

SIXTH EDITION

ESSENTIAL IMMUNOLOGY

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PREFACE

As one writes new editions of a book, one tends to fit the new advances into the overall structure of the first edition, not wishing to 'savage' the baby too severely. However, in the 17 years since the first edition appeared, my broad perception of the field has changed so much that an utterly radical reshaping of the book's structure now seemed inescapable. Thus, in many aspects this may be looked upon as a new book.

From the physiological standpoint, immunological systems evolved to provide protection against infection and it is logical therefore to begin with an account of the principal mechanisms which constitute the phylogenetically earlier innate immune system, phagocytosis, complement and other soluble microbicidal factors, and non-specific killer cells. The second chapter then shows how this innate immune system can be unbelievably improved by the operation of an adaptive or acquired system based upon lymphocytes. We note also that this system may occasionally produce unwanted effects and give rise to immunopathology. Thus, the first two chapters provide a general overview of the subject.

Another major decision was to treat B- and T-cell activities in parallel. Thus, Chapter 3, which is concerned with molecules involved in the recognition of antigen, includes not only immunoglobulins, but also the T-cell receptor and perforce the major histocompatibility complex; this also seemed the right place to discuss the generation of diversity in receptors. Chapter 4 deals with the nature of the

primary antigen recognition process with an extended discussion of what the T-cell 'sees'. The detection of this recognition process and the technical applications to which this gives rise are covered in the following chapter.

The next four chapters deal with the acquired immune response as a consequence of antigen recognition. Chapter 6 discusses the early activation of lymphocytes and the anatomy of the lymphoid system where this occurs, while Chapter 7 goes on to cover the production of effectors — maturation to antibody-forming cells, monoclonal antibodies, T-cell lymphokines affecting B-cell and macrophage responses, and cytotoxic T-cells — all involving considerable updating. The next chapter looks at control of the acquired response by antibody, T-cells and idiotypic networks and extends the discussion to networks linking the neurological and endocrine systems. A far more expanded account of the ontogeny of B- and T-cells is given in Chapter 9 and a large new section on lymphoproliferative disorders arising as unregulated developments of lymphoid cells is now included; finally, a discussion of phylogeny draws attention to the large family of recognition molecules based on the immunoglobulin domain ancestral gene.

Immunity to infection in Chapter 10 is examined from the point of view of the adversarial strategies of host and microbe engaged in unremitting warfare. Newer approaches to vaccines based on recombinant DNA technology, synthetic peptides and anti-idiotypes are looked at in Chapter 11, with the

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section on immunodeficiency including a far more expansive account of AIDS than the last edition, as might be expected.

Turning to immunopathology, the newer advances in our knowledge of IgE receptors, the biochemistry of mast cell activation and the importance of late reactions in atopic allergy are incorporated in the chapter on hypersensitivity. In the transplantation chapter (13), I highlight some of the work on T-cell anti-idiotypic suppression of allograft reactivity and on the increasing importance of cyclosporin; the developments in cancer immunotherapy based on biological response modifiers such as IL-2, inter-

ferons and colony stimulating factors are also given a good run. Autoimmunity is split into two chapters and is structured more positively. A full account of the pathogenesis of rheumatoid arthritis focuses on recent work concerning glycosylation defects in the IgG molecule which raises the issue of oligosaccharide changes as a possible factor in the causation of other disorders.

The new two-column format should make the book easier to read. The diagrams have been greatly improved by the consultant art designer, Mike Rubens, and further colour plates have been added. I hope the reader will enjoy this new edition.

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With great pleasure I acknowledge my gratitude to J.R. Batchelor, P.C.L. Beverley, J. Brostoff, A. Cooke, M. Feldmann, J.W. Hadden, F.C. Hay, P.G. Isaacson, P.M. Lydyard, D.K. Male, D.Y. Mason, J.H.L. Playfair, J. Taverne, N. Woolf for invaluable discussions and M.D. Cooper, H.M. Geysen, T. Ishisaka, H. Metzgar, G. Mitchell, S.A. Rosenberg, G.L. Smith, J.W. Yewdell for sending preprints of their articles. I am indebted also to my co-editors, J. Brostoff and D.K. Male, the publishers Gower Medical Publishing Ltd. and the following individuals for permission to utilize or modify their figures which have appeared in *Immunology*: J. Brostoff and T. Hall for Figs 1.12, 1.14 and 11.10, F.C. Hay for Figs 3.24, 3.27

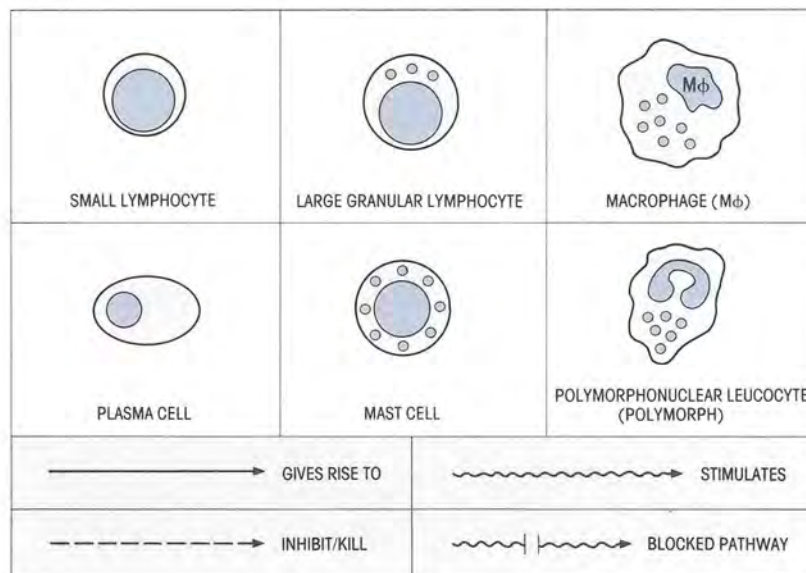
and 12.18, J. Horton for Fig. 9.16, J. Howard for Fig. 8.18, D.K. Male and D.L. Brown for Figs 1.12 and 11.10, D.K. Male for Fig. 2.14, G. Rook for Figs 10.1 and 10.5, M. Steward for Fig. 4.8, J. Taverne for Figs 10.9, 10.14, 10.16 and Table 10.2.

I am especially grateful to Mike Rubens for his creative design work on the figures, to Mary-Clare Swatman for her friendly, excellent and unflappable editorship and John Robson for his expert overall supervision. My particular gratitude goes to my secretary, Christine Meats, who was just incredibly fast, accurate and so cheerful, and to my wife, Margaret, who supported me so ably and lovingly for what must have seemed an interminable time!

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USER GUIDE

Throughout the illustrations standard forms have been used for commonly-occurring cells and pathways. A key to these is given in the figure below.



CHAPTER 1

THE BASIS OF IMMUNOLOGY

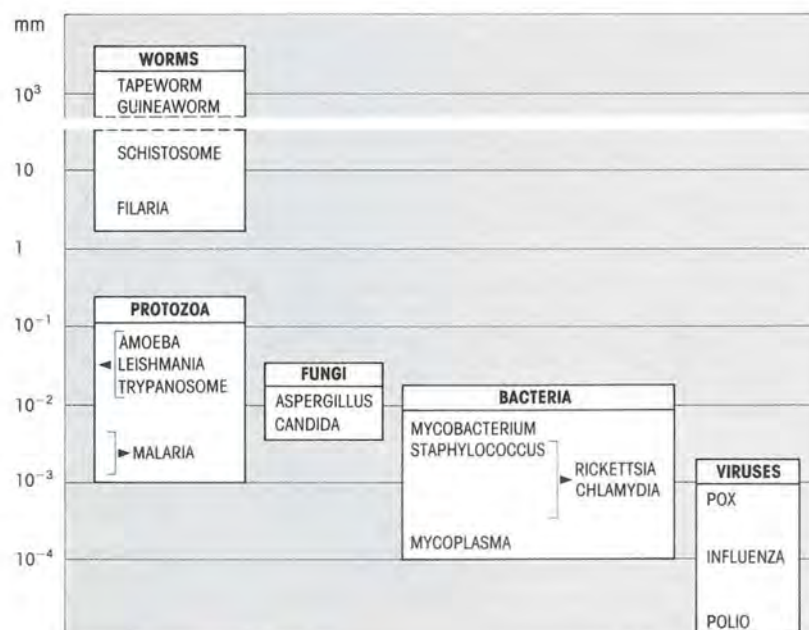
I – Innate Immunity

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 which would very happily use us as rich sanctuaries for propagating their 'selfish genes' had we not also developed a series of defence mechanisms at least their equal in effectiveness and ingenuity (except in the case of many parasitic infections where the situation is best described as an uneasy and often unsatisfactory truce). It is these defence

mechanisms which 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 which make one species innately susceptible and another resistant to certain infections, a number of non-specific anti-microbial systems (e.g. phagocytosis) have been recognized which are 'innate' in the sense that they are not intrinsically affected by

Figure 1.1. The formidable range of infectious agents which confront 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.



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.

Barriers against infection

The simplest way to avoid infection is to prevent the micro-organisms from gaining access to the body (figure 1.2). The major line of defence is of course the skin which, when intact, 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 which they generate. An exception is *Staphylococcus aureus* which often infects the relatively vulnerable hair follicles and glands.

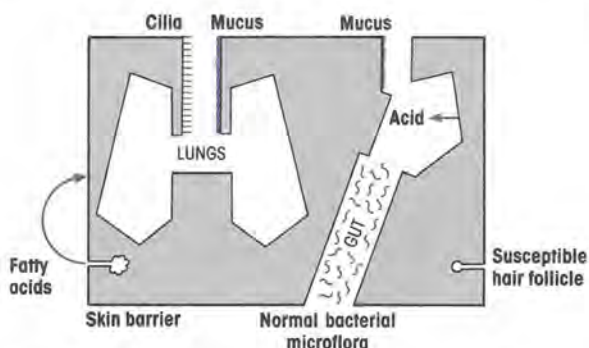


Figure 1.2. The first lines of defence against infection: protection at the external body surfaces.

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 which help protect the epithelial surfaces, one should also include the washing action of tears,

Figure 1.3 continued

neutrophil (below). (j) Mast cells 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

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. These suppress the growth of many potentially pathogenic bacteria and fungi at superficial sites by competition for essential nutrients or by production of inhibitory substances such as colicins or acid. To give one example, pathogen invasion is limited by lactic acid produced by particular species of commensal bacteria which 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.

If micro-organisms do penetrate the body, two main defensive operations come into play, the destructive effect of soluble chemical factors such as bactericidal enzymes and the mechanism of phagocytosis—literally ‘eating’ by the cell.

Phagocytic cells kill micro-organisms

‘Professional’ phagocytes

The engulfment and digestion of micro-organisms is assigned to two major cell types recognized by Metchnikoff at the turn of the century as *micro-* and *macrophages*.

The polymorphonuclear neutrophil

This cell, the smaller of the two, shares a common haemopoietic stem cell precursor with the other formed elements of the blood and is the dominant white cell in the bloodstream. It is a non-dividing short-lived cell with a multilobed nucleus and an array of granules which are virtually unstained by histological dyes such as haematoxylin and eosin, unlike those structures in the closely related eosinophil and basophil (figures 1.3 and 1.5). The neutrophil granules are of three types, the primary

dermal capillaries. The slides from which illustrations (a), (b), (d), (e), (f), (i) and (j) were reproduced were very kindly provided by Mr M. Watts of the Dept. Haematology, Middlesex Hospital Medical School; (c) was kindly supplied by Prof. J.J. Owen, (g) by Drs P. Lydyard and G. Rook, (h) by Dr Meryl Griffiths and (k) by Prof. N. Woolf.

