancet-shaped cocci that usually occur in pairs or short chains (Fig. 4.**5a**). The cells are surrounded by a thick capsule.

When cultured on blood agar, *S. pneumoniae* develop α-hemolytic colonies with a mucoid (smooth, shiny) appearance (hence "S" form, Fig. 4.**5b**). Mutants without capsules produce colonies with a rough surface ("R" form).

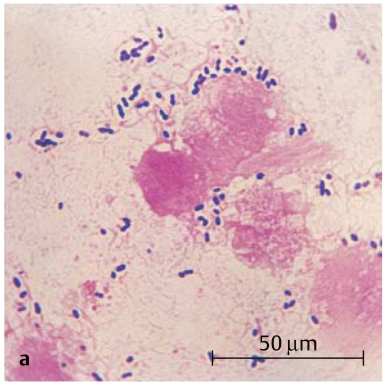
Antigen structure. Pneumococci are classified in 90 different serovars based on the fine chemical structure of the capsule polysaccharides acting as antigens. This capsule antigen can be identified using specific antisera in a reaction known as capsular swelling.

Pathogenesis and clinical pictures. The capsule protects the pathogens from phagocytosis and is the most important determinant of pneumococcal virulence. Unencapsulated variants are not capable of causing disease. Other potential virulence factors include pneumolysin with its effects on membranes and an IgA_1 protease.

The natural habitat of pneumococci is provided by the mucosa of the upper respiratory tract. About 40–70% of healthy adults are carriers. Pneumococcal infections usually arise from this normal flora (endogenous infections). Predisposing factors include primary cardiopulmonary diseases, previous infections (e.g., influenza), and extirpation of the spleen or complement system defects.

The most important pneumococcal infections are **lobar pneumonia** and **bronchopneumonia**. Other infections include acute exacerbation of chronic bronchitis, otitis media, sinusitis, meningitis, and corneal ulcer. Severe pneumococcal infections frequently involve sepsis.

Streptococcus pneumoniae



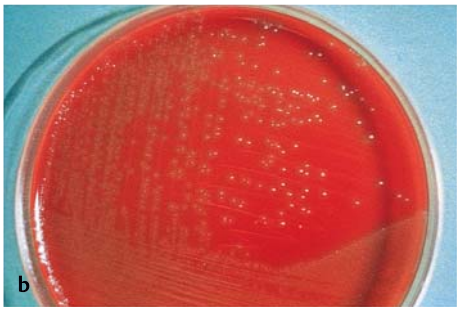


Fig. 4.5 **a** Gram staining of a preparation of middle ear secretion: gram-positive, round-oval, encapsulated cocci; clinical diagnosis: otitis media.
b Culture on blood agar: gray colonies showing little intrinsic color, often mucoid (due to capsules); a zone of greening is often observed around the colonies, caused by α-hemolysis; the shiny appearance of the colonies is caused by light reflections from their mucoid surface.

Diagnosis. The laboratory diagnosis includes detection of the pathogen in appropriate test samples by means of microscopy and culturing. Pneumococci can be differentiated from other α-hemolytic streptococci based on their greater sensitivity to optochin (ethyl hydrocuprein hydrochloride) in the disk test or their bile solubility. Bile salts increase autolysis in pneumococci.

Therapy. Penicillin is still the antibiotic of choice. There have been reports of high-frequency occurrence of strains resistant to penicillin (South Africa, Spain, Hungary, USA). These strains are still relatively rare in Germany, Switzerland, and Austria (5–10%). Macrolide antibiotics are an alternative to penicillins, but resistance to them is also possible.

Penicillin resistance is not due to penicillinase, but rather to modified penicillin-binding proteins (PBPs) to which penicillins have a lower level of affinity. PBPs are required for murein biosynthesis. Biochemically, penicillin re-

sistance extends to cephalosporins as well. However, certain cephalosporins (e.g., ceftriaxone) can be used against penicillin-resistant pneumococci due to their higher levels of activity.

Epidemiology and prophylaxis. Pneumococcal infections are endemic and occur in all seasons, more frequently in the elderly. Humans are the natural pathogen reservoir.

The vaccine product Pneumovax® is available for immunization purposes. It contains 25 mg of the purified capsule polysaccharides of each of 23 of the most frequent serovars. Eighty to ninety percent of all isolated pneumococci have antigens contained in this vaccine, which is primarily indicated in persons with predisposing primary diseases. There is also a seven-valent conjugate vaccine that is effective in children under two years of age (p. 33). Exposure prophylaxis is not necessary.

Streptococcus agalactiae (B Streptococci)

B streptococci occasionally cause infections of the skin and connective tissues, sepsis, urinary tract infections, pneumonia, and peritonitis in immunocompromised individuals. About one in 1000 neonates suffers from a sepsis with or without meningitis. These infections manifest in the first days of life (early onset type) or in the first weeks of life (late onset type). In the early onset form, the infection is caused intra partum by B streptococci colonizing the vagina. Potential predisposing factors include birth complications, premature birth, and a lack of antibodies to the capsule in mother and neonate.

Oral Streptococci

Most of the oral streptococci of the type often known as the viridans group have no group antigen. They usually cause α-hemolysis, some γ-hemolysis as well.

Oral streptococci are responsible for 50–70% of all cases of bacterial **endocarditis**, overall incidence of which is one to two cases per 100 000 annually. The origins of endocarditis lie in invasion of the vascular system through lesions in the oral mucosa. A transitory bacteremia results. The heart valves are colonized and a biofilm is formed by the organism. Predisposing factors include congenital heart defects, acute rheumatic fever, cardiac surgery, and scarred heart valves. Laboratory diagnosis of endocarditis involves isolation of the pathogen from blood cultures. Drug therapy of endocarditis is carried out with either penicillin G alone or combined with an aminoglycoside (mostly gentamicin). Bactericidal activity is the decisive parameter.

Pronounced Dental Caries

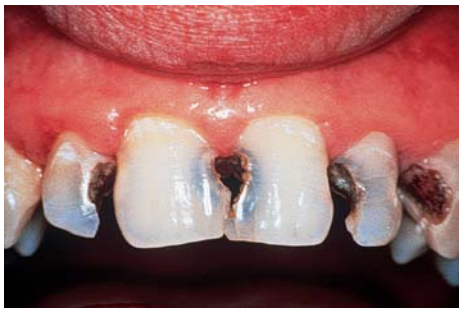


Fig. 4.**6** Certain oral streptococci (*S. mutans*) are the main culprits in tooth decay.

S. mutans, *S. sanguis*, and *S. mitis* are, besides *Actinomyces viscosus* and *A. naeslundii*, responsible for **dental caries** (Fig. 4.**6**). These streptococci can attach to the proteins covering the tooth enamel, where they then convert sucrose into extracellular polysaccharides (mutan, dextran, levan). These sticky substances, in which the original bacterial layer along with secondary bacterial colonizers are embedded, form dental plaque. The final metabolites of the numerous plaque bacteria are organic acids that breach the enamel, allowing the different caries bacteria to begin destroying the dentin.

Enterococcus (Enterococci)

Enterococci are a widespread bacterial genus (p. 220) normally found in the intestines of humans and other animals. They are nonmotile, catalase-negative, and characterized by group antigen D. They are able to proliferate at 45 °C, in the presence of 6.5 % NaCl and at pH 9, qualities that differentiate them from streptococci. As classic opportunists, enterococci show only low levels of pathogenicity. However, they are frequently isolated as components of a mixed flora in nosocomial infections (p. 343). Ninety percent of such isolates are identified as *E. faecalis*, 5–10 % as *E. faecium*. Among the most dangerous enterococcal infections is endocarditis, which must be treated with a combination of an aminopenicillin and streptomycin or gentamicin. Therapeutic success depends on the bactericidal efficacy of the combination used. The efficacy level will be insufficient in the presence of high levels of resistance to either streptomycin (MIC >1000 mg/l) or gentamicin (MIC >500 mg/l) or resistance to the aminopenicillin. Enterococci frequently develop resistance to antibiotics. Strains manifesting multiple resistance are found mainly in hospitals, in keeping with the classic opportunistic

character of these pathogens. Recently observed epidemics on intensive care wards involved strains that were resistant to all standard anti-infective agents including the glycopeptides vancomycin and teicoplanin.

Gram-Positive, Anaerobic Cocci

Gram-positive, strictly anaerobic cocci are included in the genera *Peptococcus* and *Peptostreptococcus*. The only species in the first genus is *Peptococcus niger*, whereas the latter comprises a number of species. The anaerobic cocci are commonly observed in normal human flora. In a pathogenic context they are usually only encountered as components of mixed florae together with other anaerobes or facultative anaerobes. These bacteria invade tissues through dermal or mucosal injuries and cause subacute purulent infections. Such infections are either localized in the head area (cerebral abscess, otitis media, mastoiditis, sinusitis) or lower respiratory tract (necrotizing pneumonia, pulmonary abscess, empyema). They are also known to occur in the abdomen (appendicitis, peritonitis, hepatic abscess) and female genitals (salpingitis, endometriosis, tubo-ovarian abscess). Gram-positive anaerobic cocci may also contribute to soft-tissue infections and postoperative wound infections. See p. 317ff. for clinical details of anaerobe infections.

