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IMMUNOLOGY

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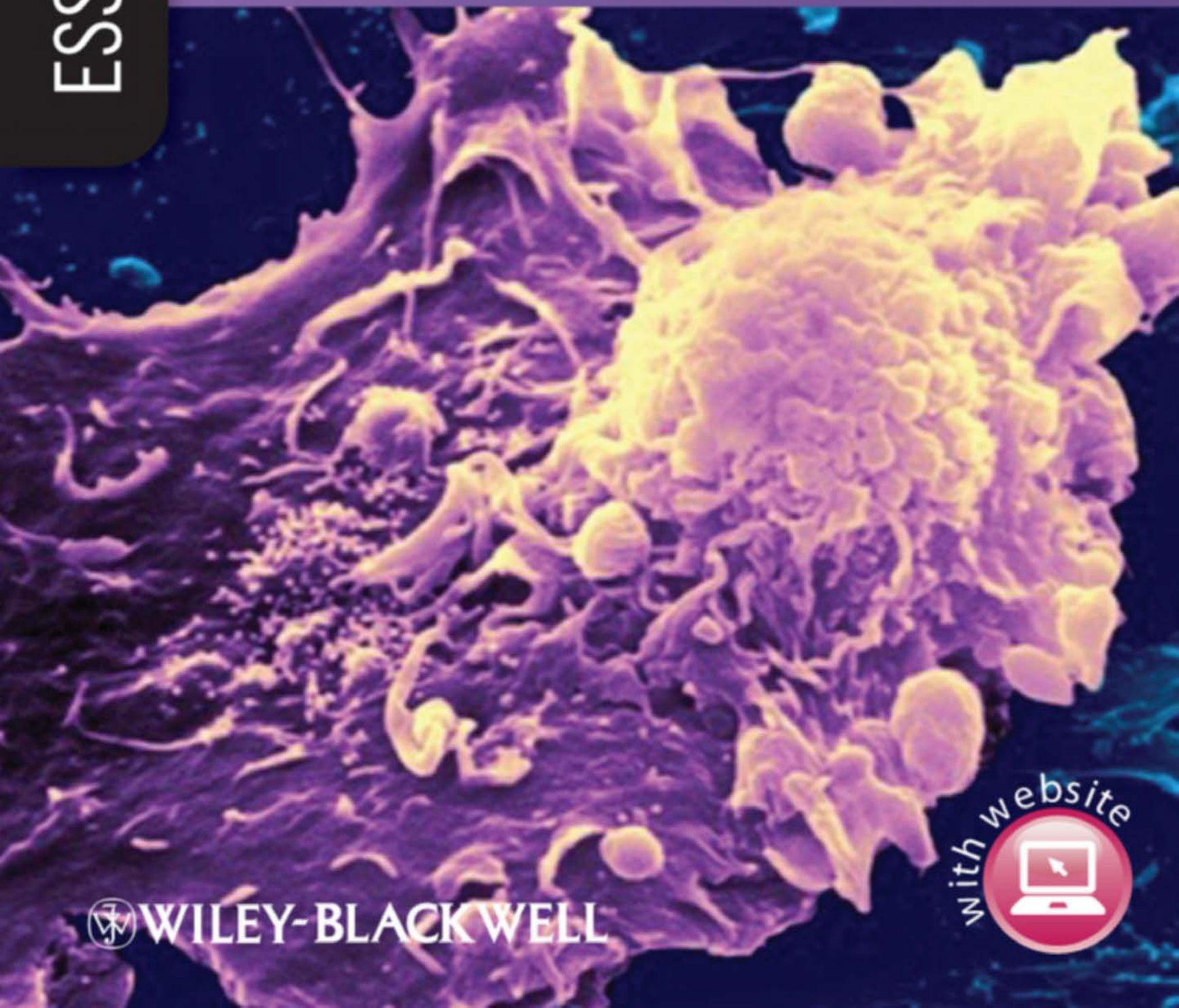

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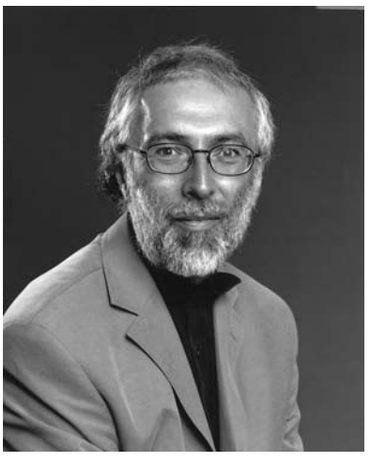


Peter J. Delves

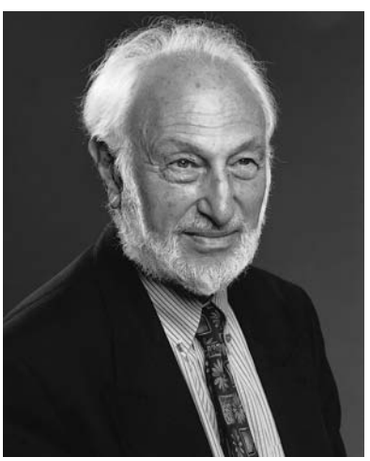
Professor Delves obtained his PhD from the University of London in 1986 and is a Professor of Immunology at UCL (University College London). His research has focused on molecular aspects of antigen recognition. He has authored and edited a number of immunology books, and teaches the subject at a broad range of levels.

Seamus J. Martin

Professor Martin received his PhD from The National University of Ireland in 1990 and trained as a post-doctoral fellow at University College London (with Ivan Roitt) and The La Jolla Institute for Allergy and Immunology, California, USA (with Doug Green). Since 1999, he is the holder of the Smurfit Chair of Medical Genetics at Trinity College Dublin and is also a Science Foundation Ireland Principal Investigator. His research is focused on various aspects of programmed cell death (apoptosis) in the immune system and in cancer and he has received several awards for his work in this area. He has previously edited two books on apoptosis and was elected as a Member of The Royal Irish Academy in 2006 and as a member of The European Molecular Biology Organisation (EMBO) in 2009.

Dennis R. Burton

Professor Burton obtained his BA in Chemistry from the University of Oxford in 1974 and his PhD in Physical Biochemistry from the University of Lund in Sweden in 1978. After a period at the University of Sheffield, he moved to the Scripps Research Institute in La Jolla, California in 1989 where he is Professor of Immunology and Molecular Biology. His research interests include antibodies, antibody responses to pathogens and vaccine design, particularly in relation to HIV.



Ivan M. Roitt

Professor Roitt was born in 1927 and educated at King Edward's School, Birmingham and Balliol College, Oxford. In 1956, together with Deborah Doniach and Peter Campbell, he made the classic discovery of thyroglobulin autoantibodies in Hashimoto's thyroiditis which helped to open the whole concept of a relationship between autoimmunity and human disease. The work was extended to an intensive study of autoimmune phenomena in pernicious anaemia and primary biliary cirrhosis. In 1983 he was elected a Fellow of The Royal Society, and has been elected to Honorary Membership of the Royal College of Physicians and appointed Honorary Fellow of The Royal Society of Medicine.

TWELFTH EDITION

Roitt's Essential Immunology

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- Podcasts to reinforce the key principles explained in the text: ideal for revision 'on the go'

Preface

Welcome to this new edition! When Ivan wrote the first edition some 40 years ago, he wanted to feel that he was chatting to the reader almost informally, rather than preaching, and it has been our intention to maintain this style. As a subject, immunology is exciting and dynamic and to persuade you that it is absolutely worthwhile for you to tackle this new edition we have made very extensive changes to update the previous edition. Accordingly, apart from the introduction of numerous new illustrations, we have:

- Expanded discussion of pathogen- and danger-associated molecular patterns (PAMPs & DAMPs)
- Introduced a new section on dendritic cells and their role in antigen processing including cross-presentation
- Updated sections on B-cell and NK receptors
- Enhanced discussion of lymphocyte trafficking
- Incorporated the latest findings on T-cell subsets, particularly Th17 and the diversity of regulatory T-cells
- Recorded newer information on NK and cytotoxic T-cell killing mechanisms
- Given more insight into the effects of aging on immune responses
- Carried out a major rewrite of the vaccine chapter with new emphasis on mechanisms of action of conventional and carbohydrate vaccines, and new approaches to vaccine development including reverse vaccinology, together with progress in malaria vaccines and adjuvant action
- Provided new information on novel genetic immunodeficiency defects, on the origin of AIDS and the ever-expanding plethora of AIDS drugs plus results from the latest HIV vaccine trials
- Clarified the recent findings on the cellular transformations leading to cancer, the manipulation of the immune system by tumors and the links between infection, inflammation and cancer
- Substantially rewritten the chapter on autoimmune diseases.

It is our fond expectation that you will enjoy and benefit from a reading of our offering.

Peter J. Delves
Seamus J. Martin
Dennis R. Burton
Ivan M. Roitt

Abbreviations

AAV	adeno-associated virus
Ab	antibody
AChR	acetylcholine receptor
ACT	adoptive cell transfer
ACTH	adrenocorticotropic hormone
ADA	adenosine deaminase
ADCC	antibody-dependent cellular cytotoxicity
AEP	asparagine endopeptidase
Ag	antigen
AID	activation-induced cytidine deaminase
AIDS	acquired immunodeficiency syndrome
AIRE	autoimmune regulator
ALBA	addressable laser bead assay
ANCA	antineutrophil cytoplasmic antibodies
APC	antigen-presenting cell
ARRE-1	antigen receptor response element-1
ARRE-2	antigen receptor response element-2
ART	antiretroviral therapy
ASFV	African swine fever virus
AZT	zidovudine (3′-azido-3′-deoxythymidine)
BAFF	B-cell-activating factor of the tumor necrosis factor family
B-cell	lymphocyte which matures in bone marrow
BCG	bacille Calmette–Guérin attenuated form of tuberculosis
BCR	B-cell receptor
BM	bone marrow
BSA	bovine serum albumin
BSE	bovine spongiform encephalopathy
Btk	Bruton's tyrosine kinase
BUDR	bromodeoxyuridine
C	complement
Cα(β/γ/δ)	constant part of TCR α(β/γ/δ) chain
CALLA	common acute lymphoblastic leukemia antigen
cAMP	cyclic adenosine monophosphate
CCP	complement control protein repeat
CD	cluster of differentiation
CDR	complementarity determining regions of Ig or TCR variable portion
CEA	carcinoembryonic antigen
CFA	complete Freund's adjuvant
cGMP	cyclic guanosine monophosphate
ChIP	chromatin immunoprecipitation
CHIP	chemotaxis inhibitory protein
$C_{H(L)}$	constant part of Ig heavy (light) chain
CLA	cutaneous lymphocyte antigen
CLIP	class II-associated invariant chain peptide
CMI	cell-mediated immunity
CML	cell-mediated lympholysis
CMV	cytomegalovirus
Cn	complement component "n"
$C\overline{n}$	activated complement component "n"
iCn	inactivated complement component "n"
Cna	small peptide derived by proteolytic activation of Cn
CpG	cytosine phosphate-guanosine dinucleotide motif
CR(n)	complement receptor "n"
CRP	C-reactive protein
CSF	cerebrospinal fluid
CSR	class switch recombination
CTLR	C-type lectin receptor
D gene	diversity minigene joining V and J segments to form variable region
DAF	decay accelerating factor
DAG	diacylglycerol
DAMP	danger-associated molecular pattern
DC	dendritic cells
DMARD	disease-modifying antirheumatic drug
DNP	dinitrophenyl
DTH	delayed-type hypersensitivity
DTP	diphtheria, tetanus, pertussis triple vaccine
EAE	experimental autoimmune (allergic) encephalomyelitis
EBV	Epstein–Barr virus
ELISA	enzyme-linked immunosorbent assay
EM	electron microscope
Eø	eosinophil
EPO	erythropoietin
ER	endoplasmic reticulum
ES	embryonic stem (cell)
ET	exfoliative toxins
F(B)	factor (B, etc.)
Fab	monovalent Ig antigen-binding fragment after papain digestion
$F(ab')_2$	divalent antigen-binding fragment after pepsin digestion
FasL	Fas-ligand
FACS	fluorescence-activated cell sorter
Fc	Ig crystallisable-fragment originally; now non-Fab part of Ig
FcγR	receptor for IgG Fc fragment
FDC	follicular dendritic cell
flt-3	flk-2 ligand
(sc)Fv	(single chain) V_H–V_L antigen binding fragment
GADS	GRB2-related adapter protein
g.b.m.	glomerular basement membrane
G-CSF	granulocyte colony-stimulating factor