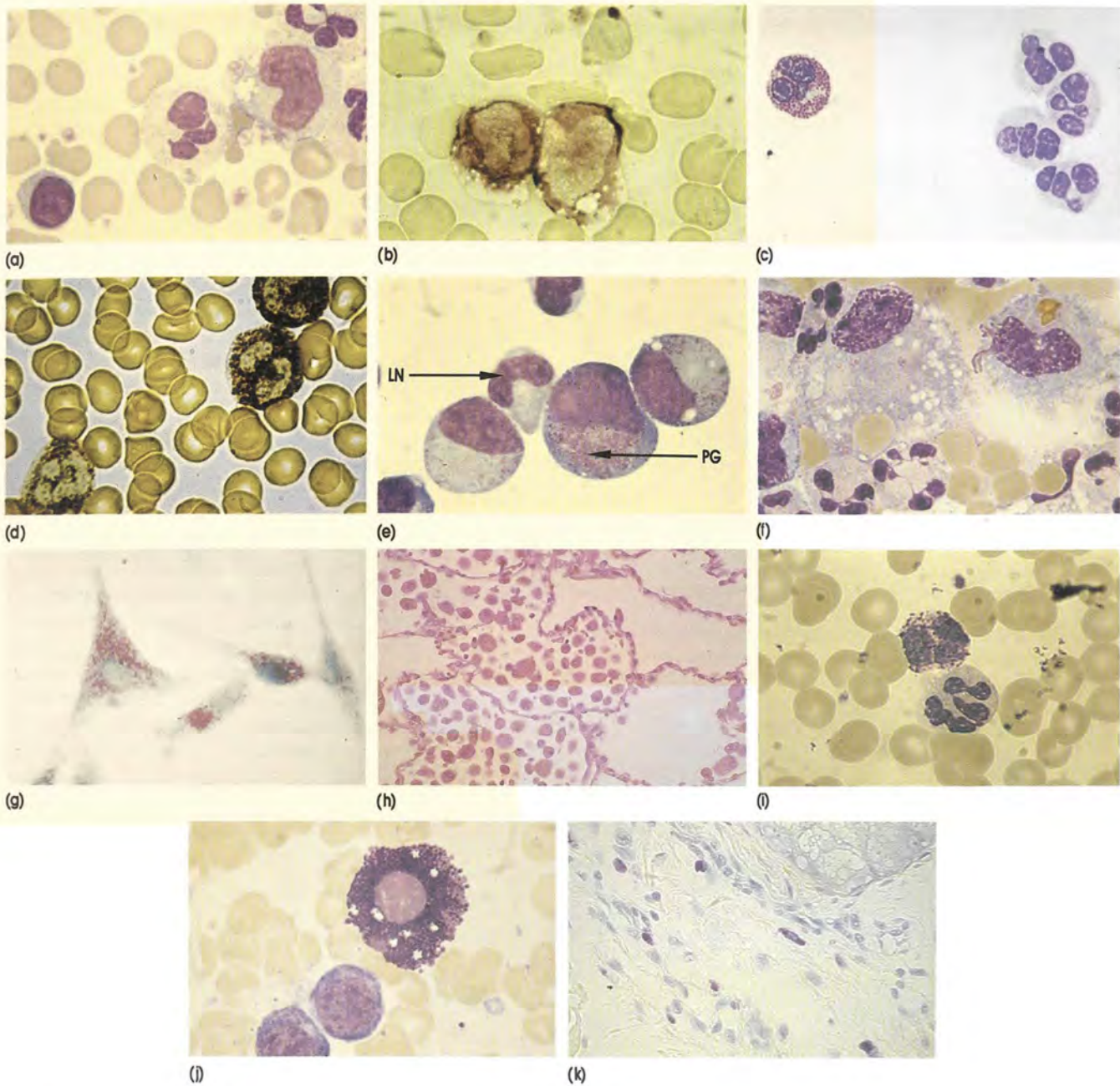


Figure 1.3. Cells involved in innate immunity. (a) Monocyte, showing 'horseshoe-shaped' nucleus and moderately abundant pale cytoplasm. Note the three multilobed polymorphonuclear neutrophils and the small lymphocyte (bottom left). Romanowsky stain. (b) Two monocytes stained for non-specific esterase with α -naphthyl acetate. Note the vacuolated cytoplasm. The small cell with focal staining at the top is a T-lymphocyte. (c) Four polymorphonuclear leucocytes (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 haemorrhage showing the large active macrophage in the centre with phagocytosed red cells and prominent vacuoles. To the right is a monocyte with horseshoe-shaped nucleus and cytoplasmic bilirubin crystals (haematoidin). 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

INNATE IMMUNITY

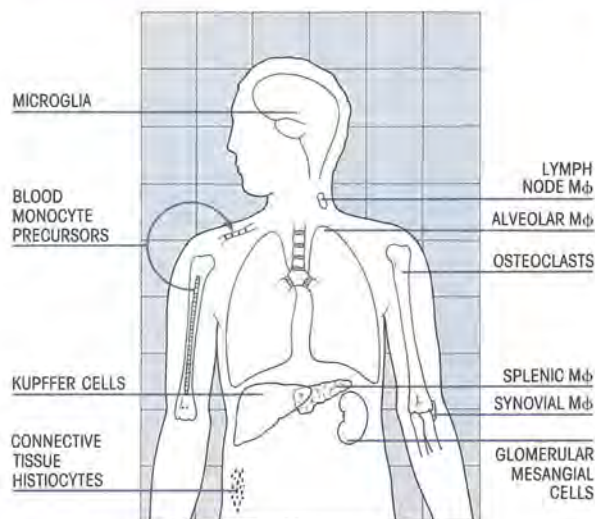


Figure 1.4. The mononuclear phagocyte system (previously included with endothelial cells and polymorphs under the term 'the reticuloendothelial system' or RES). Promonocyte precursors in the bone marrow develop into circulating blood monocytes which eventually become distributed throughout the body as mature macrophages ($M\phi$) as shown. The other major phagocytic cell, the polymorphonuclear neutrophil, is largely confined to the bloodstream except when recruited into sites of acute inflammation.

azurophilic granule containing myeloperoxidase, some lysozyme and a family of cationic proteins, the secondary 'specific' granules holding lactoferrin, lysozyme and a B_{12} -binding protein and tertiary granules akin to the conventional lysosomes with acid hydrolases. The abundant glycogen stores can be utilized by glycolysis enabling the cells to function under anaerobic conditions.

The macrophage

These cells derive from bone marrow promonocytes which, after differentiation to blood monocytes, finally settle in the tissues as mature macrophages where they constitute the *mononuclear phagocyte system* (figure 1.4). They are present throughout the connective tissue and around the basement membrane of small blood vessels and are particularly concentrated in the lung (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 the polymorphs, they are long-lived cells with significant rough-surfaced endoplasmic reticulum and mitochondria (figure 1.5) and whereas

the polymorphs provide the major defence against pyogenic (pus-forming) bacteria, as a rough generalization it may be said that macrophages are at their best in combating those bacteria (figure 1.3g), viruses and protozoa which are capable of living within the cells of the host.

Phagocytosis

Before phagocytosis can occur, the microbe must first adhere to the surface of the polymorph or macrophage, an event mediated by some rather primitive recognition mechanism likely to involve carbohydrate elements. Depending on its nature, a particle attached to the surface membrane may initiate the ingestion phase by activating an actin-myosin contractile system which extends pseudopods around the particle (figures 1.6 and 1.7); 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 1.6 and 1.8). Events are now moving smartly and within one minute the cytoplasmic granules fuse with the phagosome and discharge their contents around the imprisoned micro-organism (figure 1.8) which is subject to a formidable battery of microbicidal mechanisms.

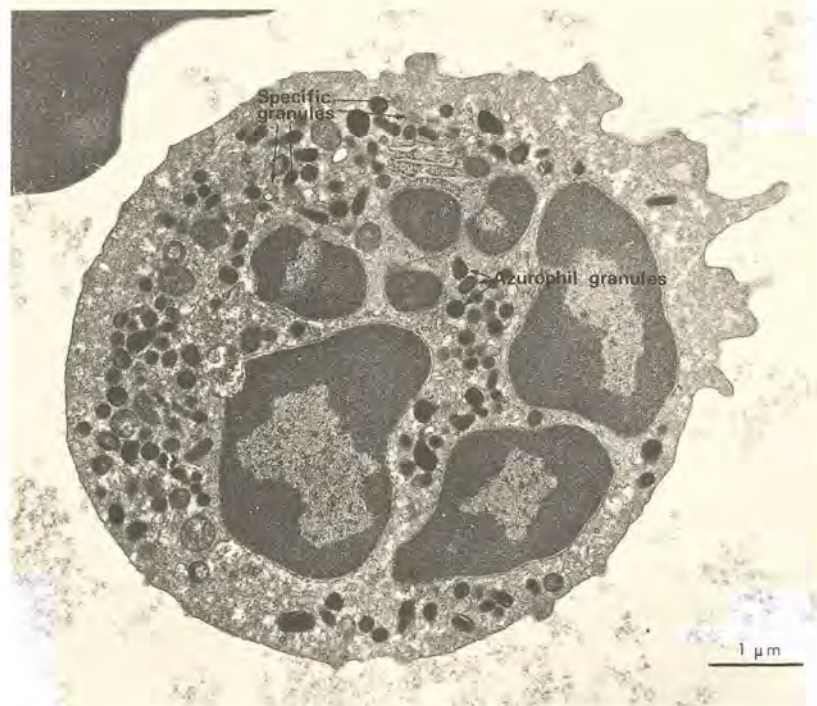
Killing

Oxygen-dependent mechanisms

Trouble starts for the invader from the moment phagocytosis is initiated. There is a dramatic increase in activity of the hexose monophosphate shunt generating NADPH. This is utilized ultimately to reduce molecular oxygen bound to a unique plasma-membrane cytochrome ($cyt\ b_{-245}$) causing a burst of oxygen consumption. As a result, oxygen is converted to superoxide anion, hydrogen peroxide, singlet O_2 and hydroxyl radicals, all of which are powerful microbicidal agents. Furthermore, the combination of peroxide, myeloperoxidase and halide ions constitutes a potent halogenating system capable of killing both bacteria and viruses (table 1.1).

Oxygen-independent mechanisms

It can also be seen from table 1.1 that the dismutation of superoxide consumes hydrogen ions and raises the pH gently so allowing the family of cationic proteins to function optimally. Although not



(a)



(b)

Figure 1.5. Ultrastructure of phagocytic cells. (a) Neutrophil. The multi-lobed nucleus and cytoplasmic granules are well displayed. (Courtesy of Dr D. McLaren.) (b) Monocyte ($\times 10\,000$). 'Horseshoe' nucleus. Phagocytic and pinocytotic vesicles, lysosomal granules, mitochondria and isolated profiles of rough-surfaced endoplasmic reticulum are evident.

fully characterized yet, they damage bacterial membranes both by neutral proteinase (cathepsin G) action and by direct transfer to the microbial surface. Low pH, lysozyme and lactoferrin constitute bactericidal or bacteriostatic factors which are oxygen independent and can function under anaerobic circumstances. Finally, the killed organisms are digested by hydrolytic enzymes and the degradation products released to the exterior (figure 1.7).

By now, the reader may be excused a little smugness as she or he shelters behind the impressive anti-microbial potential of the phagocytic cells. But there are snags to consider; our formidable array of weaponry is useless unless the phagocyte can (i) 'home onto' the micro-organism, (ii) adhere to it, and (iii) respond by the membrane activation which initiates engulfment. Some bacteria do produce chemical substances which directionally attract

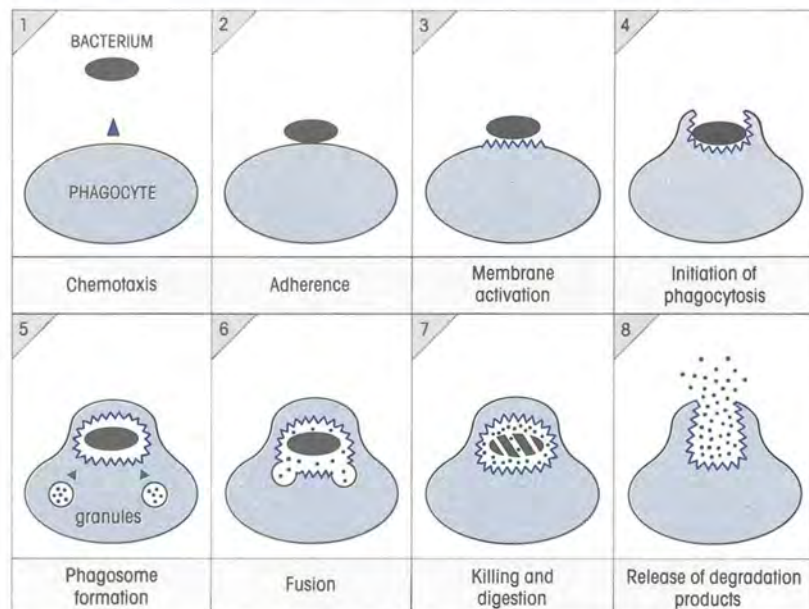


Figure 1.6. Phagocytosis and killing of a bacterium.