Bacillus

■ The natural habitat of *Bacillus anthracis*, a Gram-positive, sporing, obligate aerobic rod bacterium, is the soil. The organism causes **anthrax** infections in animals. Human infections result from contact with sick animals or animal products contaminated with the spores. Infections are classified according to the portal of entry as dermal anthrax (95% of cases), primary inhalational anthrax, and intestinal anthrax. Sepsis can develop from the primary infection focus. Laboratory diagnosis includes microscopic and cultural detection of the pathogen in relevant materials and blood cultures. The therapeutic agent of choice is penicillin G. ■

The genera *Bacillus* and *Clostridium* belong to the *Bacillaceae* family of sporing bacteria. There are numerous species in the genus *Bacillus* (e.g., *B. cereus*, *B. subtilis*, etc.) that normally live in the soil. The organism in the group that is of veterinary and human medical interest is *Bacillus anthracis*.

Bacillus anthracis (Anthrax)

Occurrence. Anthrax occurs primarily in animals, especially herbivores. The pathogens are ingested with feed and cause a severe clinical sepsis that is often lethal.

Morphology and culturing. The rods are 1 μm wide and 2–4 μm long, nonflagellated, with a capsule made of a glutamic acid polypeptide. The bacterium is readily grown in an aerobic milieu.

Pathogenesis and clinical picture. The pathogenicity of *B. anthracis* results from its antiphagocytic capsule as well as from a toxin that causes edemas and tissue necrosis. Human infections are contracted from diseased animals or contaminated animal products. Anthrax is recognized as an occupational disease.

Dermal, primary inhalational, and intestinal anthrax are differentiated based on the pathogen's portal of entry. In dermal anthrax, which accounts for 90–95% of human *B. anthracis* infections) the pathogens enter through injuries in the skin. A local infection focus similar to a carbuncle develops within two to three days. A sepsis with a foudroyant (highly acute) course may then develop from this primary focus. Inhalational anthrax (bioterrorist anthrax), with its unfavorable prognosis, results from inhalation of dust containing the pathogen. Ingestion of contaminated foods can result in intestinal anthrax with vomiting and bloody diarrheas.

Diagnosis. The diagnostic procedure involves detection of the pathogen in dermal lesions, sputum, and/or blood cultures using microscopic and culturing methods.

Therapy. The antimicrobial agent of choice is penicillin G. Doxycycline (a tetracycline) or ciprofloxacin (a fluoroquinolone) are possible alternatives. Surgery is contraindicated in cases of dermal anthrax.

Epidemiology and prophylaxis. Anthrax occurs mainly in southern Europe and South America, where economic damage due to farm animal infections is considerable. Humans catch the disease from infected animals or contaminated animal products. Anthrax is a classic zoonosis.

Prophylaxis involves mainly exposure prevention measures such as avoiding contact with diseased animals and disinfection of contaminated products. A cell-free vaccine obtained from a culture filtrate can be used for vaccine prophylaxis in high-risk persons.

Clostridium

Clostridia are 3–8 μm long, thick, Gram-positive, sporing rod bacteria that can only be cultured anaerobically. Their natural habitat is the soil. The pathogenicity of the disease-causing species in this genus is due to production of exotoxins and/or exoenzymes. The most frequent causative organism in **anaerobic cellulitis** and **gas gangrene** (clostridial myonecrosis) is *C. perfringens*. **Tetanus** is caused by *C. tetani*. This pathogen produces the exotoxin tetanospasmin, which blocks transmission of inhibitory CNS impulses to motor neurons. **Botulism** is a type of food poisoning caused by the neurotoxins of *C. botulinum*. These substances inhibit stimulus transmission to the motor end plates. **Pseudomembranous colitis** is caused by *C. difficile*, which produces an enterotoxin (A) and a cytotoxin (B). Diagnosis of clostridial infections requires identification of the pathogen (gas gangrene) and/or the toxins (tetanus, botulism, colitis). All clostridia are readily sensitive to penicillin G. Antitoxins are used in therapy of tetanus and botulism and hyperbaric O_2 is used to treat gas gangrene. The most important preventive measure against tetanus is active vaccination with tetanus toxoid.

Occurrence. Clostridia are sporing bacteria that naturally inhabit the soil and the intestinal tracts of humans and animals. Many species are apathogenic saprophytes. Under certain conditions, several species cause gas gangrene, tetanus, botulism, and pseudomembranous colitis.

Morphology and culturing. All clostridia are large, Gram-positive rod bacteria about 1 μm thick and 3–8 μm in length (Fig. 4.**7**). Many cells in older cultures show a Gram-negative reaction. With the exception of *C. perfringens*, clostridia are flagellated. Clostridia sporulate. They are best cultured in an anaerobic atmosphere at 37 °C. *C. perfringens* colonies are convex, smooth, and surrounded by a hemolytic zone. Colonies of motile clostridia have an irregular, ragged edge.

The Pathogens That Cause Gas Gangrene (Clostridial Myonecrosis) and Anaerobic Cellulitis

Pathogen spectrum. The pathogens that cause these clinical pictures include *Clostridium perfringens*, *C. novyi*, *C. septicum*, and *C. histolyticum*. Species observed less frequently include *C. sporogenes*, *C. sordellii*, and *C. bifermentans*. The most frequent causative pathogen in gas gangrene is *C. perfringens*.

Toxins, enzymes. The toxins produced by invasive clostridia show necrotizing, hemolytic, and/or lethal activity. They also produce collagenases,

Clostridium perfringens* and *sporogenes

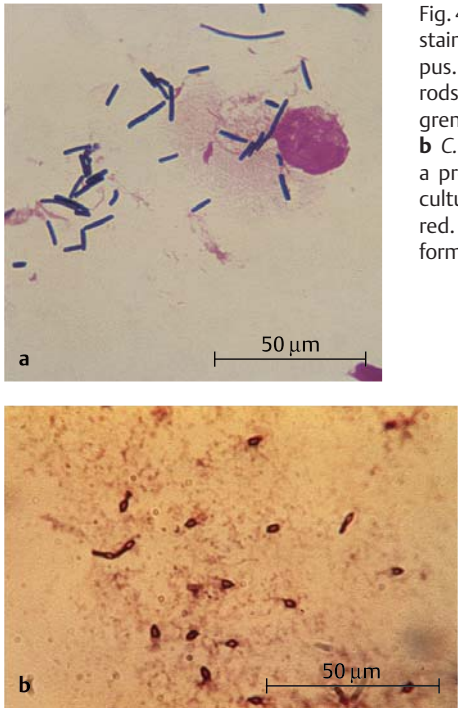


Fig. 4.7 **a** *C. perfringens*: gram staining of a preparation of wound pus. Large, thick, gram-positive rods. Clinical diagnosis: gas gangrene in a gunshot wound.
b *C. sporogenes*: Spore staining of a preparation from an aged broth culture. Thick-walled spores stained red. Occasionally "tennis racquet" forms.

proteinases, DNases, lecithinases, and hyaluronidase, all of which destroy tissue structures, resulting in accumulations of toxic metabolites.

Pathogenesis and clinical picture. Due to the ubiquitous presence of clostridia, they frequently contaminate open wounds, often together with other microorganisms. Detection of clostridia in a wound is therefore no indication of a clostridial infection. These infections develop when a low tissue redox potential makes anaerobe reproduction possible, resulting in tissue necrosis. Two such infections of differing severity are described below:

- **Anaerobic cellulitis.** Infection restricted to the fascial spaces that does not affect musculature. Gas formation in tissues causes a cracking, popping sensation under the skin known as crepitus. There is no toxemia.

Gas gangrene (clostridial myonecrosis). An aggressive infection of the musculature with myonecrosis and toxemia. The incubation period varies from hours to a few days.

Diagnosis. The diagnostic procedure includes identification of the pathogens in relevant materials by means of microscopy and culturing. Identification of anaerobically grown cultures is based on morphological and physiological characteristics.

Therapy. Primary treatment is surgical, accompanied by antibiosis (penicillins, cephalosporins). Treatment with hyperbaric O_2 in special centers has proved effective: patients breathe pure O_2 through a tube or mask in a pressure chamber (3 atm = 303 kPa) several times during two-hour periods.

Epidemiology and prevention. True gas gangrene is now a rare condition. Timely operation of contaminated wounds is the main preventive measure.

Clostridium tetani (Tetanus)

Tetanus (lockjaw) is an acute clostridial disease, its clinical manifestations do not result directly from the invasive infection, but are rather caused by a strong neurotoxin.

Toxin. Tetanospasmin (an AB toxin, p. 16) consists of two polypeptide chains linked by a disulfide bridge. The heavy chain binds specifically to neuron receptors. The light chain is a zinc-metalloprotease that is responsible for proteolysis of components of the neuroexocytosis apparatus in the synapses of the anterior horns of the spinal cord. This stops transmission of inhibitory efferent impulses from the cerebellum to the motor end plates.

Pathogenesis and clinical picture. These ubiquitous pathogens invade tissues following injuries (Fig. 4.**8a**). Given anaerobic conditions, they proliferate and produce the toxin (see above), which reaches the anterior horns of the spinal cord or brain stem via retrograde axonal transport. The clinical picture resulting from the effects of the toxin is characterized by increased muscle tone and spasms induced by visual or acoustic stimuli. The cramps often begin in the facial musculature (risus sardonicus, Fig. 4.**8b**), then spread to neck and back muscles (opisthotonus). The patient remains lucid.