GEFs	guanine-nucleotide exchange factors
GM-CSF	granulocyte–macrophage colony-stimulating factor
gp*n*	*n* kDa glycoprotein
GRB2	growth factor receptor-binding protein 2
GSK3	glycogen synthase kinase 3
g.v.h.	graft versus host
H-2	the mouse major histocompatibility complex
H-2D/K/L	main loci for classical class I (class II)
(A/E)	murine MHC molecules
HAMA	human antimouse antibodies
HATA	human anti-toxin antibody
HBsAg	hepatitis B surface antigen
hCG	human chorionic gonadotropin
HCMV	human cytomegalovirus
HEL	hen egg lysozyme
HEV	high-walled endothelium of post capillary venule
HIV	human immunodeficiency virus
HLA	the human major histocompatibility complex
HLA-A/B/C	main loci for classical class I (class II)
(DP/DQ/DR)	human MHC molecules
HMG	high mobility group
HR	hypersensitive response
HRF	homologous restriction factor
HSA	heat-stable antigen
HSC	hematopoietic stem cell
hsp	heat-shock protein
5HT	5-hydroxytryptamine
HTLV	human T-cell leukemia virus
H-Y	male transplantation antigen
IBD	inflammatory bowel disease
ICAM-1	intercellular adhesion molecule-1
Id (αId)	idiotype (anti-idiotype)
IDC	interdigitating dendritic cells
IDDM	insulin-dependent diabetes mellitus
IDO	indoleamine 2,3-dioxygenase
IEL	intraepithelial lymphocyte
IFNα	α-interferon (also IFNβ, IFNγ)
IFR	interferon-regulated factor
Ig	immunoglobulin
IgG	immunoglobulin G (also IgM, IgA, IgD, IgE)
sIg	surface immunoglobulin
Ig-α/Ig-β	membrane peptide chains associated with sIg B-cell receptor
IgSF	immunoglobulin superfamily
IL-1	interleukin-1 (also IL-2, IL-3, etc.)
iNOS	inducible nitric oxide synthase
IP_3	inositol triphosphate
ISCOM	immunostimulating complex
ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibitory motif
ITP	idiopathic thrombocytopenic purpura
IVIg	intravenous immunoglobulin
JAK	Janus kinases
J chain	polypeptide chain in IgA dimer and IgM
J gene	joining gene linking V or D segment to constant region
*K*a(d)	association (dissociation) affinity constant (usually Ag–Ab reactions)
kDa	units of molecular mass in kilo Daltons
KIR	killer immunoglobulin-like receptors
KLH	keyhole limpet hemocyanin
LAK	lymphokine-activated killer cell
LAMP	lysosomal-associated membrane proteins
LAT	linker for activation of T cells
LATS	long-acting thyroid stimulator
LBP	LPS binding protein
LCM	lymphocytic choriomeningitis virus
$Le^{a/b/x}$	$Lewis^{a/b/x}$ blood group antigens
LFA-1	lymphocyte functional antigen-1
LGL	large granular lymphocyte
LHRH	luteinizing hormone releasing hormone
LIF	leukemia inhibiting factor
LRR	leucine-rich repeat
LT(B)	leukotriene (B etc.)
LPS	lipopolysaccharide (endotoxin)
Mφ	macrophage
mAb	monoclonal antibody
MAC	membrane attack complex
MAdCAM	mucosal addressin cell adhesion molecule
MALT	mucosa-associated lymphoid tissue
MAM	*Mycoplasma arthritidis* mitogen
MAP kinase	mitogen-activated protein kinase
MAPKKK	mitogen-associated protein kinase kinase kinase
MBL	mannose binding lectin
MBP	major basic protein of eosinophils (also myelin basic protein)
MCP	membrane cofactor protein (complement regulation)
MCP-1	monocyte chemotactic protein-1
M-CSF	macrophage colony-stimulating factor
MDP	muramyl dipeptide
MHC	major histocompatibility complex
MICA	MHC class I chain-related A chain
MIDAS	metal ion-dependent adhesion site
MIF	macrophage migration inhibitory factor
MIIC	MHC class II-enriched compartments
MLA	monophosphoryl lipid A
MLR	mixed lymphocyte reaction
MMTV	mouse mammary tumor virus
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	multiple sclerosis
MSC	mesenchymal stem cell

MSH	melanocyte stimulating hormone
MTP	microsomal triglyceride-transfer protein
MuLV	murine leukemia virus
NADP	nicotinamide adenine dinucleotide phosphate
NAP	neutrophil activating peptide
NBT	nitroblue tetrazolium
NCF	neutrophil chemotactic factor
NFAT	nuclear factor of activated T-cells
NFκB	nuclear transcription factor
NK	natural killer cell
NLR	nod-like receptor
NO·	nitric oxide
NOD	Nonobese diabetic mouse
NZB	New Zealand Black mouse
NZB × W	New Zealand Black mouse × NZ White F1 hybrid
$\cdot O_2^-$	superoxide anion
OD	optical density
ORF	open reading frame
OS	obese strain chicken
Ova	ovalbumin
PAF(-R)	platelet activating factor (-receptor)
PAGE	polyacrylamide gel electrophoresis
PAMP	pathogen-associated molecular pattern
PBSCs	peripheral blood stem cells
PCA	passive cutaneous anaphylaxis
PCR	polymerase chain reaction
PERV	porcine endogenous retroviruses
PG(E)	prostaglandin (E etc.)
PHA	phytohemagglutinin
phox	phagocyte oxidase
PI3K	phosphatidylinositol 3-kinase
PIAS	protein inhibitor of activated STAT
pIgR	poly-Ig receptor
PIP_2	phosphatidylinositol diphosphate
PKC	protein kinase C
PKR	RNA-dependent protein kinase
PLC	phospholipase C
PLCγ2	phospholipase Cγ2
PMN	polymorphonuclear neutrophil
PMT	photomultiplier tube
PNH	paroxysmal nocturnal hemoglobinuria
PPAR	peroxisome proliferator-activated receptor
PPD	purified protein derivative from *Mycobacterium tuberculosis*
PRR	pattern recognition receptors
PTFE	polytetrafluoroethylene
PTK	protein tyrosine kinase
PWM	pokeweed mitogen
RA	rheumatoid arthritis
RANTES	*r*egulated upon *a*ctivation *n*ormal *T*-cell *e*xpressed and *s*ecreted chemokine
RAST	radioallergosorbent test
RF	rheumatoid factor
Rh(D)	rhesus blood group (D)
RIP	rat insulin promoter
RLR	RIG-like helicase receptor
RNAi	RNA interference
ROI	reactive oxygen intermediates
RSS	recombination signal sequence
SAP	serum amyloid P
SAP	sphingolipid activator protein
SAR	systemic acquired resistance
SARS	severe acute respiratory syndrome
SARS-CoV	SARS-associated coronavirus
SC	Ig secretory component
SCF	stem cell factor
scFv	single chain variable region antibody fragment (V_H + V_L joined by a flexible linker)
SCG	sodium cromoglycate
SCID	severe combined immunodeficiency
SDF	stromal-derived factor
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SEA(B etc.)	*Staphylococcus aureus* enterotoxin A (B etc.)
SEREX	serological analysis of recombinant cDNA expression libraries
siRNA	short-interfering RNA
SIV	Simian immunodeficiency virus
SLE	systemic lupus erythematosus
SLIT	sublingual allergen immunotherapy
SLP76	SH2-domain containing leukocyte protein of 76 kDa
SOCs	*s*uppressor of *c*ytokine *s*ignaling
SPE	streptococcal pyogenic exotoxins
SRID	single radial immunodiffusion
SSA	streptococcal superantigen
STAT	signal transducer and activator of transcription
TACI	transmembrane activator and calcium modulator and cyclophilin ligand [CAML] interactor
TAP	transporter associated with antigen processing
T-ALL	T-acute lymphoblastic leukemia
TB	tubercle bacillus
Tc	cytotoxic T-cell
T-cell	thymus-derived lymphocyte
TCF	T-cell factor
TCR1(2)	T-cell receptor with γ/δ chains (with α/β chains)
TdT	terminal deoxynucleotidyl transferase
TG-A-L	polylysine with polyalanyl side-chains randomly tipped with tyrosine and glutamic acid
TGFβ	transforming growth factor-β

Th(1/2/3/9/17)	T-helper cell (subset 1, 2, 3, 9 or 17)
THF	thymic humoral factor
Thp	T-helper precursor
TLI	total lymphoid irradiation
TLR	Toll-like receptor
TM	transmembrane
TNF	tumor necrosis factor
TNP	trinitrophenol
TPO	thrombopoietin
Treg	regulatory T-cell
Ts	suppressor T-cell
TSAb	thyroid stimulating antibodies
TSE	transmissible spongiform encephalopathy
TSH(R)	thyroid stimulating hormone (receptor)
TSLP	thymic stromal lymphopoietin
TSST	toxic shock syndrome toxin
TUNEL	TdT-mediated dUTP (deoxyuridine triphosphate) nick end labeling
Vα(β/γ/δ)	variable part of TCR α(β/γ/δ) chain
vCJD	variant Creutzfeldt–Jakob disease
VCP	valosin-containing protein
V gene	variable region gene for immunoglobulin or T-cell receptor
V_H	variable part of Ig heavy chain
VIP	vasoactive intestinal peptide
V_L	variable part of light chain
$V_{\kappa/\lambda}$	variable part of κ(λ) light chain
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial cell growth factor
VIMP	VCP-interacting membrane protein
VLA	very late antigen
VLP	virus-like particle
VNTR	variable number of tandem repeats
VP1	virus-specific peptide 1
XL	X-linked
ZAP-70	zeta chain associated protein of 70 kDa