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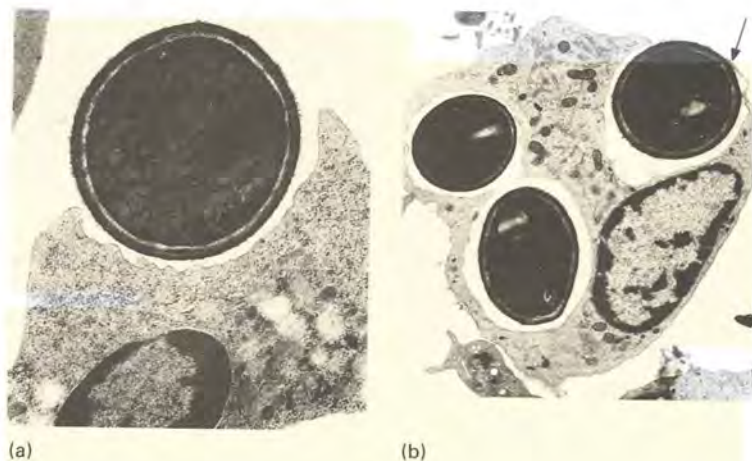


Figure 1.7. Adherence and phagocytosis. (a) Phagocytosis of *Candida albicans* by a polymorphonuclear leucocyte (neutrophil). Adherence to the surface initiates enclosure of the fungal particle within arms of cytoplasm. Lysosomal granules are abundant but mitochondria are rare ($\times 15\,000$). (b) Phagocytosis of *C. albicans* by a monocyte showing near completion of phagosome formation (arrowed) around one organism and complete ingestion of two others ($\times 5000$). (Courtesy of Dr H. Valdimarsson.)



Figure 1.8. Phagolysosome formation. (a) Neutrophil 30 minutes after ingestion of *C. albicans*. The cytoplasm is already partly degranulated and two lysosomal granules (arrowed) are fusing with the phagocytic vacuole. Two lobes of the nucleus are evident ($\times 5000$). (b) Higher magnification of (a) showing fusing granules discharging their contents into the phagocytic vacuole (arrowed). ($\times 33\,000$; courtesy of Dr H. Valdimarsson.)

OXYGEN-DEPENDENT MECHANISMS:

Glucose + NADP ⁺	hexose monophosphate shunt	pentose phosphate + NADPH	O ₂ burst + generation of superoxide anion
NADPH + O ₂	cytochrome b ₂₄₅	NADP ⁺ + O ₂ ⁻	
2O ₂ ⁻ + 2H ⁺	spontaneous dismutation	H ₂ O ₂ + ¹ O ₂	Spontaneous formation of further microbicidal agents
O ₂ ⁻ + H ₂ O ₂		.OH + OH + ¹ O ₂	
H ₂ O ₂ + Cl ⁻	myeloperoxidase	OCl ⁻ + H ₂ O	Myeloperoxidase generation of microbicidal molecules
OCl ⁻ + H ₂ O		¹ O ₂ + Cl ⁻ + H ₂ O	
2O ₂ ⁻ + 2H ⁺	superoxide dismutase	O ₂ + H ₂ O ₂	Protective mechanisms used by host + many microbes
2H ₂ O ₂	catalase	2H ₂ O + O ₂	

OXYGEN-INDEPENDENT MECHANISMS:

Cationic proteins (incl. cathepsin G)	Damage to microbial membranes
Lysozyme	Splits mucopeptide in bacterial cell wall
Lactoferrin	Deprives proliferating bacteria of iron
Proteolytic enzymes	Digestion of killed organisms
Variety of other hydrolytic enzymes	

Table 1.1. Anti-microbial systems in phagocytic vacuoles. Microbicidal species in bold letters. O₂⁻ superoxide anion; ¹O₂, singlet (activated) oxygen; OH, hydroxyl free radical.

leucocytes, a process known as chemotaxis; some organisms do adhere to the phagocyte surface and some do spontaneously provide the appropriate membrane initiation signal. However, our teeming microbial adversaries are continually mutating to produce new species which may outwit the defences by doing none of these. What then? The body has solved these problems with the effortless ease that comes with a few million years of evolution by developing the complement system.

Complement facilitates phagocytosis

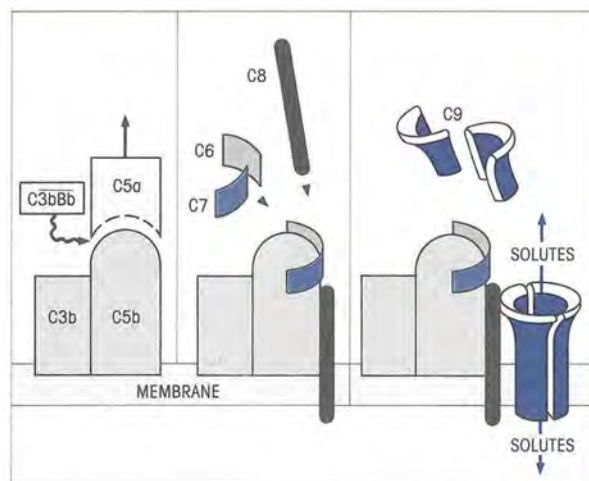
Complement and its activation

Complement is the name given to a complex series of some 20 proteins which, along with blood clotting, fibrinolysis and kinin formation, forms one of the triggered enzyme systems found in plasma. These systems characteristically produce a rapid, highly amplified response to a trigger stimulus mediated by a cascade phenomenon where the product of one reaction is the enzymic catalyst of the next.

Some of the complement components are designated by the letter 'C' followed by a number which is related more to the chronology of its discovery than to its position in the reaction sequence. The most abundant and the most pivotal component is C3 which has a molecular weight of 195 kD and is present in plasma at a concentration of around 1.2 mg/ml.

The C3 cleavage mechanism

Under normal circumstances, the internal thioester bond in C3 becomes activated at a very slow rate either through reaction with water or with trace amounts of a plasma proteolytic enzyme to form a reactive intermediate, either the split product C3b, or a functionally similar molecule. In the presence of Mg⁺⁺, this can complex with another complement component, factor B, which then undergoes cleavage by a normal plasma enzyme (factor D) to generate C3bBb. Note that conventionally, a bar over a complex denotes enzymic activity and that on cleavage of a complement component, the larger product is generally given the suffix 'b' and the smaller 'a'.



(a)



(b)

Figure 1.12. Post C3 pathway generating C5a and the C5b-9 membrane attack complex. (a) Cartoon of molecular assembly. (b) Electron micrograph of a membrane C5b-9 complex incorporated into liposomal membranes clearly showing the annular structure. The cylindrical complex is seen from the side inserted into the membrane of the liposome on the left, and end-on in that on the right (courtesy Prof. J. Trandum-Jensen and Dr S. Bhakdi).

The complement-mediated acute inflammatory reaction

We can now put together an effectively orchestrated defensive scenario initiated by activation of the alternative complement pathway (figure 1.15).

In the first act, C3bBb is stabilized on the surface of the microbe and cleaves large amounts of C3. The C3a fragment is released but C3b molecules bind copiously to the microbe. These activate the next step in the sequence to generate C5a and the membrane attack complex (although many organisms will be resistant to its action).

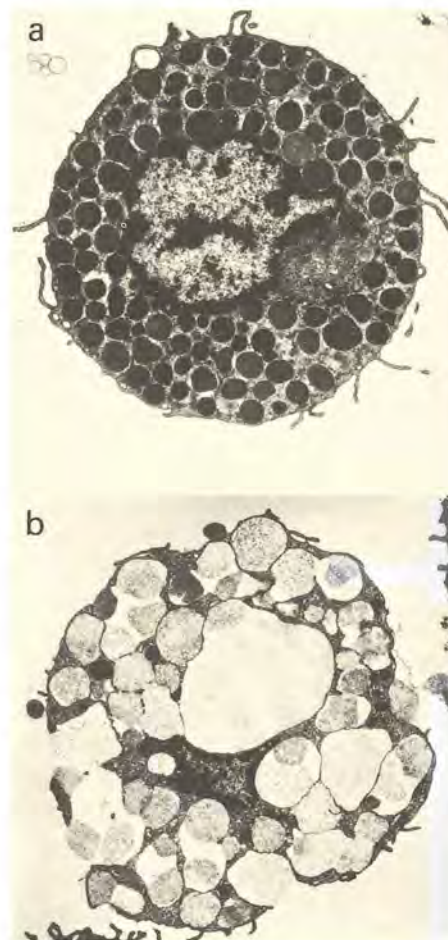


Figure 1.13. The mast cell. (a) A resting cell containing many membrane-bound granules containing pre-formed mediators. (b) A triggered mast cell. Note that the granules have released their contents and are morphologically altered, being larger and less electron dense. Although most of the altered granules remain within the circumference of the cell, they are open to the extracellular space. (Electron micrographs $\times 5400$; courtesy of Drs D. Lawson, C. Fewtrell, B. Gomperts and M. Raff; from J. Exp. Med. 1975, 142, 391.)

The next act sees C3a and C5a, together with the mediators they trigger from the mast cell, acting to recruit polymorphonuclear neutrophils and further plasma complement components to the site of microbial invasion. The relaxation induced in arteriolar walls causes increased blood flow and dilatation of the small vessels while contraction of capillary endothelial cells allows exudation of plasma proteins. Neutrophils slow down and marginate to the walls of the capillaries where they pass through gaps between the endothelial cells (diapedesis) and move up the concentration gradient of chemotactic factors until they come face to face with the

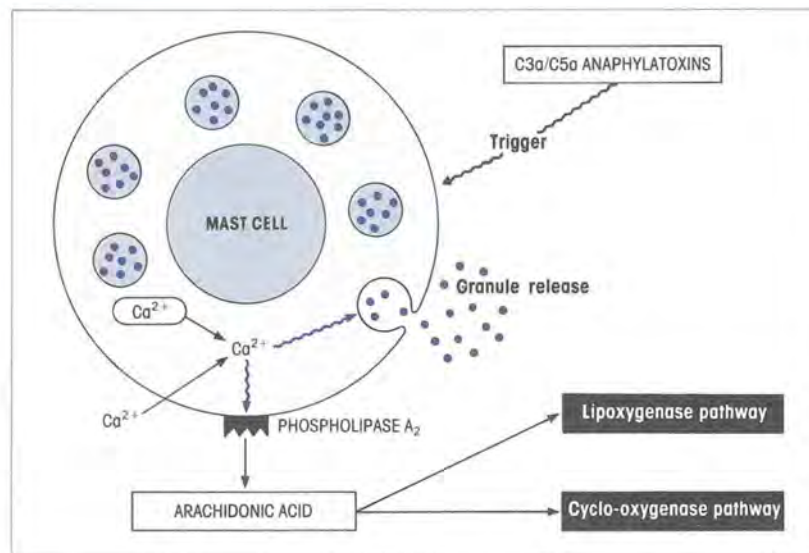


Figure 1.14. Mast cell triggering leading to release of mediators by two major pathways: (i) release of pre-formed mediators present in the granules, and (ii) the metabolism of arachidonic acid produced through activation of a phospholipase. Intracellular Ca^{2+} and cAMP are central to the initiation of these events but details are still unclear. (ECF = eosinophil chemotactic factor; NCF = neutrophil chemotactic factor. Chemotaxis refers to directed migration of granulocytes up the concentration gradient of the mediator while chemokinesis describes randomly increased motility of these cells.)