Diagnosis. The preferred method is toxin detection in wound material in an animal test (mouse) based either on neutralization or detection of the toxin gene with PCR. The pathogen is difficult to culture.

Therapy. Antitoxic therapy with immune sera is applied following a meticulous wound cleaning. The patient's musculature must also be relaxed with curare or similar agents.

Tetanus

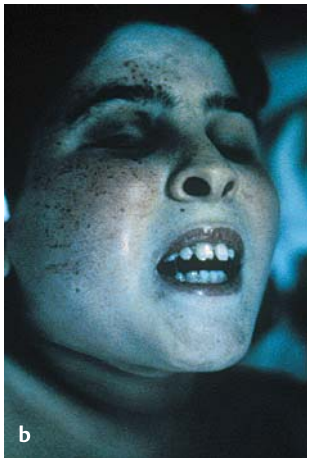


Fig. 4.**8** **a** Open lower-leg fracture following a traffic accident; the portal of entry of *C. tetani*.
b Risus sardonicus: fully manifest case of tetanus in a patient with lower-leg fracture. Patient was not vaccinated.

Epidemiology and prophylaxis. Tetanus is now rare in developed countries due to widespread vaccination practice with incidence rates of approximately one case per million inhabitants per year. The frequency of occurrence is much higher in developing or underdeveloped countries. Worldwide, about 300 000 persons contract tetanus every year, with a lethality rate of approximately 50%. Thus, the importance of the active vaccination as a protective measure can hardly be overstated (see p. 33 for vaccination schedule). A dose of Td should be administered once every 10 years to sustain protection (p. 33). A booster shot is also required in case of severe injury if the patient's last inoculation was administered longer than five years before, and in case of minor injury longer than 10 years. Human tetanus immunoglobulin (250 IU)

must be administered to severely injured persons with insufficient vaccination protection or if the basic immunization history is uncertain.

Clostridium botulinum (Botulism)

Foodborne botulism is not an infection, but rather an intoxication, that is, the toxin is ingested with food. Infant botulism involves ingestion of spores and wound botulism results from infection of a wound.

Toxin. The very strong botulinum neurotoxin is a heat-labile protein. Seven toxigenic types are differentiated, each of which produces an immunologically distinct form of botulinum toxin. Types A, B, and E cause poisoning in humans. The toxin is a metalloprotease that catalyzes the proteolysis of components of the neuroexocytosis apparatus in the motor end plates, resulting in flaccid paralysis of the musculature.

Pathogenesis and clinical picture. Classic botulism results from eating spoiled foods in which the toxin has been produced under anaerobic conditions by *C. botulinum*. The toxin is absorbed in the gastrointestinal tract, and then transported to the peripheral nervous system in the bloodstream.

Within a matter of hours or days paralysis symptoms occur, especially in the nerves of the head. Frequent symptoms include seeing double, difficulty swallowing and speaking, constipation, and dry mucosa. Lethality rates range from 25–70%, depending on the amount of toxin ingested. Death usually results from respiratory paralysis. **Wound botulism** results from wound infection by *C. botulinum* and is very rare. **Infant botulism**, first described in 1976, results from ingestion of spores with food (e.g., honey). Probably due to the conditions prevailing in the intestines of infants up to the age of six months, the spores are able to proliferate there and produce the toxin. The lethality of infant botulism is low (<1%).

Diagnosis. Based on toxin detection by means of the mouse neutralization test.

Therapy. Urgent administration of a polyvalent antitoxin.

Epidemiology and prevention. Botulism is a rare disease. Exposure to the toxin is a food hygiene problem that can be avoided by taking appropriate precautions during food production. Aerosolized botulinum toxin has been used experimentally as a bioweapon.

Clostridium difficile (Pseudomembranous Colitis)

C. difficile occurs in the fecal flora of 1–4% of healthy adults and in 30–50% of children during the first year of life. The factors that lead to development of the disease are not known with certainty. Cases of pseudomembranous colitis are observed frequently under treatment with clindamycin, aminopenicillins, and cephalosporins (hence the designation **antibiotic-associated colitis**), but also occur in persons not taking antibiotics. Occasional outbreaks are seen in hospitals. The **pathological mechanism** is based on formation of two toxins. Toxin A is an enterotoxin that causes a dysfunction characterized by increased secretion of electrolytes and fluids. Toxin B is a cytotoxin that damages the mucosa of the colon.

The **clinical course** includes fever, diarrhea, and spasmodic abdominal pains. Coloscopy reveals edematous changes in the colon mucosa, which is also covered with yellowish-whitish matter. **Laboratory diagnosis** involves culturing the pathogen from patient stool and detection of the cytotoxin in bacteria-free stool filtrates on the basis of a cytopathic effect (CPE) observed in cell cultures, which CPE is then no longer observed after neutralization with an antiserum. Toxins A and B can also be detected with immunological test kits (ELISA tests, see p. 127f.). A specific **therapy** is not required in many cases. Antibiotic treatment is indicated in severe cases. The agent of choice is currently metronidazole.

Listeria, Erysipelothrix, and Gardnerella

■ ***Listeria monocytogenes*** are diminutive Gram-positive rods with peritrichous flagellation that are quite motile at 20 °C and can be cultured aerobically on blood agar. They occur ubiquitously in nature. Human infections may result if 10^6–10^9 pathogens enter the gastrointestinal tract with food. Listeriae are classic opportunists. In immunocompetent persons, an infection will either be clinically silent or present the picture of a mild flu. In immunocompromised patients, the disease manifests as a primary sepsis and/or meningoencephalitis. More rarely, listeriae cause endocarditis. Listeriosis during pregnancy may result in spontaneous abortion or connatal listeriosis (granulomatosis infantiseptica). Penicillins (amoxicillin) and cotrimoxazole, sometimes in combination with aminoglycosides, are used in therapy. Listeriosis is a rare infection characterized by sporadic occurrence. Occasional gastrointestinal epidemics due to contaminated food may result from the coincidence of unfortunate circumstances.

Erysipelothrix rhusiopathiae, the pathogen that causes the zoonosis swine erysipelas, is the causative organism in the human infection erysipeloid, now a rare occupational disease.

Gardnerella vaginalis is usually responsible, in combination with other bacteria, for nonspecific vaginitis (vaginosis).

Listeria monocytogenes

The only listeriae that cause human disease are *L. monocytogenes* and the rare species *L. ivanovii*. The designation *L. monocytogenes* results from the observation that infections of rodents, which are much more susceptible than humans, are accompanied by a monocytosis.

Morphology and culturing. The small Gram-positive rods feature peritrichous flagellation. They show greater motility at 20 °C than at 37 °C. Culturing is most successful under aerobic conditions on blood agar. Following incubation for 18 hours, small gray colonies surrounded by inconspicuous hemolytic zones appear. The zones are caused by listeriolysin O. Listeriae can also reproduce at 5–10 °C, which fact can be used in their selective enrichment ("cold enrichment").

Pathogenesis. Studies of the molecular processes involved have used mainly systemically infected mice.

- **Adherence.** To phagocytic cells (e.g., macrophages) and nonphagocytic cells (e.g., enterocytes).
- **Invasion.** Endocytosis, induced by the protein internalin on the surface of the listeriae. Formation of the endosome.
- **Destruction of the endosome.** The virulence factor listeriolysin forms pores in the endosomal membrane, releasing the listeriae into the cytoplasm.
- **Replication** of the listeriae in the cytoplasm of infected cells.
- **Local intercellular dissemination.** Polymerization of the actin of infected cells at one pole of the listeriae to form so-called actin tails that move the listeriae toward the membrane. Formation of long membrane protuberances (known as listeriopods) containing listeriae. Neighboring cells engulf the listeriopods, whereupon the process of listeria release by means of endosome destruction is repeated.
- **Dissemination is generally** by means of hematogenous spread.

Clinical characteristics. Listeriae are classic opportunists. The course of most infections is clinically silent. Symptoms resembling a mild flu do not occur in immunocompetent persons until large numbers of pathogens (10^6–10^9) enter the gastrointestinal tract with food. Massive infections frequently cause symptoms of gastroenteritis.

Listeriosis can take on the form of a **sepsis** and/or **meningoencephalitis** in persons with T cell defects or malignancies, in alcoholics, during cortisone therapy, during pregnancy, in elderly persons and in infants.

Connatal listeriosis is characterized by sepsis with multiple abscesses and granulomas in many different organs of the infant (**granulomatosis infantiseptica**).

The lethality rate in severe cases of listeriosis varies between 10% and 40%. The incubation period can vary from one to three days to weeks.

Diagnosis requires pathogen identification by means of microscopy and culturing.

Therapy. Amoxicillin, penicillin G, or cotrimoxazole.