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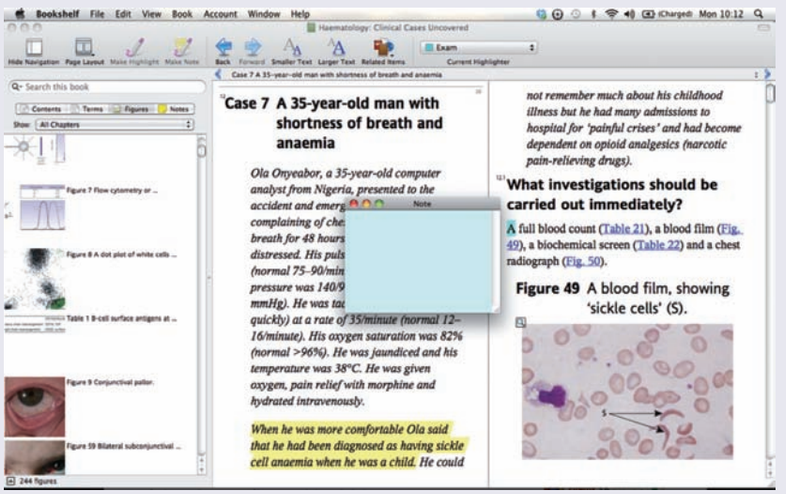

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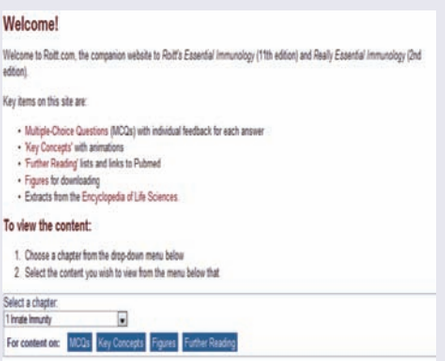

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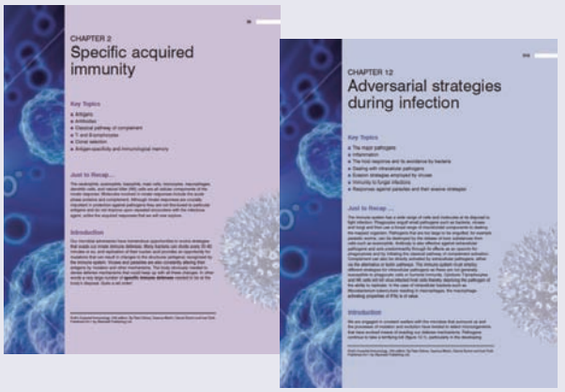

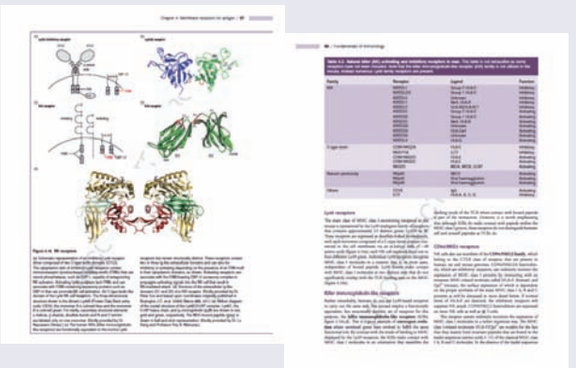

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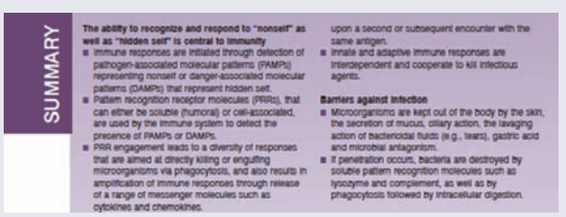

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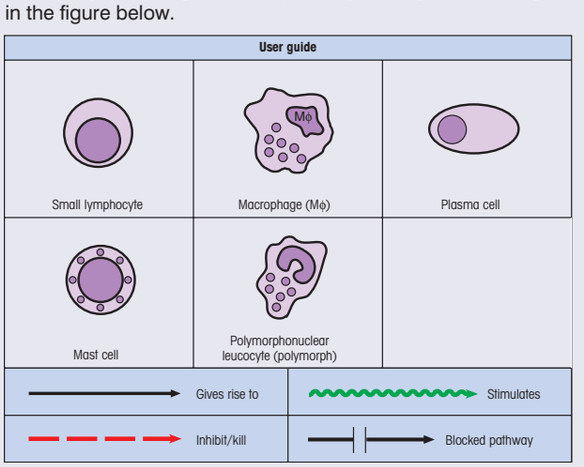

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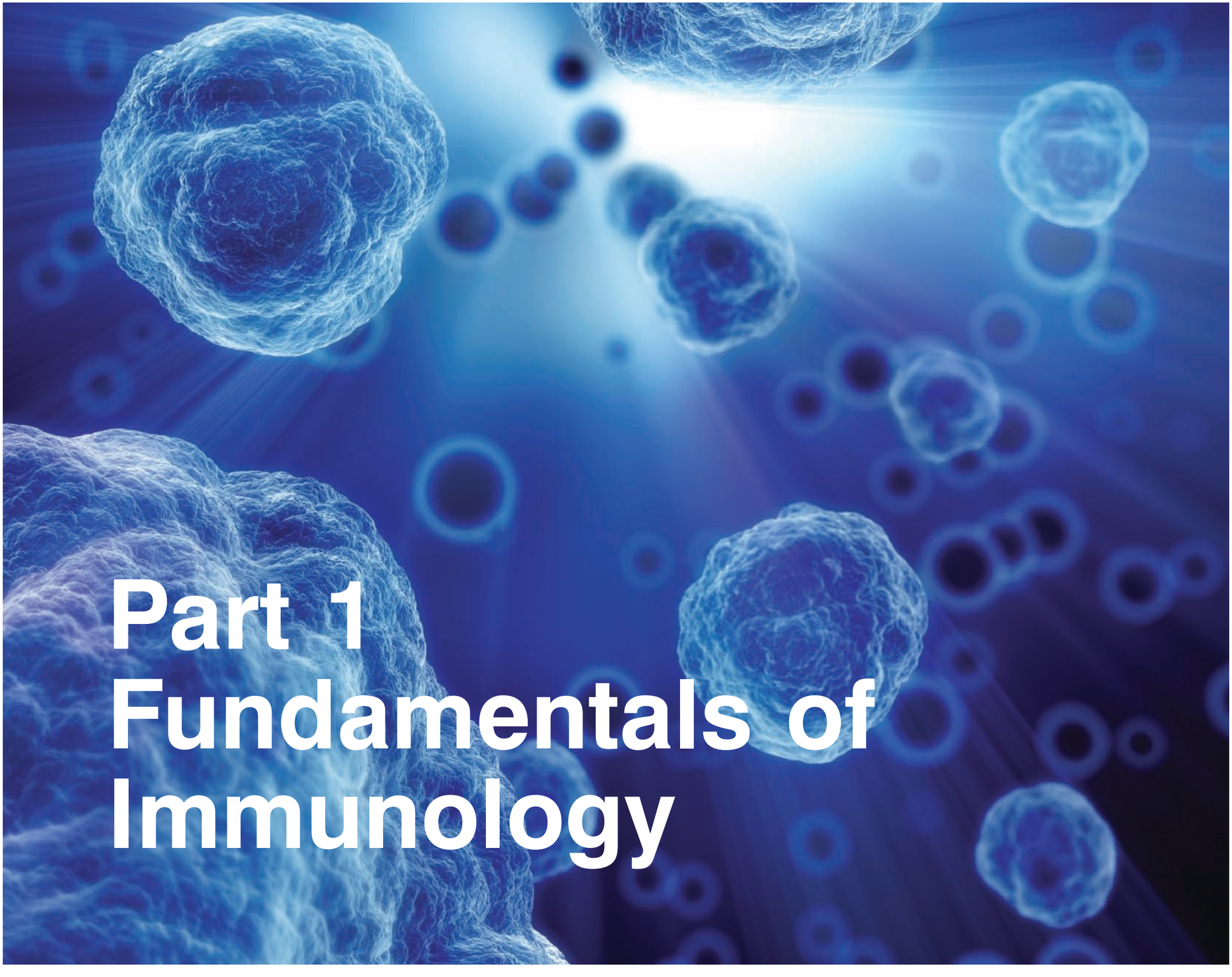

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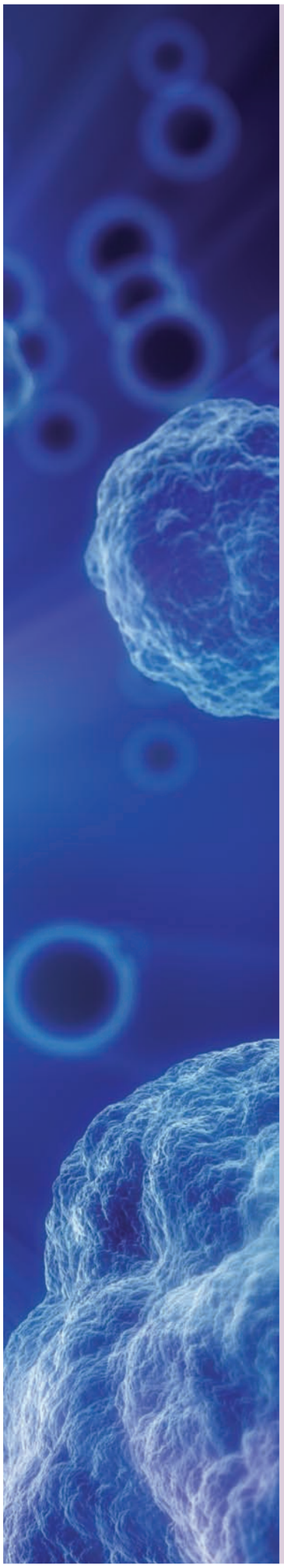


CHAPTER 1

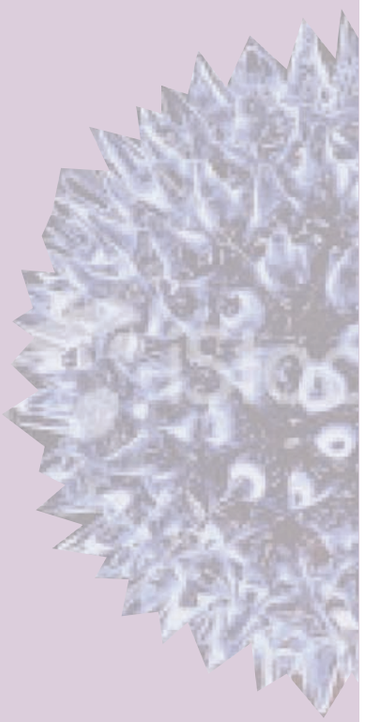
Innate immunity

Key Topics

Introduction

We live in a potentially hostile world filled with a bewildering array of infectious agents (Figure 1.1) of diverse shape, size, composition and subversive character that would very happily use us as rich sanctuaries for propagating their "selfish genes" had we not also developed a series of defense mechanisms at least their equal in effectiveness and ingenuity (except in the case of many parasitic infections in which the situation is best described as an uneasy and often unsatisfactory truce). It is these defense mechanisms that can establish a state of immunity against infection (Latin *immunitas*, freedom from) and whose operation provides the basis for the delightful subject called "Immunology."