	PRE-FORMED	EFFECT
Granule release	HISTAMINE	Vasodilation, incr. capillary permeability, chemokinesis, bronchoconstriction
	HEPARIN	Anti-coagulant
	TRYPTASE	Activates C3
	β -GLUCOSAMINIDASE	Splits off glucosamine
	ECF	Eosinophil chemotaxis
	NCF	Neutrophil chemotaxis
	PLATELET ACTIVATING FACTOR	Mediator release
	NEWLY SYNTHESIZED	EFFECT
Lipoxygenase pathway	LEUKOTRIENES C_4 AND D_4 (SRS-A) LEUKOTRIENE B_4	Vasoactive, bronchoconstriction, chemotaxis and/or chemokinesis
Cyclo-oxygenase pathway	PROSTAGLANDINS THROMBOXANES	Affect bronchial muscle, platelet aggregation and vasodilation

C3b-coated microbe. Adherence to the neutrophil C3b-receptors then takes place, C3a and C5a activate the respiratory burst and, hey presto, the slaughter of the last act can begin!

The processes of capillary dilatation (redness), exudation of plasma proteins and also of fluid (oedema) due to hydrostatic and osmotic pressure changes, and accumulation of neutrophils are collectively termed the *acute inflammatory response*.

Humoral mechanisms provide a second defensive strategy

Turning now to those defence systems which are mediated entirely by soluble factors, we recollect

that many microbes activate the complement system and may be lysed by the insert of the membrane attack complex. The spread of infection may be limited by enzymes released through tissue injury which activate the clotting system. Of the soluble bactericidal substances elaborated by the body, perhaps the most abundant and widespread is the enzyme lysozyme, a muramidase which splits the exposed peptidoglycan wall of susceptible bacteria (cf. figure 10.1).

Acute phase proteins

A number of plasma proteins collectively termed *acute phase proteins* show a dramatic increase in

concentration in response to infection or tissue injury. These include C-reactive protein (CRP), serum amyloid A protein, α_1 -anti-trypsin, α_2 -macroglobulin, fibrinogen, caeruloplasmin, C9 and factor B.

Human CRP is composed of five identical polypeptide units non-covalently arranged as a cyclic pentamer. This class of protein has been around in the animal kingdom for some time since a closely related homologue, limulin, is present in the haemolymph of the horseshoe crab, not exactly a close relative of *Homo sapiens*. A major property of CRP is its ability to bind, in a Ca-dependent fashion, to a number of micro-organisms which contain phosphorylcholine in their membranes, the complex

Interferons

These are a family of broad-spectrum anti-viral agents present in birds, reptiles and fishes as well as the higher animals, and first recognized by the phenomenon of viral interference in which an animal infected with one virus resists superinfection by a second unrelated virus. Different molecular forms of interferon have been identified, all of which have been gene cloned. There are at least 14 different α -interferons (IFN α) produced by lymphocytes while fibroblasts, and probably all cell types, synthesize IFN β . We will keep a third type (IFN γ), which is not directly induced by viruses, up our sleeves for the moment.

Cells synthesize interferon when infected by a virus and secrete it into the extracellular fluid whence it binds to specific receptors on uninfected neighbouring cells. The bound interferon now exerts its anti-viral effect in the following way. At least two genes are thought to be derepressed in the interferon-treated cell allowing the synthesis of two new enzymes. The first, a protein kinase, catalyses the phosphorylation of a ribosomal protein and an initiation factor necessary for protein synthesis, so greatly reducing mRNA translation. The other catalyses the formation of a short polymer of adenylic acid which activates a latent endonuclease; this in turn degrades both viral and host mRNA.

Whatever the precise mechanism of action ultimately proves to be, the net result is to establish a cordon of uninfected cells around the site of virus infection so restraining its spread. The effectiveness of interferon *in vivo* may be inferred from experiments in which mice injected with an antiserum to murine interferons could be killed by several hundred times less virus than was needed to kill the controls. However, it must be presumed that interferon plays a significant role in the recovery from, as distinct from the prevention of, viral infections.

As a group, the interferons may prove to have a wider biological role than the control of viral infection. It will be clear, for example, that the induced enzymes described above would act to inhibit host cell division just as effectively as viral replication. The interferons may also modulate the activity of other cells such as the natural killer (NK) cells, to be discussed in the following section.

Extracellular killing

Natural killer (NK) cells

Viruses lack the apparatus for self-renewal so it is essential for them to penetrate the cells of the infected host in order to take over its replicative machinery. It is clearly in the interest of the host to find a way to kill such infected cells *before* the virus has had a chance to reproduce. NK cells appear to do just that when studied *in vitro*.

They are large granular lymphocytes (figure 1.3) with a characteristic morphology (figure 2.6b). They are thought to recognize structures on high molecular weight glycoproteins which appear on the surface of virally infected cells and which allow them to be differentiated from normal cells. This recognition occurs through receptors on the NK cell

surface which bring killer and target into close opposition. Activation of the NK cell ensues and leads to extracellular release of granule contents into the space between the two cells. Perhaps the most important of these is a *perforin* or *cytolysin* bearing some structural relationship to C9; like that protein, it can insert itself into the membrane of the target and associate to form a transmembrane pore with an annular structure, comparable to the complement membrane attack complex, which induces cell death. Antibodies made against the cytolysin inhibit this extracellular killing. The granules also contain two serine esterases which can function as NK cytotoxic factors but the time course of their action casts some doubt over the primacy of their role in NK-mediated lysis. Chondroitin sulphate A, a protease-resistant highly negatively charged proteoglycan, is also present and may subserve the function of protecting the NK cell from autolysis by its own lethal agents (figure 1.16).

The various interferons augment NK cytotoxicity and since interferons are produced by virally infected cells, we have a nicely integrated feedback defence system.

Eosinophils

Large parasites such as helminths cannot physically be phagocytosed and extracellular killing by eosinophils would seem to have evolved to help cope with

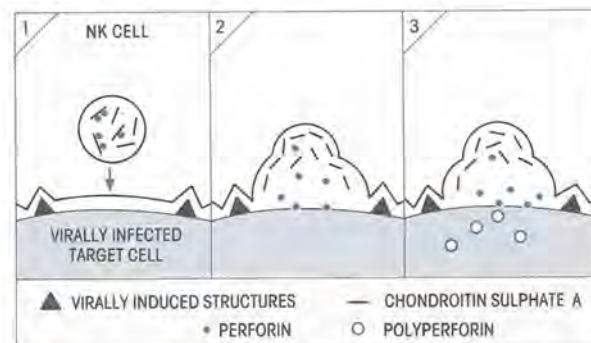


Figure 1.16. Extracellular killing of virally infected cell by natural killer (NK) cell (following diagram by Tschopp J. & Conzelmann A. In *Immunology Today* 1986, 7, 135). As the NK receptors bind to the surface of the virally infected cell, the granules release perforin molecules extracellularly; these polymerize to form transmembrane channels which bring about lysis of the target. Tschopp & Conzelmann have shown that chondroitin sulphate A binds to and inactivates the perforin particularly at the low pH within the granule and suggest that as the granule discharges, the higher pH dissociates the perforin which then acts to lyse the target cell.

this situation. These polymorphonuclear 'cousins' of the neutrophil have distinctive granules which stain avidly with acid dyes (figure 1.3) and have a characteristic appearance in the electron microscope (figure 10.15). A major basic protein (MBP) is localized in the core of the granules while an eosinophilic cationic protein together with a peroxidase have been identified in the granule matrix. Other enzymes include arylsulphatase B, phospholipase D and histaminase. They have surface receptors for C3b and on activation produce a particularly impressive respiratory burst with concomitant generation of active oxygen metabolites. As if that were

not enough, it has recently been demonstrated that one of the granule proteins can produce a trans-membrane plug in the target membrane like C9 and the NK perforin.

Most helminths can activate the alternative complement pathway, but although resistant to C9 attack, their coating with C3b allows adherence of eosinophils through their C3b receptors. Upon activation, the eosinophil launches its extracellular attack which includes the release of MBP and especially the cationic protein which damages the parasite membrane.

CHAPTER 2

THE BASIS OF IMMUNOLOGY

II – Specific Acquired Immunity

The need for specific immune mechanisms

Our microbial adversaries have tremendous opportunities through mutation to evolve strategies which evade our innate immune defences. For example, most of the *successful* parasites activate the alternative complement pathway and bind C3b, yet eosinophils which adhere are somehow not triggered into offensive action. The same holds true for many bacteria while some may so shape their exteriors as to avoid complement activation completely. The body obviously needed to 'devise' defence mechanisms which could be dovetailed individually to each of these organisms no matter how many there were. In other words a *very large number* of specific immune defences needed to be at the body's disposal. Quite a tall order!

Antibody—the specific adaptor

Evolutionary processes came up with what can only be described as a brilliant solution. This was to fashion an adaptor molecule which was intrinsically capable not only of activating the complement system *and* of stimulating phagocytic cells, but also of sticking to the offending microbe. The adaptor thus had three main regions, two concerned in communicating with complement and the phagocytes (the biological functions) and one devoted to binding to an individual micro-organism (the ex-

ternal recognition function). In most biological systems like hormones and receptors, and enzymes and substrates, recognition usually occurs through fairly accurate complementarity in shape allowing the ligands to approach so close to each other as to permit the normal intermolecular forces to become relatively strong. In the present case, each adaptor would have a recognition portion complementary in shape to some micro-organism to which it could then bind reasonably firmly. The part of the adaptor with biological function would be constant, but for each of hundreds of thousands of different organisms, a special recognition portion would be needed.

Thus the body has to make hundreds of thousands, or even millions, of adaptors with different recognition sites. The adaptor is of course the molecule we know affectionately as *antibody* (figure 2.1).

Antibody initiates a new complement pathway ('classical')

Antibody, when bound to a microbe, will link to and activate the first molecule in the so-called classical complement sequence, C1q. This molecule is polyvalent with respect to antibody binding and consists of a central collagen-like stem branching into six peptide chains each tipped by an antibody-binding subunit (resembling the blooms on a bunch of flowers). C1q is associated with two further subunits, C1r and C1s, in a Ca^{++} stabilized trimolecular complex and these are activated sequentially

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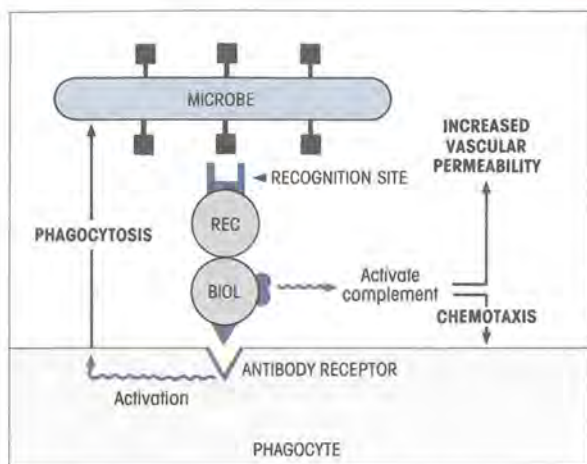


Figure 2.1. The antibody adaptor molecule. The constant part with biological function (BIOL) activates complement and the phagocyte. The portion with the recognition unit for the foreign microbe (REC) varies from one antibody to another.

by the alternative pathway. From then on things march along exactly in parallel to the post-C3 pathway with one molecule of C3b added to the C4b2b to make it into a C5-splitting enzyme with eventual production of the membrane attack complex (figure 2.2). Just as the alternative pathway C3 convertase is controlled by factors H and I, so the breakdown of C4b2b is brought about by either a C4 binding protein (C4bp) or a cell surface C3b receptor (CR1) in the presence of factor I.

The similarities between the two pathways are set out in figure 2.3 and show how antibody can supplement and even improve on the ability of the innate immune system to initiate acute inflammatory reactions. Antibody provides yet a further bonus in this respect; the class known as immunoglobulin E (see legend to figure 2.2) can sensitize mast cells through binding to their surface so that combination with antigen triggers mediator release independently of C3a or C5a, adding yet more flexibility to our defences.

Complexed antibody activates phagocytic cells

I drew attention to the fact that many C3b-coated organisms adhere to phagocytic cells yet avoid provoking their uptake. If small amounts of antibody are added the phagocyte springs into action. It does so through the recognition of two or more antibody molecules bound to the microbe, using specialized receptors on the cell surface.