Epidemiology and prevention. Listeriae occur ubiquitously in soil, surface water, plants, and animals and are also found with some frequency (10%) in the intestines of healthy humans. Despite the fact that contact with listeriae is, therefore, quite normal and even frequent, listeriosis is not at all common. The incidence of severe infections is estimated at six cases per 10^6 inhabitants per year. Occurrence is generally sporadic. Small-scale epidemics caused by food products—such as milk, milk products (cheese), meat products, and other foods (e.g., coleslaw)—contaminated with very high numbers of listeriae have been described. Preventive measures include proper processing and storage of food products in keeping with relevant hygienic principles.

Erysipelothrix rhusiopathiae

This bacterium is a slender, nonmotile, Gram-positive rod. *E. rhusiopathiae* causes a septic disease in pigs, swine erysipelas. The correlate in humans is now quite rare and is a recognized occupational disease. Following contact with infectious animal material, the pathogens enter body tissues through dermal injuries. After an incubation period of one to three days, the so-called **erysipeloid**—a hivelike, bluish-red swelling—develops at the site of entry. The lymph nodes are also affected. These benign infections often heal spontaneously and disappear rapidly under treatment with penicillin G. Laboratory diagnostic procedures involve identification of the pathogen in wound secretion using the methods of microscopy and culturing.

Gardnerella vaginalis

G. vaginalis is a Gram-variable, nonmotile, nonencapsulated rod bacterium. Its taxonomy has changed repeatedly in recent decades. It has thus also been designated as *Corynebacterium vaginalis* and *Haemophilus vaginalis*. Based on DNA hybridization, the pathogen is now classified with the regularly shaped, Gram-positive, nonsporing rod bacteria. The natural habitat of this organism is the vagina of sexually mature women. It can also cause vulvovaginitis (vaginosis). *G. vaginalis* is found in over 90 % of women showing the symptoms of this infection, usually together with other bacteria including in particular obligate anaerobes (*Mobiluncus*, *Bacteroides*, *Peptostreptococcus*). The organism can be detected in vaginal discharge by means of microscopy and culturing. In the microscopic analysis, so-called clue cells (vaginal epithelia densely covered with Gram-labile rods) provide evidence of the role played by *G. vaginalis*. This bacterium can be cultured on blood-enriched agar incubated in an atmosphere containing 5 % CO_2. The therapeutic agent of choice is metronidazole.

Corynebacterium, Actinomyces, Other Gram-Positive Rod Bacteria

Diphtheria bacteria are pleomorphic, club-shaped rod bacteria that often have polar bodies and group in V, Y, or palisade forms. They can be grown on enriched nutrient media. Their pathogenicity derives from diphtheria toxin, which binds to receptors of sensitive cells with the B fragment. Once the binding process is completed, the active A fragment invades the cell. This substance irreversibly blocks translation in the protein biosynthesis chain. The toxin gene is a component of the β prophage. Local and systemic intoxications are differentiated when evaluating the clinical picture. Local infection usually affects the tonsils, on which the diphtherial pseudomembrane develops. Systemic intoxications affect mainly the liver, kidneys, adrenal glands, cardiac muscle, and cranial nerves. Laboratory diagnosis is based on pathogen identification. The most important treatment is antitoxin therapy. Diphtheria occurs only in humans. Thanks to extensive diphtheria toxoid vaccination programs, it is now rare.

Actinomycetes are part of the normal mucosal flora. These are Gram-positive rods that often occur in the form of branched filaments in young cultures. Conglomerates of microcolonies in pus form so-called sulfur granules. Actinomycetes are obligate anaerobes. The pathogens enter body tissues through mucosa defects. Monoinfections are rare, the most frequent case being actinomycetes-dominated endogenous polyinfections. Cervicofacial

actinomycosis, caused by oral cavity colonizer *A. israelii*, is the most frequent form of actinomycosis. Treatment includes surgical procedures and antibiosis with aminopenicillins.

The group of Gram-positive, irregular (pleomorphic), nonsporing rod bacteria includes many different genera that are normal components of the skin and mucosal flora (Table 4.**3**, p. 261). Pathogens in this group cause two characteristic diseases: diphtheria, caused by *Corynebacterium diphtheriae* and actinomycosis, caused mainly by *Actinomyces israelii*.

Corynebacterium diphtheriae (Diphtheria)

Morphology and culturing. Diphtheria bacteria are Gram-positive, pleomorphic, often club-shaped rods. The individual cells tend to group in V, Y, or palisade arrangements (Fig. 4.**9**). Neisser staining reveals the polar bodies (polyphosphates stored at one end of the rod).

Löffler nutrient medium, which consists of coagulated serum and nutrient broth, is still used for the primary cultures. Selective indicator mediums containing tellurite are used in selective culturing. K tellurite is used to inhibit the accompanying flora. The K tellurite is also reduced to tellurium, coloring the colonies a brownish black.

Extracellular toxin. Diphtheria toxin consists of two functionally distinct fragments, A and B, whereby **B** stands for **binding** to receptors of target cells and **A** stands for toxic **activity**. Fragment A irreversibly blocks protein synthesis translation in the target cells, which then die. The toxin gene is always a prophage genome component (see lysogenic conversion, p. 186).

Corynebacterium diphtheriae

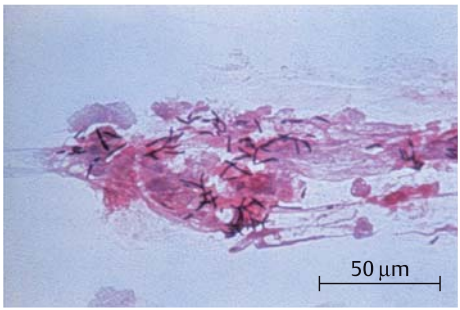


Fig. 4.**9** Gram staining of a wound secretion preparation in wound diphtheria: typical configuration of Gram-positive rods of irregular thickness, often with a clublike enlargement at one end.

Diphtheria toxin

Fragment A is an ADP ribosyl transferase. The enzyme transfers adenosine diphosphate ribose from NAD to the elongation factor eEF2, thereby inactivating it:

$$NAD + eEF2 \rightarrow ADP\ ribosyl\ eEF2 + nicotinamide + H^+$$

eEF2 "translocates" the peptidyl tRNA from the amino acid position A to the peptide position P on the eukaryotic ribosome. Although the toxin gene is integrated in a phage genome, its activity is regulated by the gene product DtxR of the *dtxR* gene of the bacterial cell's genome. DtxR combines with Fe^{2+} to become an active repressor that switches off the transcription of the toxin gene.

4

Pathogenesis and Clinical Picture

Local infection. Infection of the mucosa of tonsils, pharynx, nose, and conjunctiva (Fig. 4.**10**). Wounds and skin lesions can also be infected. The pathogens invade the host through these portals, reproduce, and produce toxin, resulting in local cell damage. The inflammatory reaction leads to collection of a grayish-white exudate, the matrix of the "diphtherial pseudomembrane" consisting of fibrin, dead granulocytes, and necrotic epithelial cells. This coating adheres quite strongly to the mucosa. It may extend into the larynx, thus eventually hindering respiration. Regional lymph nodes are highly swollen.

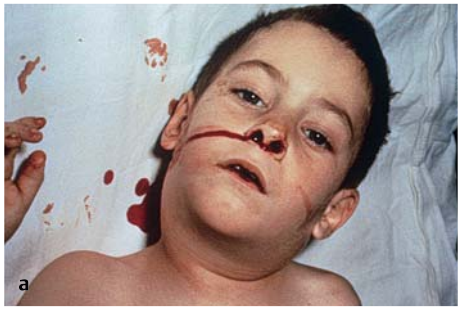
Systemic intoxication. Parenchymal degeneration in the cardiac muscle, liver, kidneys, and adrenal glands. Motor cranial nerve paralysis. Late sequel damage due to the intoxication is frequently seen after the acute infection has subsided.

Toxin-negative strains of *C. diphtheriae* are occasionally observed as pathogens in endocarditis or dermal infections. The pathogenicity of such strains corresponds to that of commensal corynebacteria (see Table 4.**3**, p. 261).

Diagnosis. The method of choice is detection and identification of the pathogen in cultures from local infection foci. The culture smear, which arrives at the laboratory in transport medium, is plated out on Löffler medium and a selective indicator medium. Identification is based on both morphological and physiological characteristics. The toxin is detected by the Elek-Ouchterlony immunodiffusion test. A molecular method is now also being used to identify the toxin gene. Toxin detection is necessary for a laboratory diagnosis of diphtheria because of the occurrence of toxin-negative strains.

Therapy. Antitoxic serum therapy is the primary treatment and it must commence as soon as possible if diphtheria is suspected. This treatment is supplemented by administration of penicillin or erythromycin.

Nose and Throat (Nasopharyngeal) Diphtheria



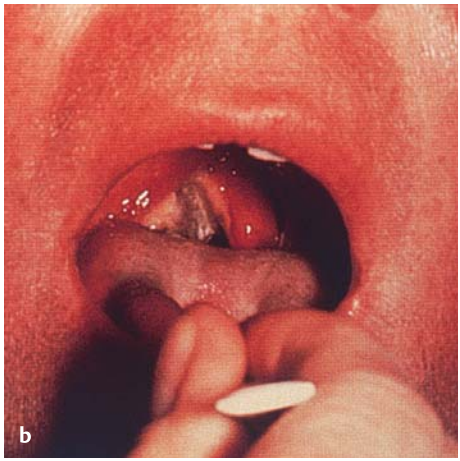


Fig. 4.**10** **a** Hemorrhaging of the nasal mucosa (endothelial damage). Pronounced cervical adenopathy and swelling, creating a bull neck appearance. **b** Thick coating (membrane) on highly swollen tonsils (so-called diphtherial pseudomembrane), causing respiratory stridor.