Aside from ill-understood constitutional factors that make one species innately susceptible and another resistant to certain infections, a number of relatively nonspecific antimicrobial systems (e.g. phagocytosis) have been recognized that are **innate** in the sense that they are not affected by prior contact with the infectious agent. We shall discuss these systems and examine how, in the state of **specific acquired immunity**, their effectiveness can be greatly increased.



Roitt's Essential Immunology, Twelfth Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt.

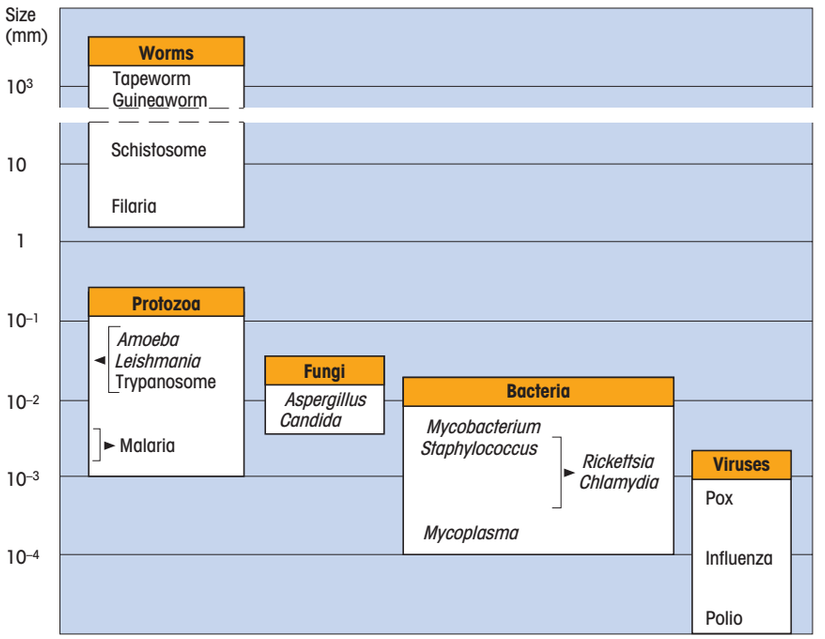


Figure 1.1. The formidable range of infectious agents that confronts the immune system.

Although not normally classified as such because of their lack of a cell wall, the mycoplasmas are included under bacteria for convenience. Fungi adopt many forms and approximate values for some of the smallest forms are given.]►, range of sizes observed for the organism(s) indicated by the arrow; ◄[, the organisms listed have the size denoted by the arrow.

Knowing when to make an immune response

The ability to recognize and respond to foreign entities is central to the operation of the immune system

The vertebrate immune system is a conglomeration of cells and molecules that cooperate to protect us from infectious agents and also provides us with a surveillance system to monitor the integrity of host tissues. Although the immune system is quite elaborate, as we shall see, its function can be boiled down to two basic roles; **recognition** of foreign substances and organisms that have entered the body, and **removal** of such agents by a diverse repertoire of cells and molecules that act in concert to eliminate the potential threat. Thus, a major role of the immune system is to be able to determine what is foreign (what immunologists often call "nonself") from what is normally present in the body (i.e. self). The cells and molecules that comprise the innate immune system are preoccupied with detecting the presence of particular **molecular patterns** that are typically associated with infectious agents (Figure 1.2). Charlie Janeway dubbed such molecules **pathogen-associated molecular patterns** (**PAMPs**).

Tissue damage can also instigate an immune response

Aside from infection, there is a growing recognition that tissue damage, leading to nonphysiological cell death, can also provoke activation of the immune system (Figure 1.3). In this situation, the molecules that activate the immune system are derived from self but are not normally present within the extracellular space. Such molecules, for which Polly Matzinger coined the term "**danger signals**," are normally safely sequestered within healthy cells and only escape when a cell dies via an uncontrolled mode of cell death, called **necrosis** (see Videoclip 1). Necrosis is typically caused by tissue trauma, burns, certain toxins, as well as other non-physiological stimuli and is characterized by rapid swelling and rupture of the plasma membranes of damaged cells. This permits the release of multiple cellular constituents that don't normally escape from healthy cells.

The precise identity of the molecules that act as danger signals—now more commonly called **danger-associated molecular patterns** (**DAMPs**) or alarmins—is an area of active investigation at present but molecules such as HMGB1, a chromatin-binding protein, as well as the immunological messenger proteins interleukin-1α (IL-1α) and IL-33, repre-

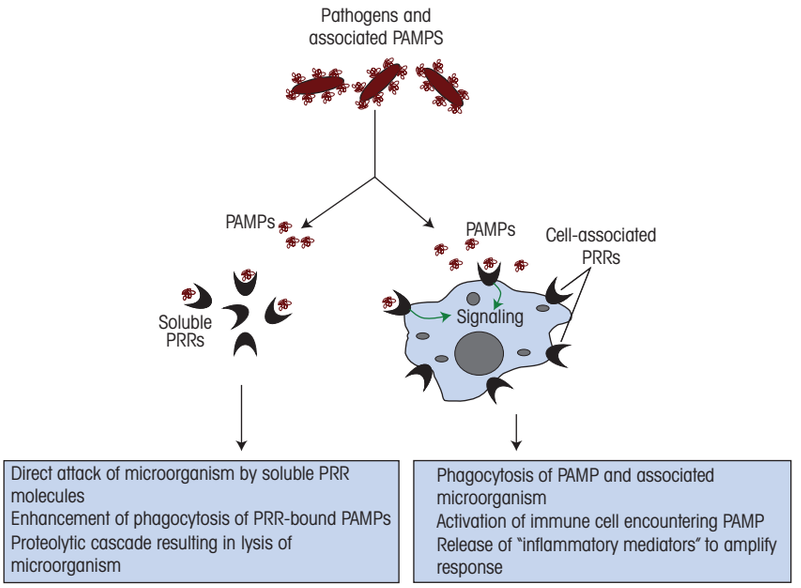


Figure 1.2. Pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs) and initiate immune responses.

PRRs can be either soluble or cell-associated and can instigate a range of responses upon encountering their appropriate ligands.

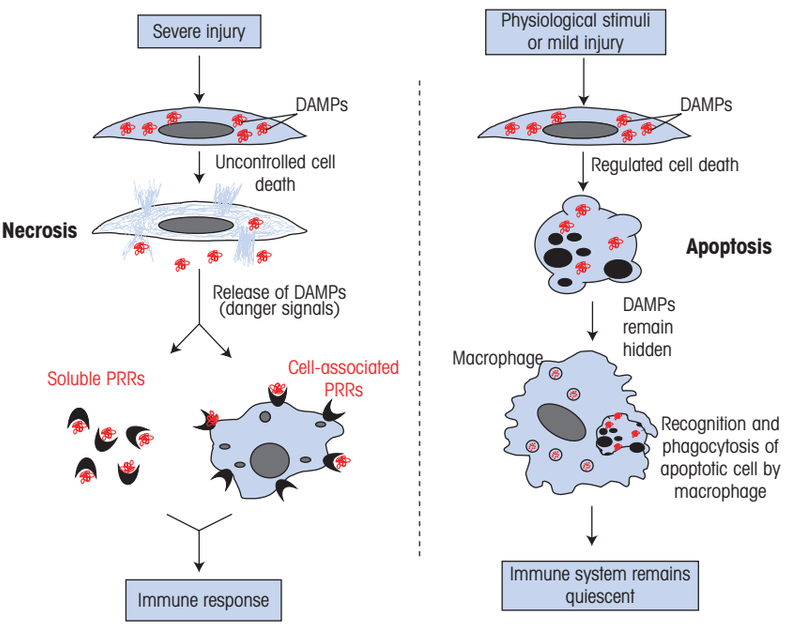


Figure 1.3. Necrotic cells release danger-associated molecular patterns (DAMPs), whereas apoptotic cells typically do not.

Stimuli that induce necrosis frequently cause severe cellular damage, which leads to rapid cell rupture with consequent release of intracellular DAMPs. DAMPs can then engage cells of the immune system and can promote inflammation. On the other hand, because stimuli that initiate apoptosis are typically physiological and relatively mild, apoptotic cells do not rupture and their removal is coordinated by macrophages and other cells of the innate immune system, before release of DAMPs can occur. For this reason, apoptosis is not typically associated with activation of the immune system.

sent good candidates. It might seem surprising that the immune system can also be activated by self-derived molecules, however, this makes good sense when one considers that events leading to necrotic cell death are often rapidly followed or accompanied by infection. Furthermore, if a pathogen manages to evade direct detection by the immune system, its presence will be betrayed if it provokes necrosis within the tissue it has invaded.

Before moving on, we should also note that there is another mode of cell death that frequently occurs in the body that is both natural and highly controlled and is not associated with plasma membrane rupture and release of intracellular contents. This mode of cell death, called **apoptosis** (see Videoclip 2), is under complex molecular control and is used to eliminate cells that have reached the end of their natural lifespans. Apoptotic cells do not activate the immune system because cells dying in this manner display molecules on their plasma membranes (e.g. phosphatidylserine) that mark these cells out for removal through phagocytosis before they can rupture and release their intracellular contents. In this way, DAMPs remain hidden

during apoptosis and such cells do not activate the immune system (Figure 1.3).

Pattern recognition receptors (PRRs) raise the alarm

To distinguish self-components from potentially dangerous microbial agents, our immune systems need to be able to discriminate between "noninfectious self and infectious nonself" as Janeway elegantly put it. Recognition of nonself entities is achieved by means of an array of **pattern recognition receptors and proteins** (collectively called pattern recognition molecules) that have evolved to detect conserved (i.e. not prone to mutation) components of infectious agents that are not normally present in the body (i.e. PAMPs).

In practice, PAMPs can be anything from carbohydrates that are not normally exposed in vertebrates, proteins only found in bacteria such as flagellin (a component of the bacterial flagellum that is used for swimming), double-stranded RNA that is typical of RNA viruses, as well as many other molecules that betray the presence of microbial agents. The cardinal rule is that a PAMP is not normally found in the body but is a common feature of many frequently encountered pathogens. Pattern recognition molecules also appear to be involved in the recognition of DAMPs released from necrotic cells.

Upon engagement of one or more of these pattern recognition molecules with an appropriate PAMP or DAMP, an immune response ensues (Figure 1.2). Fortunately, we have many ways in which an impending infection can be dealt with, and indeed it is a testament to the efficiency of our immune systems that the majority of us spend most of our lives relatively untroubled by infectious disease.