A single antibody molecule complexed to the micro-organism is not enough because it cannot cause the cross-linking of antibody receptors in the



Figure 2.2. Multiple lesions in cell wall of *Escherichia coli* bacterium caused by interaction with IgM antibody and complement. (Human antibodies are divided into five main classes, immunoglobulin M—shortened to IgM—IgG, IgA, IgE and IgD, which differ in the specialization of their 'rear ends' for different biological functions such as complement activation or mast cell sensitization.) Each lesion is caused by a single IgM molecule and shows as a 'dark pit' due to penetration by the 'negative stain'. This is somewhat of an illusion since in reality these 'pits' are like volcano craters standing proud of the surface, and are each single 'membrane attack' complexes. Comparable results may be obtained in the absence of antibody since the cell wall endotoxin can activate the alternative pathway in the presence of higher concentrations of serum. Magnification $\times 400\,000$. (Courtesy of Drs R. Dourmashkin and J.H. Humphrey.)

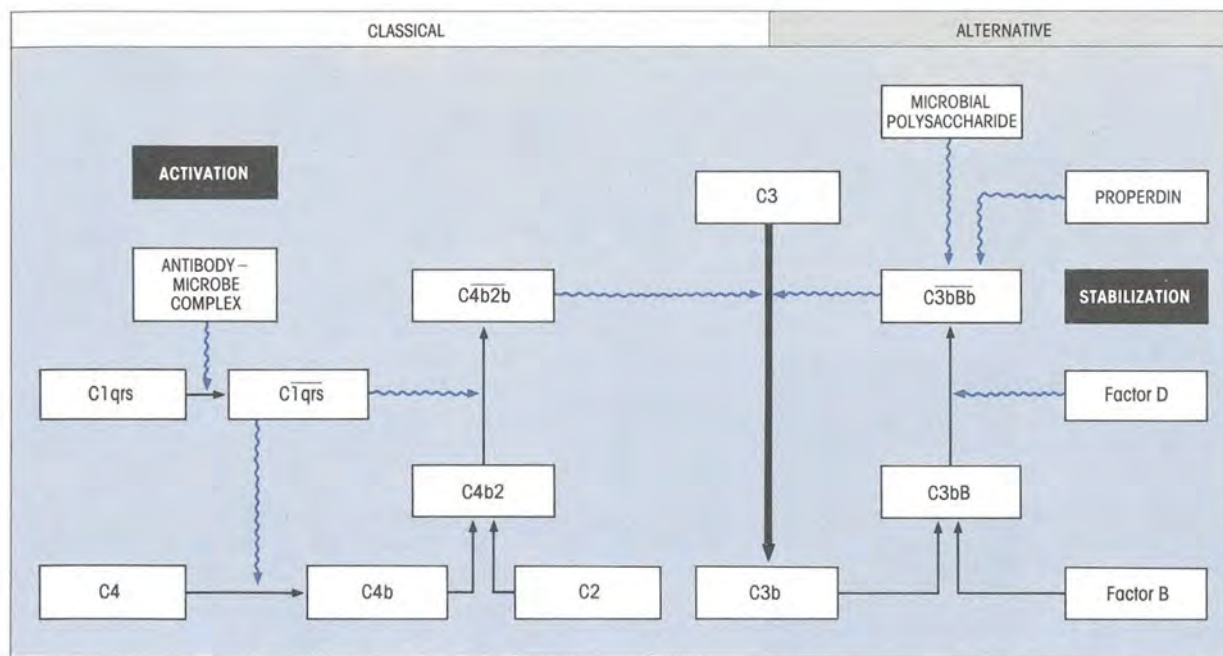


Figure 2.3. Comparison of the alternative and classical complement pathways. The classical pathway with odd

exceptions such as C-reactive protein, is antibody dependent, the alternative pathway is not.

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phagocyte surface membrane which is required to activate the cell. There is a further consideration connected with what is often called 'the bonus effect of multivalency'; for thermodynamic reasons, which will be touched on in Chapter 4, the association constant of ligands which use several rather than a single bond to react with receptors is increased geometrically rather than arithmetically. For example, three antibodies bound close together on a bacterium could be attracted to a macrophage a

thousand times more strongly than a single antibody molecule (figure 2.4).

The cellular basis of antibody production

Antibodies are made by lymphocytes

The majority of resting lymphocytes are small cells with a darkly staining nucleus due to condensed

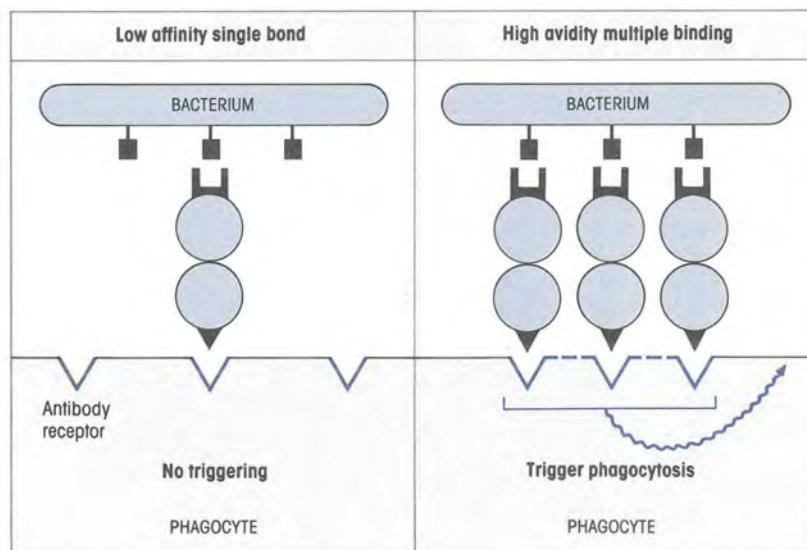
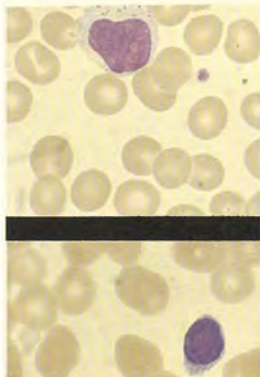
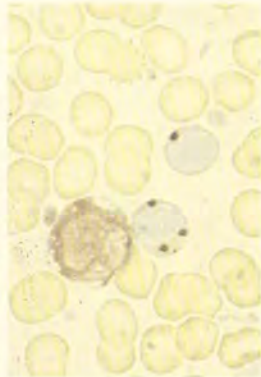


Figure 2.4. Binding of bacterium to phagocyte by multiple antibodies gives strong association forces and triggers phagocytosis by cross-linking the surface receptors for antibody.

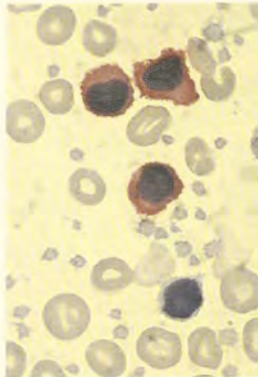
SPECIFIC ACQUIRED IMMUNITY



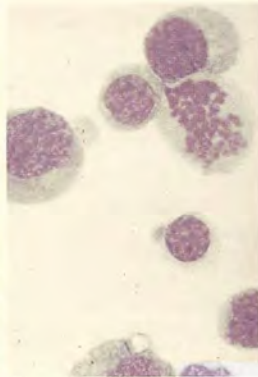
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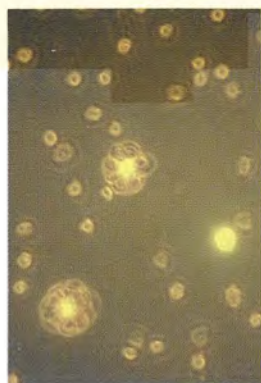
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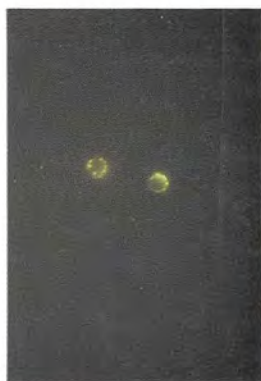
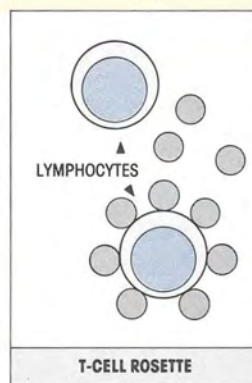
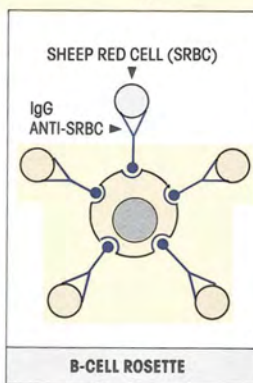
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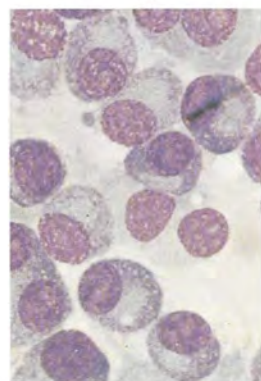
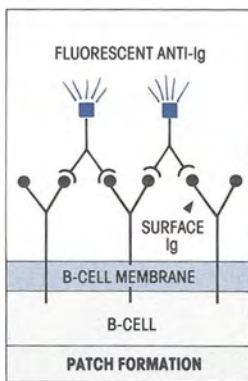
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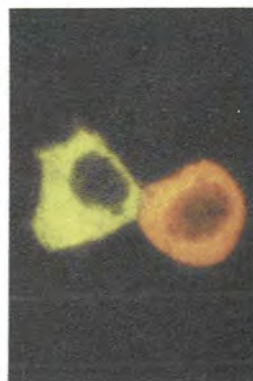
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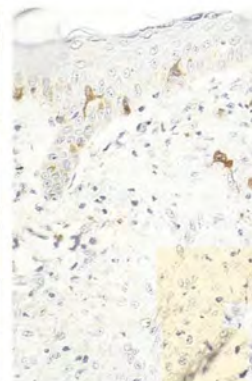
(f)



(g)



(h)



(i)

chromatin and relatively little cytoplasm containing the odd mitochondrion required for basic energy provision. Figures 2.5 and 2.6 compare the morphology of these cells with that of the minority population of large granular lymphocytes which includes the NK set referred to in Chapter 1.

The central role of the small lymphocyte in the production of antibody was established largely by the work of Gowans. He depleted rats of their lymphocytes by chronic drainage of lymph from the thoracic duct by an indwelling cannula, and showed that they had a grossly impaired ability to mount an antibody response to microbial challenge. The ability to form antibody could be restored by injecting thoracic duct lymphocytes obtained from another rat. The same effect could be obtained if, before injection, the thoracic duct cells were first incubated at 37°C for 24 hours under conditions which kill off large and medium sized cells and leave only the small lymphocytes. This shows that the small lymphocyte is necessary for the antibody response.

The small lymphocytes can be labelled if the donor rat is previously injected with tritiated thymidine; it then becomes possible to follow the fate of these lymphocytes when transferred to another rat of the same strain which is then injected with micro-organisms to produce an antibody response (figure 2.7). It transpires that after contact with the

injected microbes, some of the transferred labelled lymphocytes develop into plasma cells (figures 2.5g and 2.8) which can be shown to contain (figure 2.5h) and secrete antibody.

Antigen selects the lymphocytes which make antibody

The molecules in the micro-organisms which evoke and react with antibodies are called *antigens* (generates antibodies). It was originally envisaged that antibodies were derived from some master plastic molecule which could be moulded to the right shape by using the antigen as a template. Stimulated by the ideas of Jerne and Burnet and many others (not forgetting the incredible Ehrlich who foresaw it at the turn of the century, would you believe it?!), we now know that antibodies are formed before antigen is ever seen and that they are selected for by antigen.