Epidemiology and prevention. Humans are the sole *pathogen reservoir* for diphtheria. *Infection sources* include infected persons and carriers (rare). The disease is usually *transmitted* by droplet infection, or less frequently indirectly via contaminated objects. The *incubation period* is two to five days. Incidence levels in central Europe are low. From 1975 to 1984, only 113 cases were reported in Germany. Incidence levels are higher in other countries (Russia). *Protective immunization* with diphtheria toxoid is the most important preventive measure (see Table 1.**13**, p. 33). *Exposure prophylaxis* involves isolation of infected persons until two cultures from specimens taken at least 24 hours apart are negative.

Actinomyces

Actinomycetes are Gram-positive bacteria that tend to grow in the form of branched filaments. The resulting mycelial masses are, however, not observed in older cultures, which strongly resemble those of corynebacteria in their morphology.

Occurrence. Actinomycetes are part of the normal mucosal flora in humans and animals. They colonize mainly the oral cavity, and an actinomycosis infection is therefore always endogenous. Ninety percent of actinomycetes infections in humans are caused by *A. israelii*, with far fewer cases caused by *A. naeslundii* and other species.

Morphology and culture. Actinomycetes are Gram-positive, pleomorphic rod bacteria that sometimes also show genuine branching (Fig. 4.**11**). The yellowish **sulfur granules**, measuring 1–2 mm, can be observed macroscopically in actinomycetes pus. These particles are conglomerates of small *Actinomyces* colonies surrounded by a wall of leukocytes. Mycelial filaments extend radially from the colonies (actinium = Greek for raylike). Culturing the organism requires enriched mediums and an anaerobic milieu containing 5–10% CO_2. Mycelial microcolonies form only during the first days. Whitish macrocolonies, often with a rough surface, begin to appear after two weeks.

Pathogenesis and clinical picture. The pathogens breach mucosa (perhaps normal dermis as well) and are able to establish themselves in tissue in the presence of a low redox potential. The factors responsible for these conditions include poor blood perfusion and, above all, contributing bacterial

Actinomyces israelii

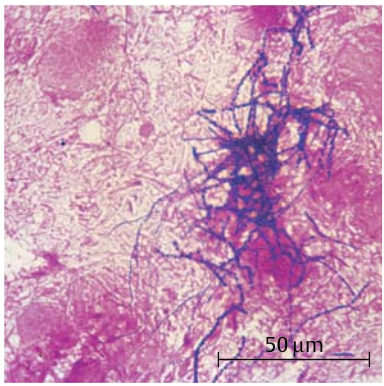


Fig. 4.**11** Gram staining of a pus preparation in cervicofacial actinomycosis: mass of Gram-positive, branched rods; next to them mixed Gram-negative flora. Tentative clinical diagnosis: actinomycosis.

pathogens. Genuine actinomycoses are actually always polymicrobial. The mixed flora found includes mainly the anaerobes of the oral cavity. *Actinobacillus actinomycetemcomitans* is frequently isolated along with various species of *Bacteroidaceae*. Facultative anaerobes such as staphylococci, streptococci, and *Enterobacteriaceae* are, however, also found among the contributing flora.

- **Cervicofacial actinomycosis.** This is the most frequent form of actinomycetes infection (>90%). The abscesses are hard and tumorlike at first, then they necrotize. They may also break through to the dermal surface to create fistulae.

- **Thoracic actinomycosis.** This rare form results from aspiration of saliva; sometimes this type also develops from an actinomycosis in the throat or hematogenous spread.

- **Abdominal actinomycosis.** This type results from injuries to the intestine or female genitals.

- **Genital actinomycosis.** May result from use of intrauterine contraceptive devices.

- **Canaliculitis.** An inflammation of the lacrimal canaliculi caused by any of several *Actinomyces* species.

- **Caries.** The *Actinomyces* species involved in caries development are *A. viscosus*, *A. naeslundii*, and *A. odontolyticus* (p. 243f.). A possible contribution to periodontitis is also under discussion.

Diagnosis involves identification of the pathogen by microscopy and culturing in pus, fistula secretion, granulation tissue, or bronchial secretion. The samples must not be contaminated with other patient flora, in particular from the oral cavity and must be transported to the laboratory in special anaerobe containers. Microscopic detection of branched rods suffices for a tentative diagnosis. Detection of mycelial microcolonies on enriched nutrient mediums after one to two weeks further consolidates this diagnosis. Final identification by means of direct immunofluorescence, cell wall analysis, and metabolic analysis requires several weeks.

Therapy. Treatment includes both surgical and antibiotic measures. The antibiotic of choice is an aminopenicillin. Antibiosis that also covers the contributing bacterial pathogens is important.

Epidemiology and prevention. Actinomycoses occur sporadically worldwide. Average morbidity (incidence) levels are between 2.5 and five cases per 100 000 inhabitants per year. Men are infected twice as often as women. Prophylactic considerations are irrelevant due to the endogenous nature of actinomycetes infections.

Other Gram-Positive Rod Bacteria

Table 4.**3** lists bacteria that are rarely involved in infections and normally infect only persons with defective immune defenses. Recent years have seen considerable changes in their classification and nomenclature—still an ongoing process. Many of these bacteria are part of the normal dermal and mucosal flora. They are frequently found in sampled materials as contaminants, but also occasionally cause infections. Some of these bacteria are designated by collective terms such as "diphtheroid rods" or "coryneform bacteria."

Table 4.3 Gram-Positive Rods with (Generally) Low-Level Pathogenicity

Actinomyces pyogenes	Cutaneous and subcutaneous purulent infections.
Arcanobacterium hemolyticum	Purulent dermal infections; pharyngitis?
Corynebacterium ulcerans	Can produce diphtheria toxin and therefore cause diphtherialike clinical symptoms
C. jeikeium	Dermal pathogen. Occasionally isolated from blood, wounds, or intravasal catheters. Often shows multiple antibiotic resistance.
C. xerosis *C. pseudodiphtheriticum*	Rare endocarditis pathogens.
Gordona bronchialis	Colonizes and infects the respiratory tract.
Rhodococcus equi	Infections of the respiratory tract in immunosuppressed persons.
Tsukamurella sp.	Infections of the respiratory tract in immunosuppressed persons; meningitis.
Turicella otitidis	Infections of the ear in predisposed persons.
Propionibacterium acnes *P. granulosum* *P. avidum*	Anaerobic or microaerophilic. Rarely involved in endocarditis. *P. acnes* is thought to be involved in the development of acne.
Eubacterium sp.	Obligate anaerobe. Normal flora of the intestinal tract. Sometimes component of an anaerobic mixed flora.
Tropheryma whipplei (nov. gen.; nov. spec.; formerly *T. whippelii)*	Causal pathogen in Whipple's disease. Culture growth of this organism has not been possible to date. Probable taxonomic classification in proximity to actinomycetes. Little is known about this organism. Rare, chronic systemic disease. Dystrophy of small intestine mucosa (100%). Also involvement of cardiovascular system (55%), respiratory tract (50%), central nervous system (25%), and eyes (10%). Primary clinical symptoms are weight loss, arthralgias, diarrhea, abdominal pain. Microscopic detection and identification in small intestine biopsies, other biopsies or cerebrospinal fluid (PAS staining) or by molecular methods (see p. 216). Cotrimoxazole is the antibiotic agent of choice.
Mobiluncus mulieri *M. curtisii*	Obligate anaerobic. Colonize the vagina; frequently isolated in cases of bacterial vaginosis together with *Gardnerella vaginalis* and other bacteria.

Mycobacterium

■ Mycobacteria are slender rod bacteria that are stained with special differential stains (Ziehl-Neelsen). Once the staining has taken, they cannot be destained with dilute acids, hence the designation acid-fast. In terms of human disease, the most important mycobacteria are the tuberculosis bacteria (TB) *M. tuberculosis* and *M. bovis* and the leprosy pathogen (LB) *M. leprae*.

TB can be grown on lipid-rich culture mediums. Their generation time is 12–18 hours. Initial droplet infection results in **primary tuberculosis**, localized mainly in the apices of the lungs. The primary disease develops with the Ghon focus (Ghon's complex), whereby the hilar lymph nodes are involved as well. Ninety percent of primary infection foci remain clinically silent. In 10% of persons infected, primary tuberculosis progresses to the **secondary** stage (reactivation or organ tuberculosis) after a few months or even years, which is characterized by extensive tissue necrosis, for example pulmonary caverns. The specific immunity and allergy that develop in the course of an infection reflect T lymphocyte functions. The allergy is measured in terms of the tuberculin reaction to check for clinically inapparent infections with TB. Diagnosis of tuberculosis requires identification of the pathogen by means of microscopy and culturing. Modern molecular methods are now coming to the fore in TB detection. Manifest tuberculosis is treated with two to four antituberculé chemotherapeutics in either a short regimen lasting six months or a standard regimen lasting nine months.