One way of dealing with unwelcome intruders involves the binding of soluble (humoral) pattern recognition molecules, such as **complement** (a series of molecules we will deal with later in this chapter), **mannose-binding lectin**, **C-reactive protein**, or **lysozyme**, to the infectious agent and this can lead directly to killing through destruction of microbial cell wall constituents and breaching of the plasma membrane due to the actions of such proteins. The latter humoral factors are also adept at coating microorganisms and enhancing their uptake and subsequent destruction by phagocytic cells. Other pattern recognition receptors are cell associated and engagement of such receptors can lead to **phagocytosis** of the microorganism followed by its destruction within phagocytic vesicles. Just as importantly, cellular PRR engagement also results in the activation of signal transduction pathways that culminate in the release of soluble messenger proteins (**cytokines**, **chemokines** and other molecules, see below) that mobilize other components of the immune system.

Cells of the immune system release messenger proteins that amplify immune responses

An important feature of the immune system is the ability of its constituent cells to communicate with each other upon encountering a pathogen. Although cells of the immune system are capable of releasing numerous biologically active molecules with diverse functions, two major categories of proteins—cytokines and chemokines—have particularly important roles in immunity. Cytokines are a diverse group of proteins that have pleiotropic effects, including the ability to activate other cells, induce differentiation and enhance microbicidal activity (Figure 1.4). Cytokines are commonly released by cells of the immune system in response to PAMPs and DAMPs, and this has the effect of altering the activation state and behaviour of other cells to galvanise them into joining the fight. Chemokines are also released upon encountering PAMPs/DAMPs and typically serve as **chemotactic factors**, helping to lay a trail that guides other cells of the immune system to the site of infection or tissue damage. Both types of messenger proteins act by diffusing away from the cells secreting them and binding to cells equipped with the appropriate plasma membrane receptors to receive such signals. Cytokines, chemokines and their respective receptors are discussed at length in Chapter 9.

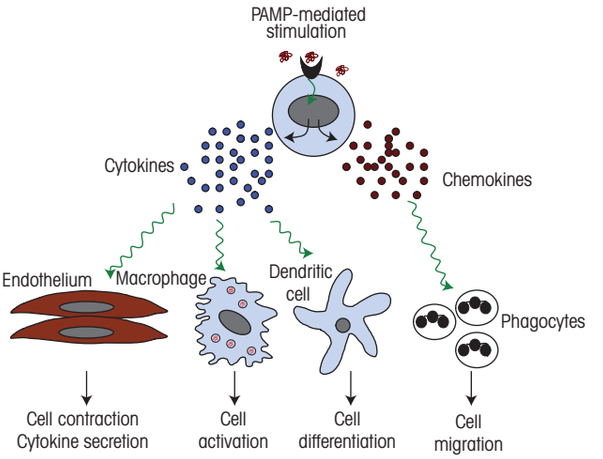


Figure 1.4. Cytokines and chemokines can have pleiotrophic effects.

Stimulation of cells of the innate immune system frequently leads to the production of inflammatory cytokines and chemokines that trigger responses from other cell types, as depicted. Note that the effects of chemokines and cytokines shown are not exhaustive.

Innate versus adaptive immunity

Three levels of immune defense

Before we get into the details, we will first take a look at how the immune system works in broad brushstrokes. The vertebrate immune system comprises three levels of defense (Figure 1.5). First, there is a **physical barrier** to infection that is provided by the skin on the outer surfaces of the body, along with the mucous secretions covering the epidermal layers of the

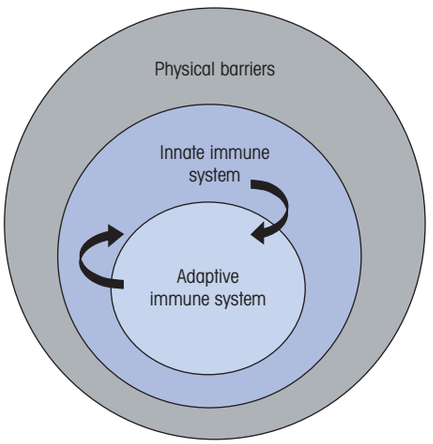


Figure 1.5. The vertebrate immune system comprises three levels of defense.

The physical barriers of the skin and mucosal surfaces comprise the first level of defense. Infectious agents that successfully penetrate the physical barriers are then engaged by the cells and soluble factors of the innate immune system. The innate immune system is also responsible for triggering activation of the adaptive immune system, as we will discuss later in this chapter. The cells and products of the adaptive immune system reinforce the defense mounted by the innate immune system.

inner surfaces of the respiratory, digestive and reproductive tracts. Any infectious agent attempting to gain entry to the body must first breach these surfaces that are largely impermeable to microorganisms; this is why cuts and scrapes that breach these physical barriers are often followed by infection. The second level of defense is provided by the **innate immune system**, a relatively broad-acting but highly effective defense layer that is largely preoccupied with trying to kill infectious agents from the moment they enter the body. The actions of the innate immune system are also responsible for alerting the cells that operate the third level of defense: the **adaptive (or acquired) immune system**. The latter cells represent the elite troops of the immune system and can launch an attack that has been specifically adapted to the nature of the infectious agent using sophisticated weapons such as antibodies.

Innate immune responses are immediate and relatively broad acting

Upon entry of a foreign entity into the body, the innate immune response occurs almost immediately. Innate immune responses do not improve upon frequent encounter with the same infectious agent. The innate immune system recognizes broadly conserved components of infectious agents, the aforementioned PAMPs, that are not normally present in the body. Upon detecting a PAMP, the innate immune system mounts an immediate attack on anything displaying such molecules by either engulfing such entities or through attacking them with destructive enzymes, such as proteases or membrane attacking proteins (Figure 1.2). The clear intent is to bludgeon the unwanted intruder into submission as quickly as possible. This makes sense when one considers the prodigious rates of proliferation that bacteria can achieve—many bacterial species are capable of dividing every 20 minutes or so—particularly in the nutrient-rich environment our bodies provide. Key players in the innate immune response include **macrophages**, **neutrophils** and soluble bactericidal (i.e. bacterial killing) proteins such as **complement** and **lysozyme**. Although highly effective, innate immune responses are not always sufficient to completely deal with the threat, particularly if the infectious agent is well adapted to avoid the initial attack.

Adaptive immune responses are delayed but highly specific

Adaptive immune responses take longer to achieve functional significance, typically 4–5 days after the innate immune response, but are specifically tailored to the nature of the infectious agent (how this is achieved will be discussed at length in later chapters, but for now, let's not trouble ourselves with the details). Importantly, adaptive immune responses improve upon each encounter with a particular infectious agent, a feature called **immunological memory**, which underpins the concept of vaccination. The adaptive immune response is mediated primarily by **T- and B-lymphocytes** and these cells display specific receptors on their plasma membranes that can be tailored to recognize an almost limitless range of structures. By definition, molecules that are recognized by T- and B-lymphocytes are called **antigens**. Recognition of antigen by a lymphocyte triggers proliferation and differentiation of such cells and this has the effect of greatly increasing the numbers of lymphocytes capable of recognizing the particular antigen that triggered the response in the first place. This rapidly swells the ranks of lymphocytes capable of dealing with the infectious agent bearing the specific antigen and results in a **memory response** if the same antigen is encountered at some time in the future. We will look in detail at the receptors used by T- and B-cells to see antigen in Chapter 4.

Innate and adaptive immune responses are interdependent

The innate and adaptive immune systems work in tandem to identify and kill infectious agents (Figure 1.5). The innate immune system uses hard-wired (i.e. germline encoded, which means that such genes are passed in essentially identical form from parent to offspring) receptors and molecules that respond to **broad categories** of foreign molecules (i.e. PAMPs) that are commonly expressed on microorganisms. Because the receptors of the innate immune system are encoded by the germline, innate immune responses are quite similar between individuals of the same species. In contrast, the adaptive immune system uses randomly generated receptors that are **highly specific** for each infectious agent that the immune system comes into

contact with. Therefore, adaptive immune responses are highly variable between individuals within a species and reflect the range of pathogens a particular individual has encountered.

Thus, when an infection occurs, **the innate immune system serves as a rapid reaction force** that deploys a range of relatively nonspecific weapons to eradicate the infectious agent, or at the very least to keep the infection contained. This gives time for the initially sluggish adaptive immune system to select and clonally expand cells with receptors that are capable of making a much more specific response that is uniquely tailored to the infectious agent. The adaptive immune response to an infectious agent reinforces and adds new weapons to the attack mounted by the innate immune system.

While it was once fashionable to view the innate immune system as somewhat crude and clumsy when compared to the relative sophistication of the adaptive immune system, an explosion of new discoveries over the past 5–10 years has revealed that the innate immune system is just as highly adapted and sophisticated as the adaptive immune system. Moreover, it has also become abundantly clear that **the adaptive immune system is highly dependent on cells of the innate immune system for the purposes of knowing when to respond, how to respond and for how long**. Exactly why this is so will be discussed later in this chapter, but for now let us consider the external barriers to infection in a little more detail.

External barriers against infection

As mentioned above, the simplest way to avoid infection is to prevent the microorganisms from gaining access to the body (Figure 1.6). When intact, the skin is impermeable to most infectious agents; when there is skin loss, as for example in burns, infection becomes a major problem. Additionally, most bacteria fail to survive for long on the skin because of the direct inhibitory effects of lactic acid and fatty acids in sweat and sebaceous secretions and the low pH that they generate. An exception is *Staphylococcus aureus*, which often infects the relatively vulnerable hair follicles and glands.

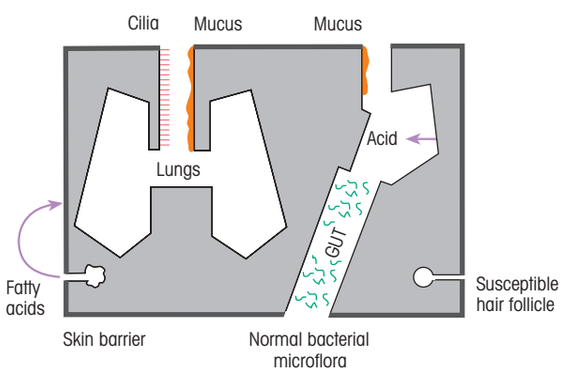


Figure 1.6. The first lines of defense against infection: protection at the external body surfaces.

Mucus, secreted by the membranes lining the inner surfaces of the body, acts as a protective barrier to block the adherence of bacteria to epithelial cells. Microbial and other foreign particles trapped within the adhesive mucus are removed by mechanical stratagems such as ciliary movement, coughing and sneezing. Among other mechanical factors that help protect the epithelial surfaces, one should also include the washing action of tears, saliva and urine. Many of the secreted body fluids contain bactericidal components, such as acid in gastric juice, spermine and zinc in semen, lactoperoxidase in milk and lysozyme in tears, nasal secretions and saliva.

A totally different mechanism is that of microbial antagonism associated with the normal bacterial flora of the body (i.e. commensal bacteria). This suppresses the growth of many potentially pathogenic bacteria and fungi at superficial sites by competition for essential nutrients or by production of inhibitory substances. To give one example, pathogen invasion is limited by lactic acid produced by particular species of commensal bacteria that metabolize glycogen secreted by the vaginal epithelium. When protective commensals are disturbed by antibiotics, susceptibility to opportunistic infections by *Candida* and *Clostridium difficile* is increased. Gut commensals may also produce colicins, a class of bactericidins that bind to the negatively charged surface of susceptible bacteria and insert a hydrophobic helical hairpin into the membrane; the molecule then undergoes a "Jekyll and Hyde" transformation to become completely hydrophobic and forms a voltage-dependent channel in the membrane that kills by destroying the cell's energy potential. Even at this level, survival is a tough game.