It works in the following way. Each lymphocyte of a subset called the B-lymphocytes, because they differentiate in the bone marrow, is programmed to make one, and only one, antibody and it places this antibody on its outer surface to act as a receptor. This can be detected by using fluorescent probes and in figure 2.5f one can see the molecules of antibody on the surface of a human B-lymphocyte stained with a fluorescent rabbit antiserum raised against

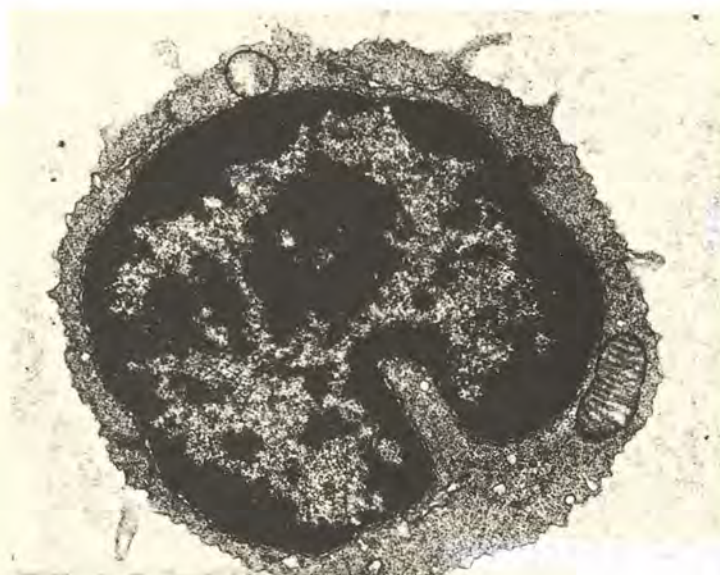
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Figure 2.5. Cells involved in the acquired immune response. (a) Small lymphocytes. Condensed chromatin gives rise to heavy staining of the nucleus. The cell on the bottom is a typical T-cell with receptors for IgM with a thin rim of cytoplasm. The upper nucleated cell is a large granular lymphocyte (LGL); it has more cytoplasm and azurophilic granules are evident. Isolated platelets are visible. B-lymphocytes range from small to intermediate in size and lack granules. Giemsa stain. (b) Small T-cells with characteristic cytoplasmic dot(s) in cytoplasm stained for acid esterase; compare with diffuse distribution in cytoplasm of monocyte above. (c) T-lymphocytes from buffy coat stained with a monoclonal anti-T using the alkaline phosphatase immunoenzymatic method (cf. figure 5.21). Note the single unstained non-T lymphocyte on the bottom. (d) Transformed lymphocytes (lymphoblasts) following stimulation of lymphocytes in culture with a polyclonal activator. The large lymphoblasts with their relatively high ratio of cytoplasm to nucleus may be compared in size with the isolated small lymphocyte. One cell is in mitosis. May-Grunewald-Giemsa. (e) Identification of lymphocytes by rosette formation with red cells visualized in U.V. light after staining with Acridine Orange which makes the lymphocyte nucleus fluorescent green. T-cell rosettes are formed by spontaneous binding to sheep erythrocytes. B-cell rosettes are developed through binding of IgG-coated

erythrocytes to specific cell surface receptors.

(f) Immunofluorescent staining of B-lymphocyte surface immunoglobulin using fluorescein-conjugated (■) anti-Ig. Provided the reaction is carried out in the cold to prevent pinocytosis, the labelled antibody cannot penetrate to the interior of the viable lymphocytes and reacts only with surface components. Patches of aggregated surface Ig are seen which are beginning to form a cap in the right-hand lymphocyte. During cap formation, submembranous myosin becomes redistributed in association with the surface Ig and induces locomotion of the previously sessile cell in a direction away from the cap. (g) Plasma cells. The nucleus is eccentric. The cytoplasm is strongly basophilic due to high RNA content. The juxta-nuclear lightly stained zone corresponds with the Golgi region. May-Grunewald-Giemsa. (h) Plasma cells stained to show intracellular immunoglobulin using a fluorescein-labelled anti-IgG (green) and a rhodamine-conjugated anti-IgM (red). (i) Langerhans cells in human epidermis in leprosy, increased in the sub-epidermal zone, possibly as a consequence of the disease process. Stained by the immunoperoxidase method with S-100 antibodies. Material for (a), (b) and (c) was kindly supplied by Mr M. Watts of the Dept. Haematology, Middlesex Hospital Medical School, (d), (e) and (f) by Dr P. Lydyard, (g) and (h) by Prof. C. Grossi and (i) by Dr Marian Ridley.



(a)



(b)

Figure 2.6. (a) Small T-lymphocyte with receptors for IgM. Indented nucleus with condensed chromatin, sparse cytoplasm: single mitochondrion shown and many free ribosomes but otherwise few organelles ($\times 13\,000$). B-lymphocytes are essentially similar with slightly more cytoplasm and occasional elements of rough-surfaced endoplasmic reticulum. (b) Large granular lymphocyte ($\times 7500$). The more abundant cytoplasm contains several mitochondria (M), free ribosomes (R) with some elements of rough-surfaced endoplasmic reticulum (ER), prominent Golgi apparatus (Go) and characteristic membrane-bound electron-dense granules (Gr). The nuclear chromatin is less condensed than that of the T_M cell. (Courtesy of Drs A. Zicca and C.E. Grossi.)

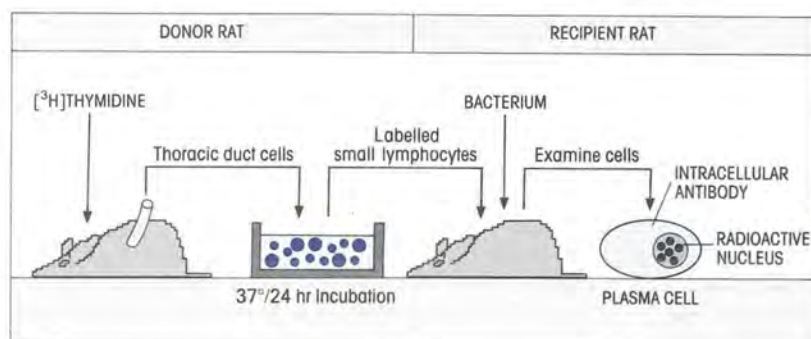


Figure 2.7. Labelled small lymphocytes become antibody-forming plasma cells. Transferred cell with radioactive nucleus shown by autoradiography. Intracellular antibody revealed by staining with fluorescent probes (cf. figure 2.5h).

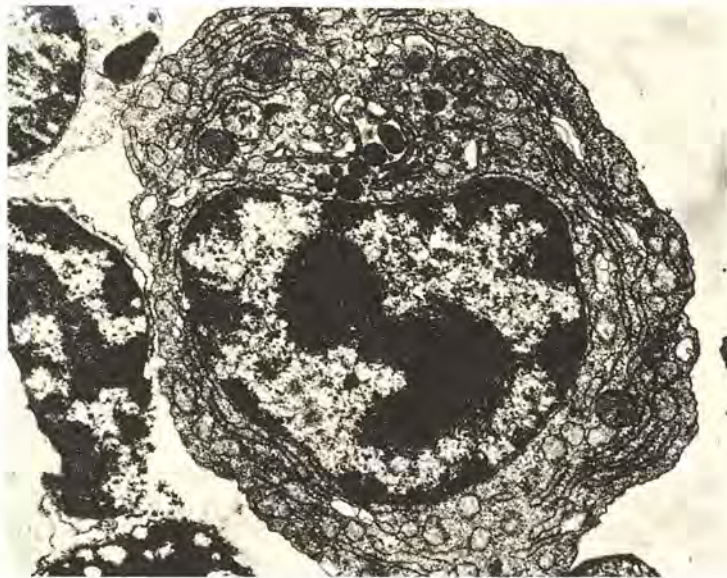


Figure 2.8. Plasma cell ($\times 10\,000$). Prominent rough-surfaced endoplasmic reticulum associated with the synthesis and secretion of Ig.

a preparation of human antibodies. Each lymphocyte has of the order of 10^5 antibody molecules on its surface.

When an antigen enters the body, it is confronted by a dazzling array of lymphocytes all bearing different antibodies each with its own individual recognition site. The antigen will only bind to those receptors with which it makes a good fit. Lymphocytes whose receptors have bound antigen receive a triggering signal and develop into antibody-forming plasma cells and since the lymphocytes are programmed to make only one antibody, that secreted by the plasma cell will be identical with that originally acting as the lymphocyte receptor, i.e. it will bind well to the antigen. In this way, antigen selects for the antibodies which recognize it effectively (figure 2.9).

The need for clonal expansion means humoral immunity must be acquired

Because we can make hundreds of thousands, maybe even millions, of different antibody molecules, it is not feasible for us to have too many lymphocytes producing each type of antibody; there just would not be enough room in the body to accommodate them. To compensate for this, lymphocytes which are triggered by contact with antigen undergo successive waves of proliferation (figure 2.5d) to build up a large clone of plasma cells which will be making antibody of the kind for which the parent lymphocyte was programmed. By this system of

clonal selection, large enough concentrations of antibody can be produced to combat infection effectively (figure 2.10).

The importance of proliferation for the development of a significant antibody response is highlighted by the ability of anti-mitotic drugs to abolish antibody production to a given antigen stimulus completely.

Because it takes time for the proliferating clone to build up its numbers sufficiently, it is usually several days before antibodies are detectable in the serum following primary contact with antigen. The newly formed antibodies are a consequence of antigen exposure and it is for this reason that we speak of the *acquired immune response*.

Acquired memory

When we make an antibody response to a given infectious agent, by definition that micro-organism must exist in our environment and we are likely to meet it again. It would make sense then for the immune mechanisms alerted by the first contact with antigen to leave behind some memory system which would enable the response to any subsequent exposure to be faster and greater in magnitude.

Our experience of many common infections tells us that this must be so. We rarely suffer twice from such diseases as measles, mumps, chicken-pox, whooping cough and so forth. The first contact clearly imprints some information, imparts some

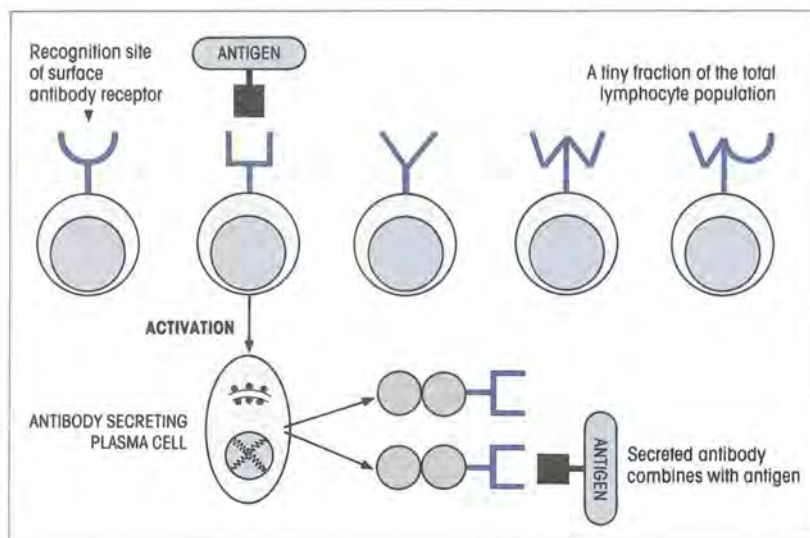


Figure 2.9. Antigen activates those B-cells whose surface antibody receptors it can combine with firmly.

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memory, so that the body is effectively prepared to repel any later invasion by that organism and a state of immunity is established.

Secondary antibody responses

By following the production of antibody on the first and second contacts with antigen we can see the basis for the development of immunity. For example, when we inject a bacterial product such as tetanus toxoid into a rabbit, for reasons already discussed, several days elapse before antibodies can be detected in the blood; these reach a peak and then fall (figure 2.11). If we now allow the animal to rest and then give a second injection of toxoid, the course of events is dramatically altered. Within two to three days the antibody level in the blood rises steeply to reach much higher values than were observed in the *primary response*. This *secondary response* then is characterized by a more rapid and more abundant production of antibody resulting from the 'tuning up' or priming of the antibody-forming system.

With our knowledge of lymphocyte function, it is perhaps not surprising to realize that these are the cells which provide memory. This can be demonstrated by adoptive transfer of lymphocytes to another animal, an experimental system frequently employed in immunology (cf. figure 2.7). In the present case, the immunological potential of the transferred cells is expressed in a recipient treated with X-rays which destroy its own lymphocyte population; thus any immune response will be of

donor not recipient origin. In the experiment described in figure 2.12, small lymphocytes are taken from an animal given a primary injection of tetanus toxoid and transferred to an irradiated host which is then boosted with the antigen; a rapid, intense production of antibody characteristic of a secondary response is seen. To exclude the possibility that the first antigen injection might exert a *non-specific* stimulatory effect on the lymphocytes, the boosting injection includes influenza haemagglutinin as a control antigen. Furthermore, a 'criss-cross' control group primed with influenza haemagglutinin must also be included to ensure that this antigen is capable of giving a secondary booster response. I have explained the design of the experiment at some length to call attention to the need for careful selection of controls.