In contrast to TB, the LB pathogens do not lend themselves to culturing on artificial nutrient mediums. **Leprosy** is manifested mainly in skin, mucosa, and nerves. In clinical terms, there is a (malignant) lepromatous type leprosy and a (benign) tuberculoid type. Nondifferential forms are also frequent. Humans are the sole infection reservoir. Transmission of the disease is by close contact with skin or mucosa. ■

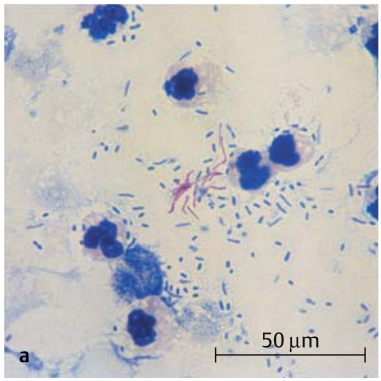
The genus *Mycobacterium* belongs to the *Mycobacteriaceae* family. This genus includes saprophytic species that are widespread in nature as well as the causative pathogens of the major human disease complexes tuberculosis and leprosy. Mycobacteria are Gram-positive, although they do not take gram staining well. The explanation for this is a cell wall structure rich in lipids that does not allow the alkaline stains to penetrate well. At any rate, once mycobacteria have been stained (using radical methods), they resist destaining, even with HCl-alcohol. This property is known as **acid fastness**.

Tuberculosis Bacteria (TB)

History. The tuberculosis bacteria complex includes the species *Mycobacterium tuberculosis*, *M. bovis*, and the rare species *M. africanum*. The clinical etiology of tuberculosis, a disease long known to man, was worked out in 1982 by R. Koch based on regular isolation of pathogens from lesions. Tuberculosis is unquestionably among the most intensively studied of all human diseases. In view of the fact that tuberculosis can infect practically any organ in the body, it is understandable why a number of other clinical disciplines profit from these studies in addition to microbiology and pathology.

Morphology and culturing. TB are slender, acid-fast rods, 0.4 µm wide, and 3–4 µm long, nonsporing and nonmotile. They can be stained with special agents (Ziehl-Neelsen, Kinyoun, fluorescence, p. 212f.) (Fig. 4.**12a**).

Mycobacterium Tuberculosis



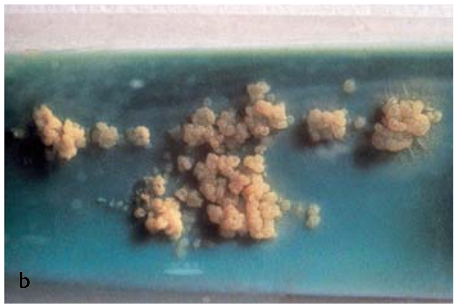


Fig. 4.**12** **a** Ziehl-Neelsen staining of a urine preparation: Fine, red, acid-fast rods, which tend to stick together. Clinical diagnosis: renal tuberculosis.
b Culture of *M. tuberculosis* on egg nutrient substrate according to Löwenstein-Jensen: after four weeks of incubation rough, yellowish, cauliflowerlike colonies.

TB are obligate anaerobes. Their reproduction is enhanced by the presence of 5–10% CO_2 in the atmosphere. They are grown on culture mediums with a high lipid content, e.g., egg-enriched glycerol mediums according to Löwenstein-Jensen (Fig. 4.**12b**). The generation time of TB is approximately 12–18 hours, so that cultures must be incubated for three to six or eight weeks at 37 °C until proliferation becomes macroscopically visible.

Cell wall. Many of the special characteristics of TB are ascribed to the chemistry of their cell wall, which features a murein layer as well as numerous lipids, the most important being the glycolipids (e.g., lipoarabinogalactan), the mycolic acids, mycosides, and wax D.

Glycolipids and wax D.
- Responsible for **resistance** to chemical and physical noxae.
- **Adjuvant effect** (wax D), i.e., enhancement of antigen immunogenicity.
- **Intracellular persistence** in nonactivated macrophages by means of inhibition of phagosome-lysosome fusion.
- **Complement resistance**.
- **Virulence.** Cord factor (trehalose 6,6-dimycolate).

Tuberculoproteins.
- **Immunogens.** The most important of these is the 65 kDa protein.
- **Tuberculin.** Partially purified tuberculin contains a mixture of small proteins (10 kDa). Tuberculin is used to test for TB exposure. Delayed allergic reaction.

Polysaccharides. Of unknown biological significance.

Pathogenesis and clinical picture. It is necessary to differentiate between primary and secondary tuberculosis (reactivation or postprimary tuberculosis) (Fig. 4.**13**). The clinical symptoms are based on reactions of the cellular immune system with TB antigens.

Primary tuberculosis. In the majority of cases, the pathogens enter the lung in droplets, where they are phagocytosed by alveolar macrophages. TB bacteria are able to reproduce in these macrophages due to their ability to inhibit formation of the phagolysosome. Within 10–14 days a reactive inflammatory focus develops, the so-called primary focus from which the TB bacteria move into the regional hilar lymph nodes, where they reproduce and stimulate a cellular immune response, which in turn results in clonal expansion of specific T lymphocytes and attendant lymph node swelling. The Ghon's complex (primary complex, PC) develops between six and 14 weeks after infection. At the same time, granulomas form at the primary infection site and in the affected lymph nodes, and macrophages are activated by the cytokine MAF (macrophage activating factor). A tuberculin allergy also develops in the macroorganism.

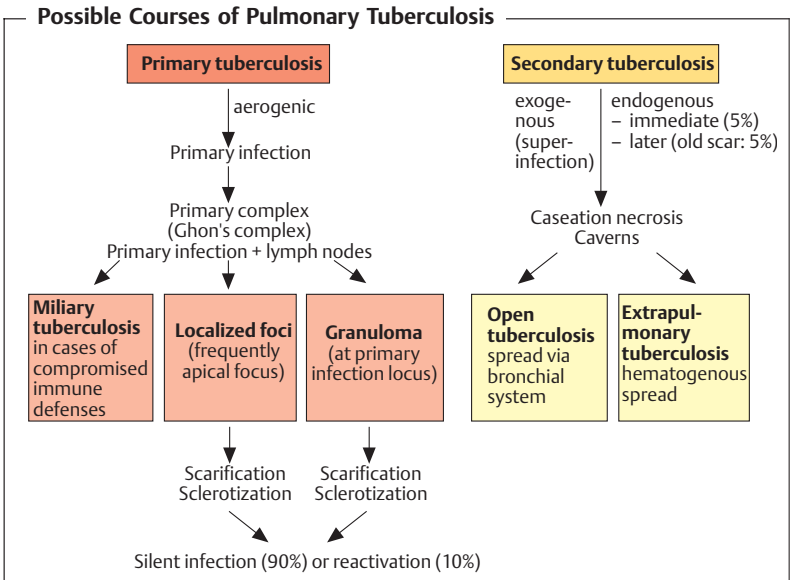


Fig. 4.**13** The primary tuberculosis that develops immediately post infection is clinically silent in most cases thanks to an effective immune response. However, in 10% of cases a so-called secondary tuberculosis develops, either immediately or years later, and may spread to the entire bronchial system or other organ systems.

The further course of the disease depends on the outcome of the battle between the TB and the specific cellular immune defenses. Postprimary dissemination foci are sometimes observed as well, i.e., development of local tissue defect foci at other localizations, typically the apices of the lungs. Mycobacteria may also be transported to other organs via the lymph vessels or bloodstream and produce dissemination foci there. The host eventually prevails in over 90% of cases: the granulomas and foci fibrose, scar, and calcify, and the infection remains clinically silent.

■ **Secondary tuberculosis.** In about 10% of infected persons the primary tuberculosis reactivates to become an organ tuberculosis, either within months (5%) or after a number of years (5%). Exogenous reinfection is rare in the populations of developed countries. Reactivation begins with a caseation necrosis in the center of the granulomas (also called tubercles) that may progress to cavitation (formation of caverns). Tissue destruction is caused by cytokines, among which tumor necrosis factor α (TNFα) appears

to play an important role. This cytokine is also responsible for the cachexia associated with tuberculosis. Reactivation frequently stems from old foci in the lung apices.

The body's immune defenses have a hard time containing necrotic tissue lesions in which large numbers of TB cells occur (e.g., up to 10^9 bacteria and more per cavern); the resulting lymphogenous or hematogenous dissemination may result in infection foci in other organs. Virtually all types of organs and tissues are at risk for this kind of secondary TB infection. Such infection courses are subsumed under the term extrapulmonary tuberculosis.

Immunity. Humans show a considerable degree of genetically determined resistance to TB. Besides this inherited faculty, an organism acquires an (incomplete) specific immunity during initial exposure (first infection). This acquired immunity is characterized by localization of the TB at an old or new infection focus with limited dissemination (Koch's phenomenon). This immunity is solely a function of the T lymphocytes. The level of immunity is high while the body is fending off the disease, but falls off rapidly afterwards. It is therefore speculated that resistance lasts only as long as TB or the immunogens remain in the organism (= infection immunity).