If microorganisms do penetrate the body, the innate immune system comes into play. Innate immunity involves two main defensive strategies to deal with a nascent infection: the destructive effect of soluble factors such as bactericidal enzymes and the mechanism of **phagocytosis**—literally "eating" by the cell (see Milestone 1.1). Before we discuss these strategies in more detail, let us first consider the stereotypical order of events that occur upon infection.

The beginnings of an immune response

A major player in the initiation of immune responses is the **macrophage**. These cells are relatively abundant in most tissues (approaching 10–15% of the total cell number in some areas of the body) and act as sentinels for infectious agent through an array of pathogen recognition receptors (PRRs) borne on their plasma membranes as well as other cellular compartments such as endosomes. Tissue macrophages are relatively quiescent cells, biding their time sampling the environment around them through continuous phagocytosis. However, upon entry of a microorganism that engages one or more of their PRRs (such as a Toll-like receptor or a NOD-like receptor), a startling transition occurs. Engagement of the PRR on the macrophage switches on a battery of genes that equip it to carry out a number of new functions.

Milestone 1.1—Phagocytosis

The perceptive Russian zoologist, Elie Metchnikoff (1845–1916; Figure M1.1.1), recognized that certain specialized cells mediate defense against microbial infections (Figure M1.1.2), so fathering the whole concept of cellular immunity. He was intrigued by the motile cells of transparent starfish larvae and made the critical observation that, a few hours after the introduction of a rose thorn into these larvae, they became surrounded by these motile cells. A year later, in 1883, he observed that fungal spores can be attacked by the blood cells of *Daphnia*, a tiny metazoan that, also being transparent, can be studied directly under the microscope. He went on to extend his investigations to mammalian leukocytes, showing their ability to engulf microorganisms, a process that he termed **phagocytosis**.

Because he found this process to be even more effective in animals recovering from infection, he came to a somewhat polarized view that phagocytosis provided the main, if not the only, defense against infection. He went on to define the existence of two types of circulating phagocytes: the polymorphonuclear leukocyte, which he termed a "microphage," and the larger "macrophage."

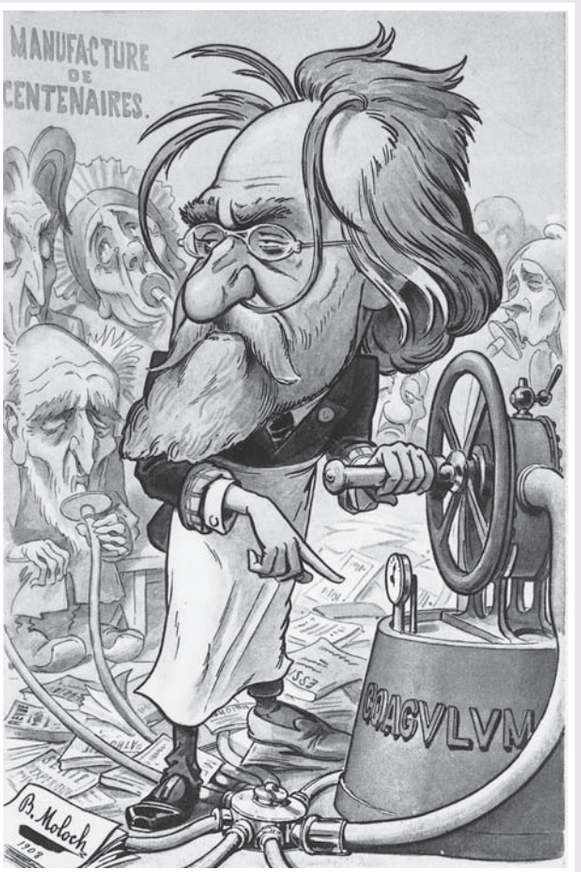


Figure M1.1.1. Caricature of Professor Metchnikoff. From *Chanteclair*, 1908, No. 4, p. 7. (Reproduction kindly provided by The Wellcome Institute Library, London, UK.)

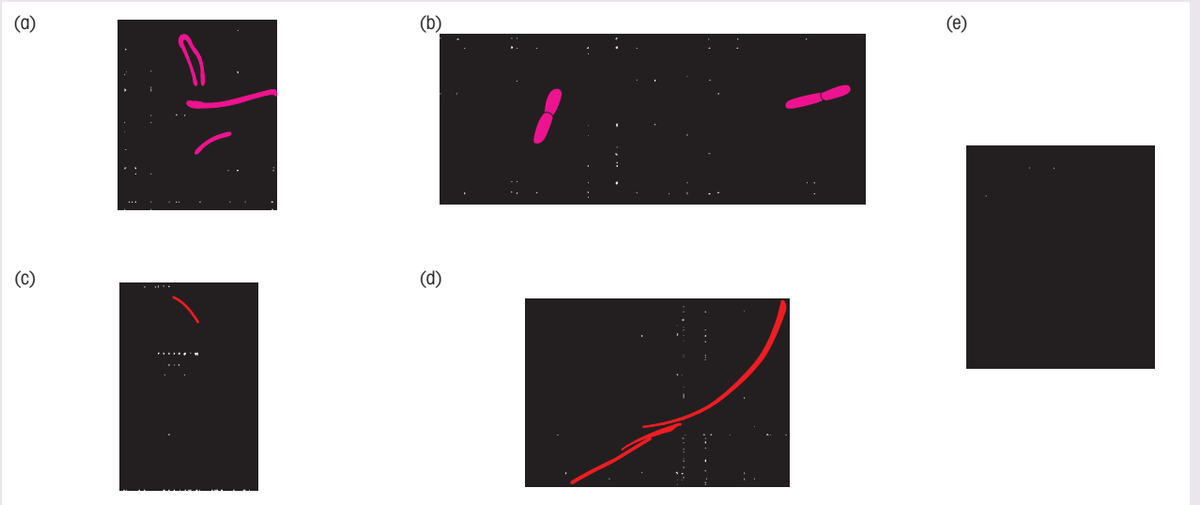


Figure M1.1.2. Reproductions of some of the illustrations in Metchnikoff's book, *Comparative Pathology of Inflammation* (1893). (a) Four leukocytes from the frog, enclosing anthrax bacilli; some are alive and unstained, others, which have been killed, have taken up the vesuvine dye and have been colored; (b) drawing of an anthrax bacillus, stained by vesuvine, in a leukocyte of the frog; the two figures represent two phases of movement of the same frog leukocyte which contains stained anthrax bacilli within its phagocytic vacuole; (c and d) a foreign body (colored) in a starfish larva surrounded by phagocytes that have fused to form a multinucleate plasmodium shown at higher power in (d); (e) this gives a feel for the dynamic attraction of the mobile mesenchymal phagocytes to a foreign intruder within a starfish larva.

First, the macrophage is put on a state of high alert (i.e. becomes activated) and is now better at engulfing and killing any microorganisms it encounters (this will be discussed in detail in the next section). Second, the macrophage begins to secrete cytokines and chemokines that have effects on nearby endothelial cells lining the blood capillaries; this makes the capillaries in this area more permeable than they would normally be. In turn, the increased vascular permeability permits two other things to happen. Plasma proteins that are normally largely restricted to blood can now invade the tissue at the point of infection and many of these proteins have microbicidal properties. A second consequence of increased vascular permeability is that another type of innate immune cell, the **neutrophil**, can now gain access to the site of infection. Neutrophils, like macrophages, are also adept at phagocytosis but are normally not permitted to enter tissues due to their potentially destructive behavior. Upon entry into an infected tissue, activated neutrophils proceed to attack and engulf any microorganisms they encounter with gusto. We will now deal with some of these events in more detail.

Pattern recognition receptors (PRRs) on phagocytic cells recognize and are activated by pathogen-associated molecular patterns (PAMPs)

Because the ability to discriminate friend from foe is of paramount importance for any self-respecting phagocyte, these cells are fairly bristling with receptors capable of recognizing diverse PAMPs. Several of these pattern recognition receptors are lectin-like and bind multivalently with considerable specificity to exposed microbial surface sugars with their characteristic rigid three-dimensional geometric configurations. They do not bind appreciably to the array of galactose or sialic acid groups that are commonly the penultimate and ultimate sugars that decorate mammalian surface polysaccharides so providing the molecular basis for discriminating between self and nonself microbial cells. Other PRRs detect nucleic acids derived from bacterial and viral genomes by virtue of modifications not commonly found within vertebrate nucleic acids or conformations not normally found in the cytoplasm (e.g. double-stranded RNA). PRRs are a diverse group of receptors that can be subdivided into at least five distinct families (TLRs, CTLRs, NLRs, RLRs and scavenger receptors) based upon structural features. Multiple receptors also exist in each class with the result that in excess of 50 distinct PRRs may be expressed by a phagocyte at any given time. Because this topic is an area of active investigation at present, it is likely that many additional PRRs will be identified in the near future. Let us now look at the five known families of PRRs in more detail.

Toll-like receptors (TLRs)

A major subset of the PRRs belong to the class of so-called **Toll-like receptors (TLRs)** because of their similarity to the Toll receptor in the fruit fly, *Drosophila*, which in the adult triggers an intracellular cascade generating the expression of antimicrobial peptides in response to microbial infection. A series of cell surface TLRs acting as sensors for extracellular infections have been identified (Figure 1.7) that are activated by microbial elements such as peptidoglycan, lipoproteins, mycobacterial lipoarabinomannan, yeast zymosan, flagellin, as well as other pathogen-derived ligands.

Although many TLRs are displayed on the cell surface, some, such as TLR3 and TLR7/8/9 that are responsive to intracellular viral RNA and unmethylated bacterial DNA, are located in endosomes and become engaged upon encounter with phagocytosed material (Figure 1.7). Engagement of TLRs with their respective ligands drives activation of nuclear factor κB (NFκB) and several members of the interferon-regulated factor (IRF) family of transcription factors, depending on the specific TLR. Combinatorial activation of TLRs is also possible, for example TLR2 is capable of responding

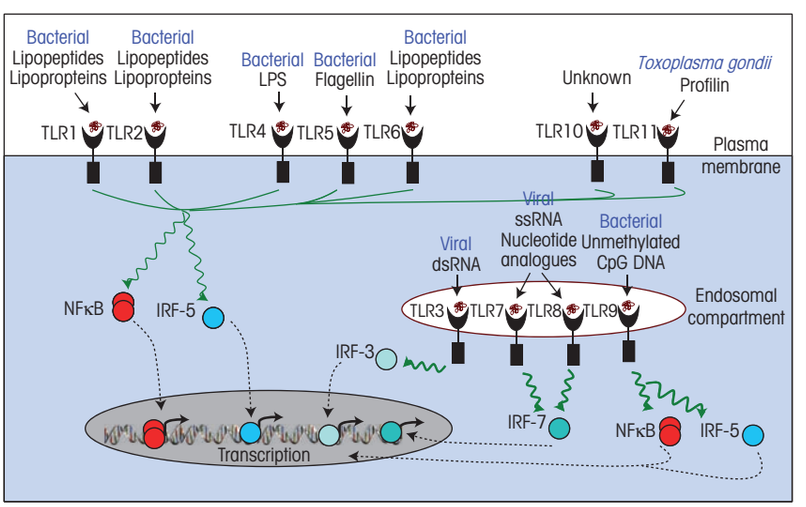


Figure 1.7. A family of Toll-like receptors (TLRs) act as sensors for pathogen-associated molecular patterns (PAMPs).