The higher response given by a primed lymphocyte population can be ascribed mainly to an expansion of the numbers of cells capable of being stimulated by the antigen (figure 2.13) although we shall see later that there are some qualitative differences in the cells as well.

The specificity of acquired immunity

The establishment of memory or immunity by one organism does not confer protection against another unrelated organism. After an attack of measles we are immune to further infection but are susceptible to other agents such as the polio or mumps viruses. Acquired immunity then shows *specificity*

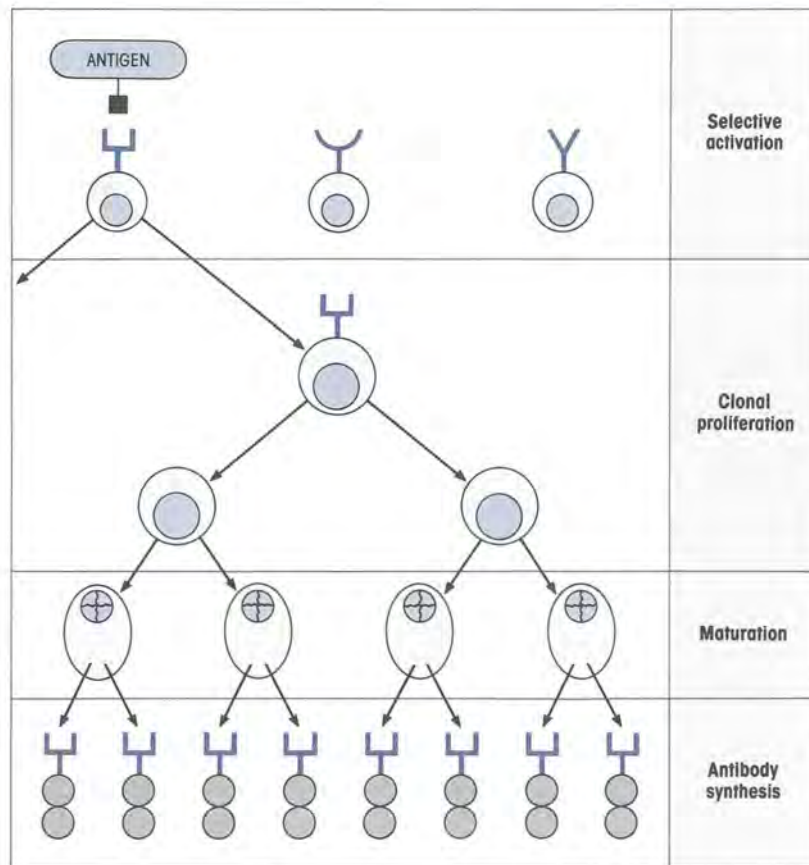


Figure 2.10. Clonal selection. The cell selected by antigen undergoes many divisions during the clonal proliferation and the progeny mature to give an expanded population of antibody-forming cells. The antibody response is particularly vulnerable to anti-mitotic agents at the proliferation stage.

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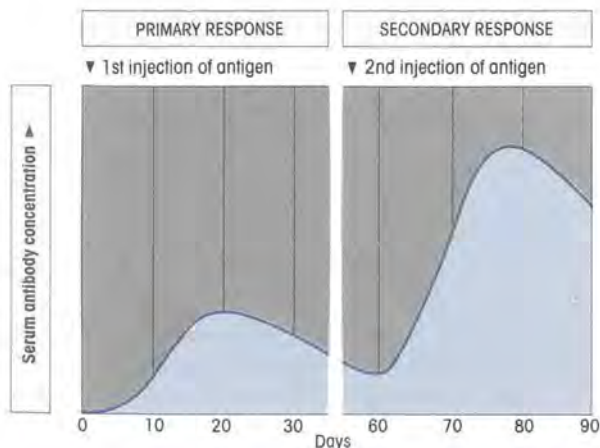


Figure 2.11. Primary and secondary response. A rabbit is injected on two separate occasions with tetanus toxoid. The antibody response on the second contact with antigen is more rapid and more intense.

tetanus toxoid evoked memory for that antigen but not for influenza and vice versa.

The basis for this lies of course in the ability of the recognition sites of the antibody molecules to distinguish between antigens; antibodies which react with the toxoid do not bind to influenza and, *mutatis mutandis* as they say, anti-influenza is not particularly smitten with the toxoid.

This ability to recognize one antigen and distinguish it from another goes even further. The individual must also recognize what is foreign, i.e. what is 'non-self'. The failure to discriminate between 'self' and 'non-self' could lead to the synthesis of antibodies directed against components of the subject's own body (*autoantibodies*) which in principle could prove to be highly embarrassing. On purely theoretical grounds it seemed to Burnet and Fenner that the body must develop some mechanism whereby 'self' and 'non-self' could be distinguished, and they postulated that those circulating body components which were able to reach the developing lymphoid system in the perinatal period could in some way be 'learnt' as 'self'. A permanent

and the immune system can differentiate specifically between the two organisms. A more formal experimental demonstration of this discriminatory power was seen in figure 2.12 where priming with

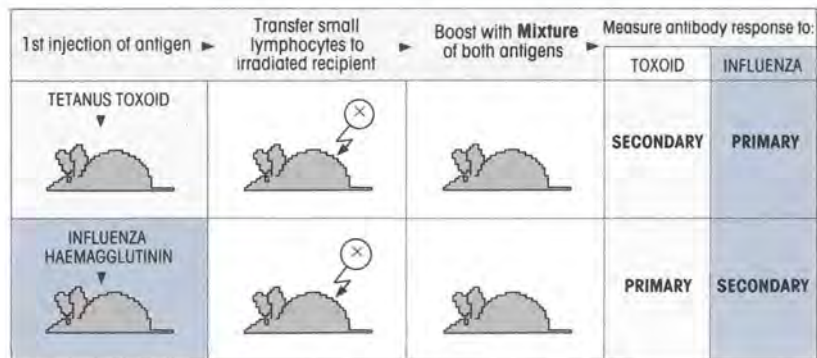


Figure 2.12. Memory for a primary response can be transferred by small lymphocytes. Recipients are treated with a dose of X-rays which directly kill lymphocytes (highly sensitive to radiation) but only affect other body cells when they divide; the recipient thus functions as a living 'test-tube' which permits the function of the donor cells to be followed.

The reasons for the design of the experiment are given in the text. In practice, because of the possibility of interference between the two antigens, it would be wiser to split each of the primary antigen-injected groups into two, giving a separate boosting antigen to each to avoid using a mixture.

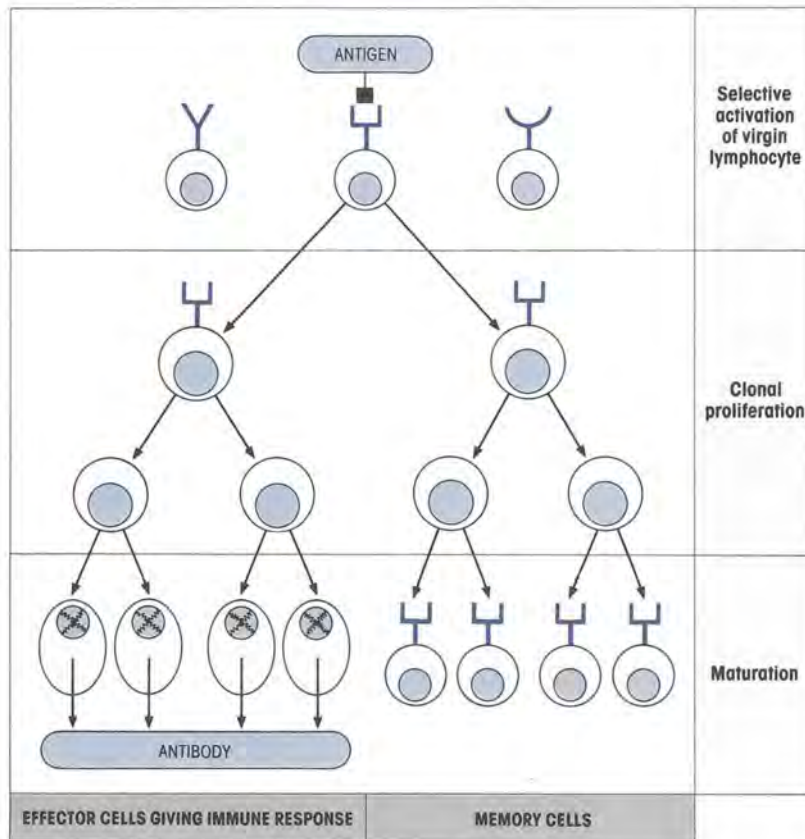


Figure 2.13. The cellular basis for the generation of effector and memory cells after primary contact with antigen. A fraction of the progeny of the original antigen-reactive lymphocytes become non-dividing memory cells and others the effector cells of humoral or, as we shall see subsequently, cell-mediated immunity. Memory cells require fewer cycles before they develop into effectors and this shortens the reaction time for the secondary response. The expanded clone of cells with memory for the original antigen provides the basis for the greater secondary relative to the primary immune response. Priming with low doses of antigen can often stimulate effective memory without producing very adequate antibody synthesis.

unresponsiveness or tolerance would then be created so that as immunological maturity was reached there would normally be an inability to respond to 'self' components. As we shall see later, these predictions have been amply verified.

Vaccination

Nearly 200 years ago, Edward Jenner carried out the remarkable studies which mark the beginning of immunology as a systematic subject. Noting the

pretty pox-free skin of the milkmaids, he reasoned that deliberate exposure to the pox virus of the cow, which is not virulent for the human, might confer protection against the related human smallpox organism. Accordingly, he inoculated a small boy with cowpox and was delighted—and I hope relieved—to observe that he was now protected against a subsequent exposure to smallpox (what would today's ethical committees have said about that?!). By injecting a harmless form of a disease organism, Jenner had utilized the specificity and memory of the acquired immune response to lay the foundations for modern vaccination (Latin *vacca*, cow).

The essential strategy is to prepare an innocuous form of the infectious organism or its toxins which still substantially retains the antigens responsible for establishing protective immunity. This has been done by using killed or live attenuated organisms, purified microbial components or chemically modified antigens (figure 2.14).

Cell-mediated immunity protects against intracellular organisms

Many micro-organisms live inside host cells where it is impossible for humoral antibody to reach them. Obligate intracellular parasites like viruses *have* to replicate inside cells; facultative intracellular parasites like mycobacteria and leishmania can replicate within cells, particularly macrophages, but *don't have to*; they like the intracellular life because of the protection it affords. A totally separate acquired immunity system has evolved to deal with this situation based on a distinct lymphocyte subpopulation made up of T-cells, designated thus because, unlike the B-lymphocytes, they differentiate within the milieu of the thymus gland. Because they are specialized to operate against cells bearing intracellular organisms, T-cells only recognize antigen when it is on the surface of a body cell. Accordingly, its surface receptors, which are different from the antibody molecules used by B-lymphocytes, recognize antigen *plus* a surface marker which informs the T-lymphocyte that it is making contact with another cell. These cell markers belong to an important group of molecules known as the major histocompatibility complex (MHC) identified originally through their ability to evoke powerful transplantation reactions in other members of the same species.

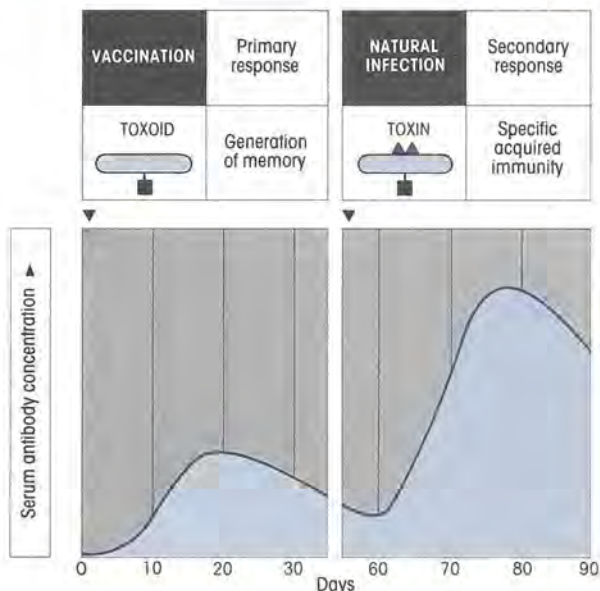


Figure 2.14. The basis of vaccination illustrated by the response to tetanus toxoid. Treatment of the bacterial toxin with formaldehyde destroys its toxicity (associated with ▲) but retains antigenicity. Exposure to toxin in a subsequent natural infection boosts the memory cells producing high levels of neutralizing antibody which are protective.