Tuberculin reaction. Parallel to this specific immunity, an organism infected with TB shows an altered reaction mechanism, the tuberculin allergy, which also develops in the cellular immune system only. The tuberculin reaction, positive six to 14 weeks after infection, confirms the allergy. The tuberculin proteins are isolated as purified tuberculin (PPD = purified protein derivative). Five tuberculin units (TU) are applied intracutaneously in the tuberculin test (Mantoux tuberculin skin test, the "gold standard"). If the reaction is negative, the dose is sequentially increased to 250 TU. A positive reaction appears within 48 to 72 hours as an inflammatory reaction (induration) at least 10 mm in diameter at the site of antigen application. A positive reaction means that the person has either been infected with TB or vaccinated with BCG. It is important to understand that a positive test is not an indicator for an active infection or immune status. While a positive test person can be assumed to have a certain level of specific immunity, it will by no means be complete. One-half of the clinically manifest cases of tuberculosis in the population are secondary reactivation tuberculoses that develop in tuberculin-positive persons.

Diagnosis requires microscopic and cultural identification of the pathogen or pathogen-specific DNA.

Traditional method

■ **Workup of test material**, for example with *N*-acetyl-L-cysteine-NaOH (NALC-NaOH method) to liquefy viscous mucus and eliminate rapidly prolif-

erating accompanying flora, followed by centrifugation to enrich the concentration.

- **Microscopy.** Ziehl-Neelsen and/or auramine fluorescent staining (p. 212). This method produces rapid results but has a low level of sensitivity ($>10^4$–10^5/ml) and specificity (acid-fast rods only).

- **Culture** on special solid and in special liquid mediums. Time requirement: four to eight weeks.

- **Identification.** Biochemical tests with pure culture if necessary. Time requirement: one to three weeks.

- **Resistance test** with pure culture. Time requirement: three weeks.

Rapid methods. A number of different rapid TB diagnostic methods have been introduced in recent years that require less time than the traditional methods.

- **Culture.** Early-stage growth detection in liquid mediums involving identification of TB metabolic products with highly sensitive, semi-automated equipment. Time requirement: one to three weeks. Tentative diagnosis.

- **Identification.** Analysis of cellular fatty acids by means of gas chromatography and of mycolic acids by means of HPLC. Time requirement: 12 days with a pure culture.

- **DNA probes.** Used to identify *M. tuberculosis* complex and other mycobacteria. Time requirement: several hours with a pure culture.

- **Resistance test.** Use of semi-automated equipment (see above). Proliferation/nonproliferation determination in liquid mediums containing standard antituberculotic agents (Table 4.**4**). Time requirement: 7–10 days.

Table 4.**4** Scheme for Chemotherapy of Tuberculosis

	Standard scheme	Months	Short scheme *	Months
Initial phase	isoniazid (INH) rifampicin (RMP) ethambutol (EMB)	2	isoniazid rifampicin ethambutol pyrazinamide (PZA)	2
Continuation phase	isoniazid rifampicin	7	isoniazid rifampicin	4

* Alternative in cases of confirmed INH sensitivity or mild clinical picture: initial treatment with a combination of fixed INH + RMP + PZA for two months

Direct identification in patient material. Molecular methods used for direct detection of the *M. tuberculosis* complex in (uncultured) test material. These methods involve amplification of the search sequence.

Therapy. The previous method of long-term therapy in sanatoriums has been replaced by a standardized chemotherapy (see Table 4.**4** for examples), often on an outpatient basis.

Epidemiology and prevention. Tuberculosis is endemic worldwide. The disease has become much less frequent in developed countries in recent decades, where its **incidence** is now about five to 15 new infections per 100 000 inhabitants per year and **mortality** rates are usually below one per 100 000 inhabitants per year. Seen from a worldwide perspective, however, tuberculosis is still a major medical problem. It is estimated that every year approximately 15 million persons contract tuberculosis and that three million die of the disease. The main **source of infection** is the human carrier. There are no healthy carriers. Diseased cattle are not a significant source of infection in the developed world. **Transmission** of the disease is generally direct, in most cases by droplet infection. Indirect transmission via dust or milk (udder tuberculosis in cattle) is the exception rather than the rule. The **incubation period** is four to 12 weeks.

Exposure prophylaxis. Patients with open tuberculosis must be isolated during the secretory phase. Secretions containing TB must be disinfected. Tuberculous cattle must be eliminated.

Disposition prophylaxis. An active vaccine is available that reduces the risk of contracting the disease by about one-half. It contains the live vaccine BCG (lyophilized bovine TB of the Calmette-Guérin type). Vaccination of tuberculin-negative persons induces allergy and (incomplete) immunity that persist for about five to 10 years. In countries with low levels of tuberculosis prevalence, the advisory committees on immunization practices no longer recommend vaccination with BCG, either in tuberculin-negative children at high risk or in adults who have been exposed to TB. Preventive chemotherapy of clinically inapparent infections (latent tuberculosis bacteria infection, LTBI) with INH (300 mg/d) over a period of six months has proved effective in high-risk persons, e.g., contact persons who therefore became tuberculin-positive, in tuberculin-positive persons with increased susceptibility (immunosuppressive therapy, therapy with corticosteroids, diabetes, alcoholism) and in persons with radiologically confirmed residual tuberculosis. Compliance with the therapeutic regimen is a problem in preventive chemotherapy.

Leprosy Bacteria (LB)

Morphology and culture. *Mycobacterium leprae* (Hansen, 1873) is the causative pathogen of leprosy. In morphological terms, these acid-fast rods are identical to tuberculosis bacteria. They differ, however, in that they cannot be grown on nutrient mediums or in cell cultures.

Pathogenesis. The pathomechanisms of LB are identical to those of TB. The host organism attempts to localize and isolate infection foci by forming granulomas. Leprous granulomas are histopathologically identical to tuberculous granulomas. High counts of leprosy bacteria are often found in the macrophages of the granulomas.

Immunity. The immune defenses mobilized against a leprosy infection are strictly of the cellular type. The lepromin skin test can detect a postinfection allergy. This test is not, however, very specific (i.e., positive reactions in cases in

Tuberculoid Leprosy

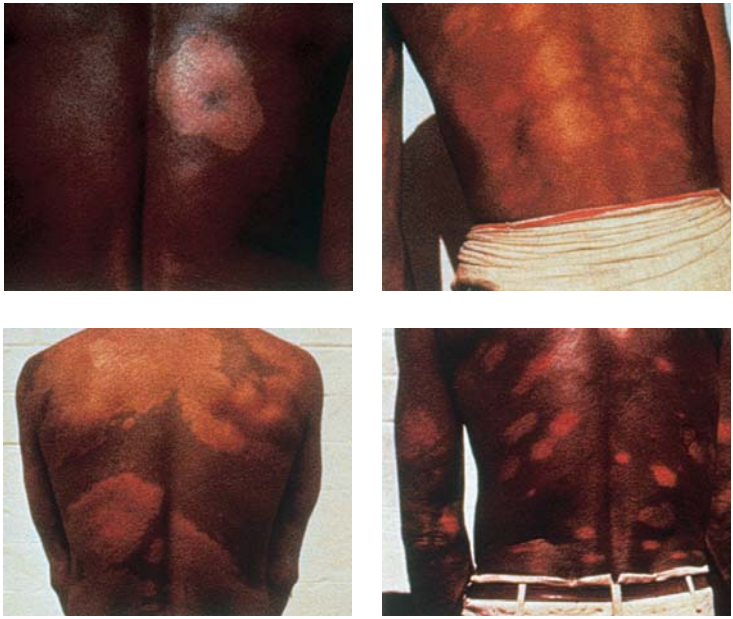
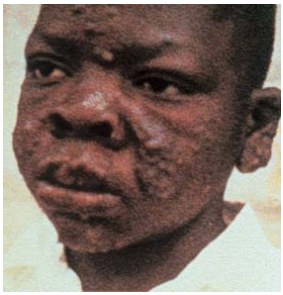
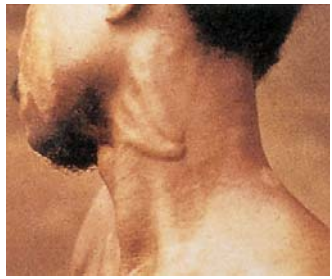


Fig. 4.**14** Tuberculoid leprosy is the benign, nonprogressive form of the disease, characterized by spotty dermal depigmentations.

Lepromatous Leprosy





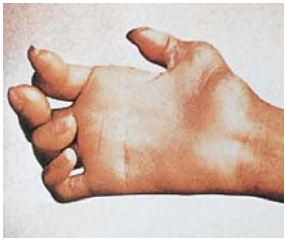


Fig. 4.**15** In lepromatous leprosy, nodular dermal and mucosal lesions develop. Nerve inflammation and neuroparalysis follow, eventually resulting in mutilations.

which no leprosy infection is present). The clinically differentiated infection course forms observed are probably due to individual immune response variants.

Clinical picture. Leprosy is manifested mainly on the skin, mucosa, and peripheral nerves.

A clinical differentiation is made between tuberculoid leprosy (TL, Fig. 4.**14**) and lepromatous leprosy (LL, Fig. 4.**15**). There are many intermediate forms. TL is the benign, nonprogressive form characterized by spotty dermal lesions. The LL form, on the other hand, is characterized by a malignant, progressive course with nodular skin lesions and cordlike nerve thickenings that finally lead to neuroparalysis. The inflammatory foci contain large numbers of leprosy bacteria.