TLRs reside within plasma membrane or endosomal membrane compartments, as shown. Upon engagement of the TLR ectodomain with an appropriate PAMP (some examples are shown), signals are propagated into the cell that activate the nuclear factor κB (NFκB) and/or interferon-regulated factor (IRF) transcription factors, as shown. NFκB and IRF transcription factors then direct the expression of numerous anti-microbial gene products, such as cytokines and chemokines, as well as proteins that are involved in altering the activation state of the cell.

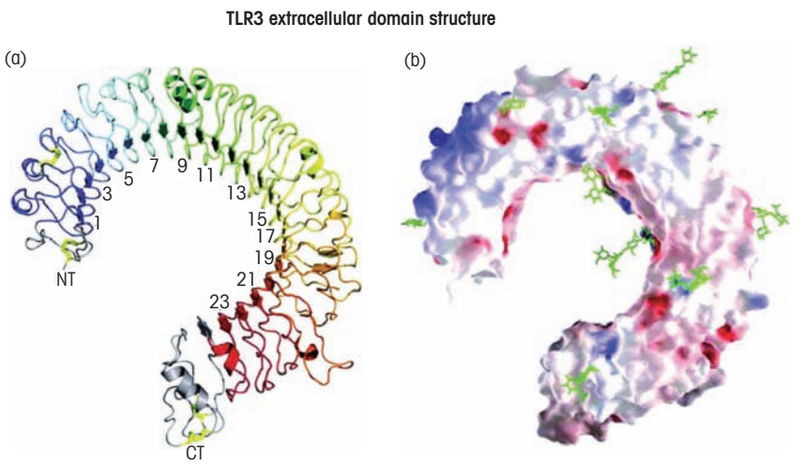


Figure 1.8. Toll-like receptor (TLR) structure.

TLR3 ectodomain structure. (a) Ribbon diagram of TLR3 ectodomain. Leucine-rich repeats (LRRs) are colored from blue to red beginning at LRR1 and proceeding to LRR23, as indicated. NT, N-terminus; CT, C-terminus. (b) Electrostatic potential surface shows positive (blue) and negative (red) charges at neutral pH. The *N*-linked glycans are shown as green ball-and-stick. (Reproduced from Bell J.K. *et al.* (2005) *Proceedings of the National Academy of Sciences USA* **102**, 10976–10980, with permission.)

to a wide diversity of PAMPs and typically functions within heterodimeric TLR2/TLR1 or TLR2/TLR6 complexes.

All TLRs have the same basic structural features, with multiple N-terminal leucine-rich repeats (LRRs) arranged in a horseshoe or crescent-shaped solenoid structure that acts as the PAMP-binding domain (Figure 1.8). Upon binding of a PAMP, TLRs transduce signals into the cell via C-terminal motifs called TIR domains which can recruit adaptor proteins within the cytoplasm (such as MyD88 or Mal) that possess similar TIR motifs. The latter adaptors propagate the signal downstream, culminating in activation of NFκB and IRF family transcription factors (Figures 1.7 and 1.9).

C-type lectin receptors (CTLRs)

Phagocytes also display another set of PRRs, the cell-bound **C-type (calcium-dependent) lectins**, of which the macrophage mannose receptor is an example. These transmembrane proteins possess multiple carbohydrate recognition domains whose engagement with their cognate microbial PAMPs generates an intracellular activation signal. The CTLR family is highly diverse and the ligands for many receptors in this category remain the subject of ongoing research.

NOD-like receptors (NLRs)

Turning now to the sensing of infectious agents that have succeeded in gaining access to the interior of a cell, microbial products can be recognized by the so-called NOD-like receptors. Unlike TLRs and CTLRs that reside within the plasma membrane or intracellular membrane compartments, NLRs are soluble proteins that reside in the cytoplasm where they also act as receptors for pathogen-derived molecular patterns. Although a diverse family of receptors, NLRs typically contain an N-terminal protein–protein interaction motif that enables these proteins to recruit proteases or kinases upon activation, followed by a central oligomerization domain and C-terminal leucine-rich repeats (LRRs) that appear to act as the sensor for pathogen products. NLRs are thought to exist in an autoinhibited state with their N-terminal domains folded back upon their C-terminal LRRs, a conformation that prevents the N-terminal region from interacting with its binding partners in the cytoplasm. Activation of these receptors is most likely triggered through direct binding of a PAMP to the C-terminal LRRs which has the effect of disrupting the interaction between the N- and C-termini of the NLR and permits oligomerization into an complex that is now capable of recruiting either an NFκB-activating kinase (such as RIP-2) or members of the caspase family of proteases that can proteolytically process and activate the IL-1β precursor into the mature biologically active cytokine. The latter complex, called **the inflammasome**, is assembled in response to a number of PAMPs and is important for the production of IL-1β as well as IL-18.

RIG-like helicase receptors (RLRs)

The RIG-like helicases are a very recently discovered group of proteins that act as intracellular sensors for viral-derived products. Similar to the NLRs, RLRs are found in the cytoplasm and all appear to be activated in response to double-stranded RNA and are capable of directing the activation of NFκB and IRF3/4 that cooperatively induce antiviral type I interferons (IFNα and β).

Scavenger receptors

Scavenger receptors represent yet a further class of phagocytic receptors that recognize a variety of anionic polymers and acetylated low-density proteins. The role of the CD14 scavenger molecule in the handling of Gram-negative LPS (lipopolysaccharide endotoxin) merits some attention, as failure to do so can result in septic shock. The biologically reactive lipid A moiety of LPS is recognized by a plasma LPS-binding protein, and the complex that is captured by the CD14 scavenger molecule on the phagocytic cell then activates TLR4.

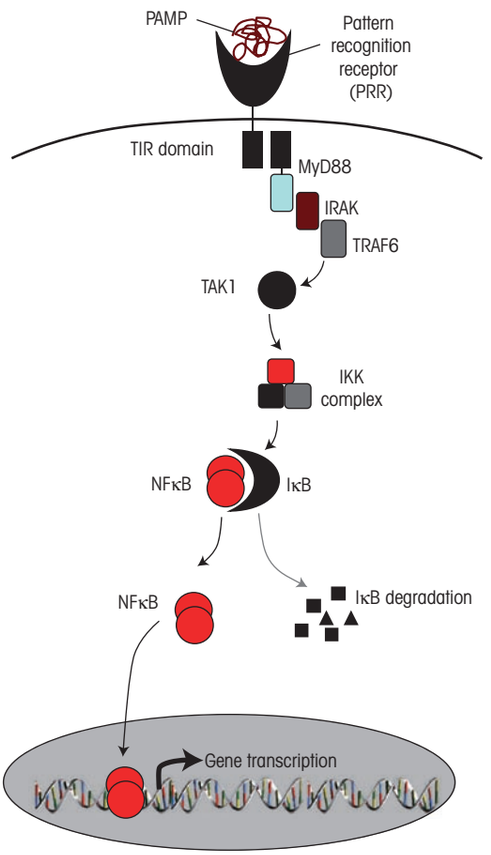


Figure 1.9. Toll-like receptors promote NFκB-dependent transcription through activation of the IκB kinase (IKK) complex.

Upon engagement of a TLR with its appropriate ligand, a series of adaptor proteins (as shown) are recruited to the TLR receptor *T*oll and *I*L-1 *r*eceptor-like (TIR) domain. Collectively, these proteins activate the IKK complex, which in turn phosphorylates the *I*nhibitor of NF*κB* (IκB), a protein that binds and tethers NFκB in the cytosol. IκB phosphorylation targets the latter for degradation, liberating NFκB which can then translocate into the nucleus and initiate transcription of multiple genes.

Pattern recognition receptor (PRR) engagement results in cell activation and pro-inflammatory cytokine production

Upon encountering ligands of any of the aforementioned PRRs, the end result is a switch in cell behavior from a quiescent state to an activated one. Activated macrophages and neutrophils are capable of phagocytosing particles that engage their PRRs and in this state they also release a range of cytokines and chemokines that amplify the immune response further.

As we have noted above, engagement of many of the above PPRs results in a signal transduction cascade culminating in activation of NFκB, a transcription factor that controls the expression of numerous immunologically important molecules such as cytokines and chemokines (Figures 1.7 and 1.9). In resting cells, NFκB is sequestered in the cytoplasm by its inhibitor IκB which masks a nuclear localization signal on the former. Upon binding of a PAMP to its cognate PRR, NFκB is liberated from IκB due to the actions of a kinase that phosphorylates IκB and promotes its destruction. NFκB is now free to translocate to the nucleus, seek out its target genes and initiate transcription (Figure 1.9).

Other transcription factor cascades, involving most notably the **interferon-regulated factors** (IRFs), are also activated downstream of the PRRs (Figure 1.7). Some of the most important inflammatory mediators synthesized and released in response to PRR engagement include the antiviral **interferons** (cf. p. 25), the small protein cytokines interleukin-1β (IL-1β), IL-6, IL-12, and tumor necrosis factor α (TNFα) (cf. p. 229), which activate other cells through binding to specific receptors, and chemokines, such as IL-8, which represent a subset of chemoattractant cytokines. Collectively, these molecules amplify the immune response further and have effects on the local blood capillaries that permit extravasation of neutrophils which come rushing into the tissue to assist the macrophage in dealing with the situation.

Dying cells also release molecules capable of engaging PRRs

As we have mentioned earlier, cells undergoing necrosis (but not apoptosis) are also capable of releasing molecules (i.e. DAMPs) that are capable of engaging PRRs (Figure 1.3). The identity of these molecules is only slowly emerging but includes HMGB1, members of the S100 calcium-binding protein family, HSP60 and the classical cytokines IL-1α and IL-33. Certain DAMPs appear to be able to bind to members of the TLR family (i.e. HMGB1 has been suggested to signal via TLR4), while others such as IL-1α and IL-33 bind to specific cell surface receptors that possess similar intracellular signaling motifs to the TLR receptors.

DAMPs are involved in amplifying immune responses to infectious agents that provoke cell death and also play a role in the phenomenon of **sterile injury**, where an immune response occurs in the absence of any discernable infectious agent (e.g. the bruising that occurs in response to a compression injury that doesn't breach the skin barrier represents an innate immune response). Indeed, Polly Matzinger has proposed that robust immune responses are only seen when nonself is detected in combination with tissue damage (i.e. a source of DAMPs). The thinking here is that the immune system does not need to respond if an infectious agent is not causing any harm. Thus, PAMPs and DAMPs may act synergistically to provoke more robust and effective immune responses than would occur in response to either alone.