Diagnosis. Detection of the pathogens in skin or nasal mucosa scrapings under the microscope using Ziehl-Neelsen staining (p. 212). Molecular confirmation of DNA sequences specific to leprosy bacteria in a polymerase chain reaction is possible.

Therapy. Paucibacillary forms are treated with dapson plus rifampicin for six months. Multibacillary forms require treatment with dapson, rifampicin, and clofazimine over a period of at least two years.

Epidemiology and prevention. Leprosy is now rare in socially developed countries, although still frequent in developing countries. There are an estimated 11 million victims worldwide. Infected humans are the only source of infection. The details of the transmission pathways are unknown. Discussion of the topic is considering transmission by direct contact with skin or mucosa injuries and aerogenic transmission. The incubation period is 2–5–20 years. Isolation of patients under treatment is no longer required. An effective epidemiological reaction requires early recognition of the disease in contact persons by means of periodical examinations every six to 12 months up to five years following contact.

Nontuberculous Mycobacteria (NTM)

Mycobacteria that are neither tuberculosis nor leprosy bacteria are categorized as atypical mycobacteria (old designation), nontuberculous mycobacteria (NTM) or MOTT (mycobacteria other than tubercle bacilli).

Morphology and culture. In their morphology and staining behavior, NTM are generally indistinguishable from tuberculosis bacteria. With the exception of the rapidly growing NTM, their culturing characteristics are also similar to TB. Some species proliferate only at 30 °C. NTM are frequent inhabitants of the natural environment (water, soil) and also contribute to human and animal mucosal flora. Most of these species show resistance to the antituberculoid agents in common use.

Clinical pictures and diagnosis. Some NTM species are apathogenic, others can cause mycobacterioses in humans that usually follow a chronic course (Table 4.**5**). NTM infections are generally rare. Their occurrence is encouraged by compromised cellular immunity. Frequent occurrence is observed together with certain malignancies, in immunosuppressed patients and in AIDS patients, whereby the NTM isolated in 80% of cases are *M. avium* or *M. intercellulare*. As a rule, NTM infections are indistinguishable from tuberculous lesions in clinical, radiological, and histological terms. Diagnosis therefore requires culturing and positive identification. The clinical significance of a positive result is difficult to determine due to the ubiquitous occurrence of these pathogens. They are frequent culture contaminants. Only about 10% of all persons in whom NTM are detected actually turn out to have a mycobacteriosis.

Therapy. Surgical removal of the infection focus is often indicated. Chemotherapy depends on the pathogen species, for instance a triple combination (e.g., INH, ethambutol, rifampicin) or, for resistant strains, a combination of four or five antituberculoid agents.

Table 4.5 Infections Caused by Nontuberculous Mycobacteria

Disease	Frequent species	Rare species
Chronic pulmonary disease (adults)	*M. kansasii* *M. avium/M. intracellulare (M. avium complex)* *M. abscessus*	*M. malmoense* *M. xenopi* *M. scrofulaceum* *M. fortuitum* *M. chelonae* and others
Local lymphadenitis (children, adolescents)	*M. avium complex*	*M. kansasii,* *M. malmoense* *M. fortuitum*
Skin and soft tissue infections	*M. marinum* *M. fortuitum* *M. chelonae* *M. ulcerans*	*M. haemophilum* *M. smegmatis* *M. hansasii*
Bone, joint, tendon infections	*M. kansasii* *M. avium complex* *M. fortuitum* *M. abscessus*	*M. smegmatis* *M. chelonae* *M. marinum* *M. malmoense*
Disseminated diseases in immunocompromised patients	*M. kansasii* *M. avium complex*	*M. fortuitum* *M. chelonae* *M. genavense* *M. xenopi* and others

Nocardia

Occurrence. The genus *Nocardia* includes species with morphology similar to that of the actinomycetes, differing from them in that the natural habitat of these obligate aerobes is the soil and damp biotopes. The pathogens known for involvement in nocardioses, a generally very rare type of infection, include *N. asteroides*, *N. brasiliensis*, *N. farcinia*, *N. nova*, and *N. otitidiscaviarum*.

Morphology and culture. Nocardia are Gram-positive, fine, pleomorphic rods that sometimes show branching. They can be cultured on standard nutrient mediums and proliferate particularly well at 30 °C. Nocardia are obligate aerobes.

Pathogenesis and clinical picture. Nocardia penetrate from the environment into the macroorganism via the respiratory tract or dermal wounds. An infection develops only in patients with predisposing primary diseases directly

affecting the immune defenses. Monoinfections are the rule. There are no typical clinical symptoms. Most cases of infection involve pyogenic inflammations with central necroses. The following types have been described: **pulmonary nocardioses** (bronchial pneumonia, pulmonary abscess), **systemic nocardioses** (sepsis, cerebral abscess, abscesses in the kidneys and musculature), and **surface nocardioses** (cutaneous and subcutaneous abscesses, lymphocutaneous syndrome).

Actinomycetomas are tumorlike processes affecting the extremities, including bone. An example of such an infection is Madura foot, caused by *Nocardia* species, the related species *Actinomadura madurae*, and *Streptomyces somaliensis*. Fungi (p. 355) can also be a causal factor in this clinical picture.

Diagnosis. Detection of the pathogen by means of microscopy and culturing techniques is required in materials varying with the specific disease. Due to the long generation time of these species, cultures have to be incubated for at least one week. Precise identification to differentiate pathogenic and apathogenic species is desirable, but difficult.

Therapy. The anti-infective agents of choice are sulfonamides and cotrimoxazole. Surgery may be required.

Epidemiology and prevention. Nocardioses are rare infections. Annual incidence levels range from about 0.5 to 1 case per 1 000 000 inhabitants. The pathogens, which are present in the natural environment, are carried by dust to susceptible patients. There are no practicable prophylactic measures.

Neisseria, Moraxella, and Acinetobacter

■ Neisseria are Gram-negative, aerobic cocci that are often arranged in pairs. They are typical mucosal parasites that die rapidly outside the human organism. Culturing on enriched nutrient mediums is readily feasible.

Neisseria gonorrheae is the pathogen responsible for gonorrhea ("clap"). Infection results from sexual intercourse. The organisms adhere to cells of the urogenital tract by means of attachment pili and the protein Opa, penetrate into the organism using parasite-directed endocytosis and cause a pyogenic infection, mainly of the urogenital epithelium. An infection is diagnosed mainly by means of microscopy and culturing of purulent secretions. The therapeutic of choice is penicillin G. Alternatives for use against penicillinase-positive gonococci include third-generation cephalosporins and 4-quinolones.

N. meningitidis is a parasite of the nasopharyngeal mucosa. These meningococci cause meningitis and sepsis. Diagnosis involves detection of the

pathogens in cerebrospinal fluid and blood. The disease occurs sporadically or in the form of minor epidemics in children, youths, and young adults. The antibiotics of choice are penicillin G and third-generation cephalosporins.

The family *Neisseriaceae* includes aerobic, Gram-negative cocci and rods (see Table 3.**9**, p. 222), the most important of which are the human pathogens *N. gonorrheae* and *N. meningitidis*. Other species in the genus *Neisseria* are elements of the normal mucosal flora.

Neisseria gonorrheae (Gonorrhea)

Morphology and culture. Gonococci are Gram-negative, coffee-bean-shaped cocci that are usually paired and have a diameter of approximately 1 µm (Fig. 4.**16**). Attachment pili on the bacterial cell surface are responsible for their adhesion to mucosal cells.

Gonococci can be grown on moist culture mediums enriched with protein (blood). The atmosphere for primary culturing must contain 5–10% CO_2.

Pathogenesis and clinical picture. Gonorrhea is a sexually transmitted disease. The pathogens penetrate into the urogenital mucosa, causing a local purulent infection. In men, the prostate and epididymis can also become infected. In women, the gonococci can also cause salpingitis, oophoritis, or even peritonitis. Gonococci reaching the conjunctival membrane may cause a purulent conjunctivitis, seen mainly in newborn children. Gonococci can also infect the rectal or pharyngeal mucosa. Hematogenously disseminated gonococci may also cause arthritis or even endocarditis.

Determinants of the Pathogenicity of Gonococci

Attachment pili on the surface and the outer membrane protein Opa are responsible for adhesion to cells of the urogenital tract. Opa also directs the invasion process by means of endocytosis. Immune defenses against granulocytes are based on the outer membrane porin Por that prevents the phagosome from fusing with lysosomes, resulting in the survival—and proliferation—of phagocytosed gonococci in granulocytes. The lipo-oligosaccharide (LOS) in the outer membrane is responsible for resistance to complement (serum resistance) as well as for the inflammatory tissue reaction in a manner analogous to the more complexly structured LPS of enterobacteria. Gonococci can capture iron from the siderophilic proteins lactoferrin and transferrin, accumulating it inside the bacterial cells to facilitate their rapid proliferation. An IgA_1 protease produced by the gonococci hydrolyzes secretory antibodies in the mucosal secretions. The pronounced antigen variability of the attachment pili (p. 14) and the Opa protein make it possible for gonococci to thwart specific immune defense mechanisms repeatedly.