Phagocytic cells engulf and kill microorganisms

Macrophages and neutrophils are dedicated "professional" phagocytes

The engulfment and digestion of microorganisms are assigned to two major cell types recognized by Elie Metchnikoff at the turn of the last century as microphages and macrophages.

The macrophage

These cells derive from bone marrow promonocytes that, after differentiation to blood monocytes (Figure 1.10a), finally settle in the tissues as mature macrophages where they constitute the **mononuclear phagocyte system** (Figure 1.11). They are present throughout the connective tissue and around the basement membrane of small blood vessels and are particularly concentrated in the lung (Figure 1.10h; alveolar macrophages), liver (Kupffer cells) and lining of spleen sinusoids and lymph node medullary sinuses where they are strategically placed to filter off foreign material. Other examples are mesangial cells in the kidney glomerulus, brain microglia and osteoclasts in bone. Unlike neutrophils, macrophages are long-lived cells with significant rough-surfaced endoplasmic reticulum and mitochondria and, whereas neutrophils provide the major defense against pyogenic (pus-forming) bacteria, as a rough generalization it may be said that macrophages are at their best in combating those bacteria (Figure 1.10g), viruses and protozoa that are capable of living within the cells of the host.

The polymorphonuclear neutrophil

This cell, the smaller of the two, shares a common hematopoietic stem cell precursor with the other formed elements of the blood and is the dominant white cell in the bloodstream. It is a nondividing short-lived cell with a multilobed nucleus and an array of granules (Figure 1.12), which are virtually unstained by histologic dyes such as hematoxylin and eosin, unlike those structures in the closely related eosinophil and basophil (Figure 1.10c and 1.10i). These neutrophil granules are of two main types: (i) the **primary azurophil granule** that develops early (Figure 1.10e), has the typical lysosomal morphology and contains myeloperoxidase together with most of the nonoxidative antimicrobial effectors including defensins, bactericidal permeability increasing (BPI) protein and cathepsin G (Figure 1.12); and (ii) the peroxidase-negative **secondary specific granules** containing lactoferrin, much of the lysozyme, alkaline phosphatase (Figure 1.10d) and membrane-bound cytochrome b_{558} (Figure 1.12). The abundant glycogen stores can be utilized by glycolysis enabling the cells to function under anerobic conditions.

Microbes are engulfed by activated phagocytic cells

After adherence of the microbe to the surface of the neutrophil or macrophage through recognition of a PAMP (Figure 1.13.2), the resulting signal (Figure 1.13.3) initiates the ingestion phase by activating an actin–myosin contractile system that extends pseudopods around the particle (Figures 1.13.4 and 1.14); as adjacent receptors sequentially attach to the surface of the microbe, the plasma membrane is pulled around the particle just like a "zipper" until it is completely enclosed in a vacuole (phagosome; Figures 1.13.5 and 1.14). Events are now moving smartly and, within 1 minute, the cytoplasmic granules fuse with the phagosome and discharge their contents around the imprisoned microorganism (Figures 1.13.7 and 1.15) which is subject to a formidable battery of microbicidal mechanisms.

There is an array of killing mechanisms

Killing by reactive oxygen intermediates

Trouble starts for the invader from the moment phagocytosis is initiated. There is a dramatic increase in activity of the hexose monophosphate shunt generating reduced nicotinamide adenine dinucleotide phosphate (NADPH). Electrons pass from the NADPH to a flavine adenine dinucleotide (FAD)-containing membrane flavoprotein and thence to a unique plasma membrane **cytochrome (cyt b_{558})**. This has the very low midpoint redox potential of −245 mV that allows it to reduce molecular oxygen directly to superoxide anion (Figure 1.16a). Thus the key reaction catalyzed by this NADPH oxidase, which initiates the formation of reactive oxygen intermediates (ROI), is:

$$\mathrm{NADPH} + \mathrm{O_2} \rightarrow \mathrm{NADP^+} + \cdot\mathrm{O_2^-}$$

(superoxide anion).

The superoxide anion undergoes conversion to hydrogen peroxide under the influence of superoxide dismutase, and subsequently to hydroxyl radicals (·OH). Each of these products has remarkable chemical reactivity with a wide range of molecular targets, making them formidable microbicidal agents; ·OH in particular is one of the most reactive free radicals known. Furthermore, the combination of peroxide, myeloperoxidase and halide ions constitutes a potent halogenating system capable of killing both bacteria and viruses (Figure 1.16a). Although H_2O_2 and the halogenated compounds are not as active as the free radicals, they are more stable and therefore diffuse further, making them toxic to microorganisms in the extracellular vicinity.

Killing by reactive nitrogen intermediates

Nitric oxide surfaced prominently as a physiologic mediator when it was shown to be identical with endothelium-derived relaxing factor. This has proved to be just one of its many roles (including the mediation of penile erection, would you believe it!), but of major interest in the present context is its formation by an inducible NO· synthase (iNOS) within most cells, but particularly macrophages and human neutrophils, thereby generating a powerful antimicrobial system (Figure 1.16b).

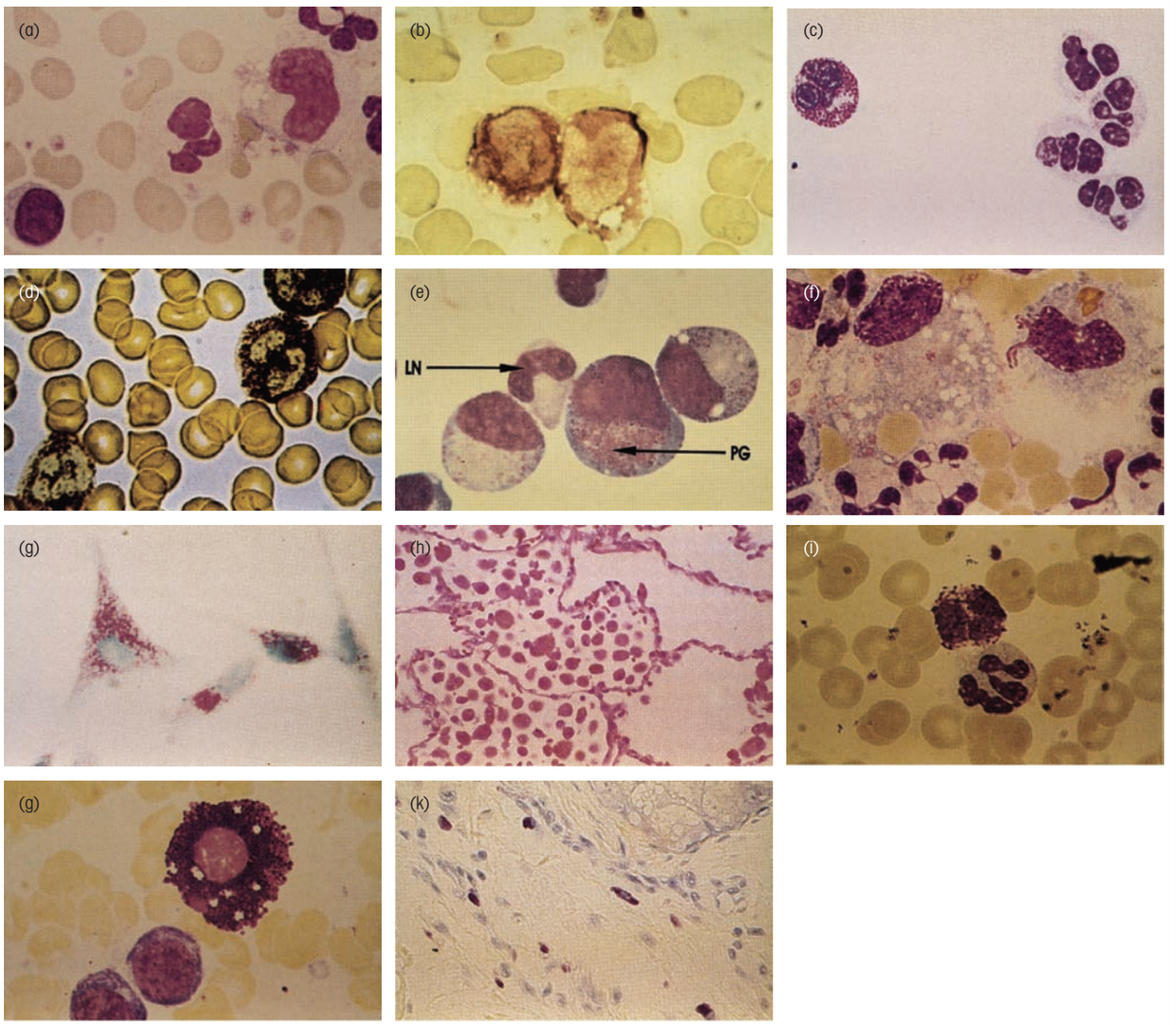


Figure 1.10. Cells involved in innate immunity.

(a) Monocyte, showing "horseshoe-shaped" nucleus and moderately abundant pale cytoplasm. Note the three multilobed polymorphonuclear neutrophils and the small lymphocyte (bottom left). Romanowsky stain. (b) Two monocytes stained for nonspecific esterase with α-naphthyl acetate. Note the vacuolated cytoplasm. The small cell with focal staining at the top is a T-lymphocyte. (c) Four polymorphonuclear neutrophils and one eosinophil. The multilobed nuclei and the cytoplasmic granules are clearly shown, those of the eosinophil being heavily stained. (d) Polymorphonuclear neutrophil showing cytoplasmic granules stained for alkaline phosphatase. (e) Early neutrophils in bone marrow. The primary azurophilic granules (PG), originally clustered near the nucleus, move towards the periphery where the neutrophil-specific granules are generated by the Golgi apparatus as the cell matures. The nucleus gradually becomes lobular (LN). Giemsa. (f) Inflammatory cells from the site of a brain hemorrhage showing the large active macrophage in the center with phagocytosed red cells and prominent vacuoles. To the right is a monocyte with horseshoe-shaped nucleus and cytoplasmic bilirubin crystals (hematoidin). Several multilobed neutrophils are clearly delineated. Giemsa. (g) Macrophages in monolayer cultures after phagocytosis of mycobacteria (stained red). Carbol-fuchsin counterstained with malachite green. (h) Numerous plump alveolar macrophages within air spaces in the lung. (i) Basophil with heavily staining granules compared with a neutrophil (below). (j) Mast cell from bone marrow. Round central nucleus surrounded by large darkly staining granules. Two small red cell precursors are shown at the bottom. Romanowsky stain. (k) Tissue mast cells in skin stained with toluidine blue. The intracellular granules are metachromatic and stain reddish purple. Note the clustering in relation to dermal capillaries. (The slides from which illustrations (a), (b), (d–f), (i) and (j) were reproduced were very kindly provided by Mr. M. Watts of the Department of Haematology, Middlesex Hospital Medical School; (c) was kindly supplied by Professor J.J. Owen; (g) by Professors P. Lydyard and G. Rook; (h) by Dr. Meryl Griffiths; and (k) by Professor N. Woolf.)