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Lymphokine-producing T-cells help macrophages to kill intracellular parasites

These organisms only survive inside macrophages through their ability to subvert the innate killing mechanisms of these cells. None the less, they cannot prevent the macrophage from processing small antigenic fragments (possibly of organisms which have spontaneously died) and placing them on the host cell surface. A subpopulation of T-lymphocytes called T-helper cells, if primed to that antigen, will recognize and bind to the combination of antigen with class II MHC molecules on the macrophage surface and produce a variety of soluble factors called *lymphokines*. These include γ -interferon and other macrophage activating factors which switch on the previously subverted microbicidal mechanisms of the macrophage and bring about the death of the intracellular micro-organisms (figure 2.15).

Killing of virally infected cells by cytotoxic T-cells

We have already discussed the advantage to the host of killing virally infected cells before the virus begins to replicate and have seen that large granular lymphocytes with NK activity (p. 13) can subserve a

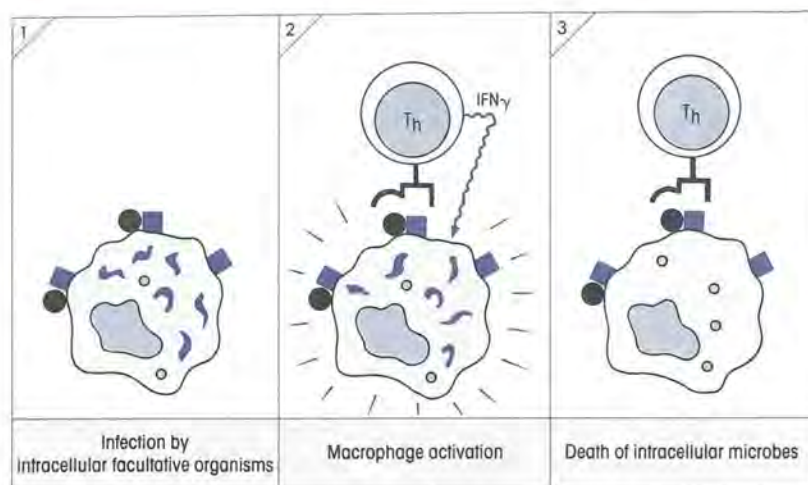


Figure 2.15. Intracellular killing of micro-organisms by macrophages activated by lymphokines released from T-helpers binding to surface microbial antigen (●) complexed with class II MHC molecules (■).

cytotoxic function. However, NK cells have a limited range of specificities and in order to improve their efficacy, this range needs to be expanded.

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One way in which this can be achieved is by coating the target cell with antibodies specific for the virally coded surface antigens because NK cells have receptors for the constant part of the antibody molecule, rather like phagocytic cells. Thus antibodies will bring the NK cell very close to the target by forming a bridge, and the NK cell being activated by the complexed antibody molecules is able to kill the virally infected cell by its extracellular mechanisms (figure 2.16). This system, termed 'antibody-dependent cell-mediated cytotoxicity' (ADCC) is very impressive when studied *in vitro* but it has proved difficult to establish to what extent it operates within the body.

On the other hand, a subset of cytotoxic T-cells has evolved for which there is evidence of *in vivo* activity. These cells have a very wide range of antigen specificities because they clonally express a large number of different surface receptors similar to, but not identical with, the surface antibody receptors on the B-lymphocytes. Again, each lymphocyte is programmed to make only one receptor and, like the T-helper cell, recognizes antigen only in association with a cell marker, in this case class I MHC molecules (figure 2.16). Through this recognition of surface antigen, the cytotoxic cell comes into intimate contact with its target and administers the 'kiss of death'. It also releases γ -interferon which would help to reduce the spread of virus to adjacent cells, particularly in cases where the virus itself may prove to be a weak inducer of $\text{IFN}\alpha$ or β .

In an entirely analogous fashion to the B-cell, T-cells are selected and activated by combination

with antigen, expanded by clonal proliferation and mature to give T-helper and cytotoxic T-effectors together with an enlarged population of memory cells. Thus both T- and B-cells provide specific acquired immunity with a variety of mechanisms which in most cases operate to extend the range of effectiveness of innate immunity and confer the valuable advantage that a first infection prepares us to withstand further contact with the same micro-organism.

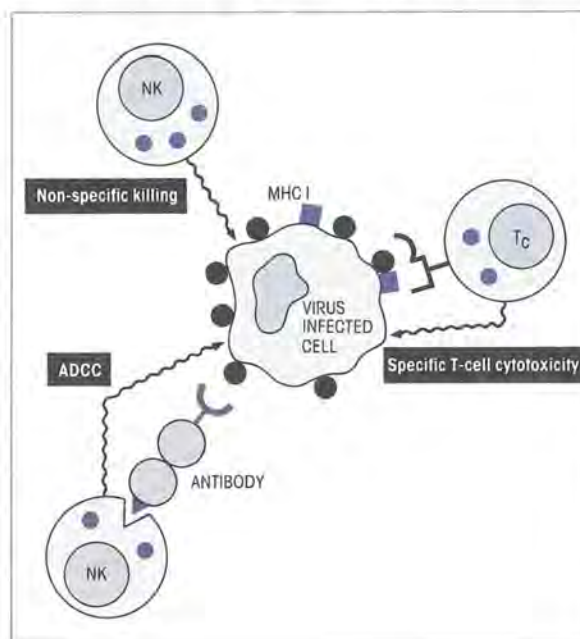


Figure 2.16. Killing virally infected cells. The non-specific killing mechanism of the NK cell can be focused on the target by antibody to produce antibody-dependent cell-mediated cytotoxicity (ADCC). The cytotoxic T-cell homes onto its target specifically through receptor recognition of surface antigen in association with MHC class I molecules.

Immunopathology

The immune system is clearly 'a good thing', but like mercenary armies, it can turn to bite the hand that feeds it, and cause damage to the host.

Thus where there is an especially heightened response or persistent exposure to exogenous antigens, tissue damaging or *hypersensitivity* reactions may result. Examples are allergy to grass pollens, blood dyscrasias associated with certain drugs, immune complex glomerulonephritis occurring after streptococcal infection, and chronic granulomas produced during tuberculosis or schistosomiasis.

In other cases, the ability to maintain non-reactivity against self-components may break down giving rise to a wide variety of diseases with an *autoimmune* component, such as thyrotoxicosis, myasthenia gravis and many of the rheumatological disorders.

Another immunopathological reaction of some consequence is *transplant rejection* where the major histocompatibility complex antigens on the donor graft may well provoke a fierce reaction. Lastly, one should consider the by no means infrequent occurrence of inadequate functioning of the immune system—immunodeficiency. I would like to think that at this stage the reader would have no difficulty in predicting that the major problems in this condition relate to persistent infection, the type of infection being related to the elements of the immune system which are defective.

SUMMARY

A wide range of innate immune mechanisms operate which do not improve with repeated exposure to infection. Micro-organisms are kept out of the body by the skin, the secretion of mucus, ciliary action, the lavaging action of bactericidal fluids (e.g. tears), gastric acid and microbial antagonism. If penetration occurs, bacteria are destroyed by soluble factors such as lysozyme and by phagocytosis with intracellular digestion.

The main phagocytic cells are polymorphonuclear neutrophils and macrophages. Organisms adhere to their surface, activate the engulfment process and are taken inside the cell where they fuse with cytoplasmic granules. A formidable array of oxygen-dependent and oxygen-independent microbicidal mechanisms then come into play.

The complement system, a multicomponent triggered enzyme cascade, is used to attract phagocytic

cells to the microbes and engulf them. The most abundant component, C3, is split by a convertase enzyme formed from its own cleavage product C3b and factor B and stabilized against breakdown caused by factors H and I, through association with the microbial surface. As it is formed, C3b becomes linked covalently to the micro-organism. The next component, C5, is activated yielding a small peptide, C5a; the residual C5b binds to the surface and assembles the terminal components C6–9 into a membrane attack complex which is freely permeable to solutes and can lead to osmotic lysis. C5a is a potent chemotactic agent for polymorphs and greatly increases capillary permeability. C3a and C5a act on mast cells causing the release of further mediators such as histamine and leukotriene B₄ with effects on capillary permeability and neutrophil chemotaxis; they also activate neutrophils. The neutrophils bind to the C3b-coated microbes by their surface C3b receptors. The influx of polymorphs and the increase in vascular permeability constitute the potent anti-microbial *acute inflammatory response* (figure 2.17).

Other humoral defences involve the acute phase proteins such as C-reactive protein.

Recovery from viral infections can be effected by the interferons which block viral replication. Virally infected cells can be killed by large granular lymphocytes with natural killer (NK) activity.

Extracellular killing by C3b-bound eosinophils may be responsible for the failure of many large parasites to establish a foothold in potential hosts.

The antibody molecule evolved as a specific adaptor to attach to micro-organisms which either fail to activate the alternative complement pathway or prevent activation of the phagocytic cells. The antibody fixes to the antigen by its specific recognition site and its constant structure regions activate complement through the classical pathway (binding C1 and generating a C4b2b convertase to split C3) and phagocytes through their antibody receptors. This supplementary route into the acute inflammatory reaction is enhanced by antibodies which sensitize mast cells (figure 2.17).

Antibodies are made by plasma cells derived from B-lymphocytes each of which is programmed to make only one antibody which is placed on the cell surface as a receptor. Antigen binds to the cell with a complementary antibody, activates it and causes clonal proliferation and finally maturation to antibody-forming cells and memory cells. Thus the antigen brings about clonal selection of the cells making antibody to itself. The increase in memory

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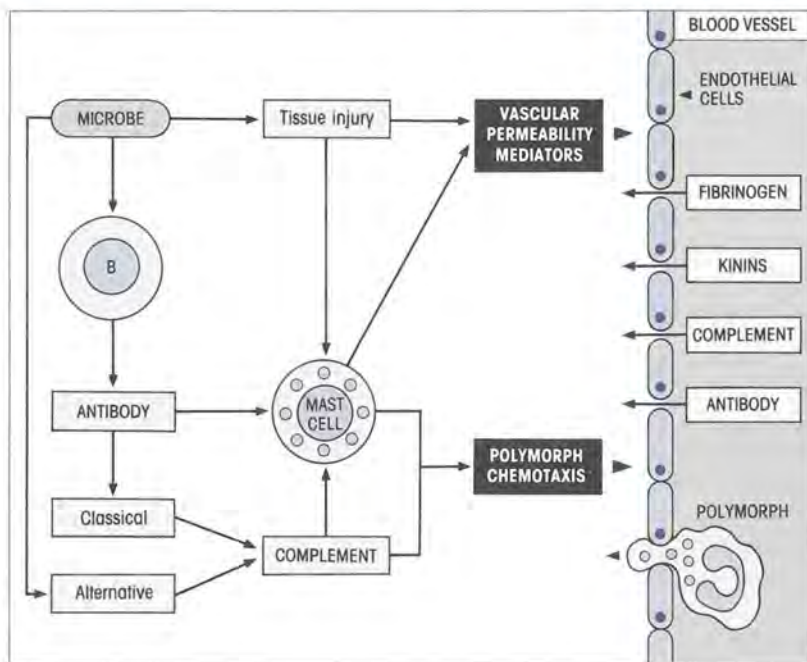


Figure 2.17. Production of a protective acute inflammatory reaction by microbes either (i) through tissue injury (e.g. bacterial toxin) or direct activation of the alternative complement pathway or (ii) by antibody-dependent triggering of the classical complement pathway or mast cell degranulation (a special type of antibody does this).

cells after priming means that the acquired secondary response is faster and greater providing the basis for vaccination, using a harmless form of the infective agent for the initial injection.

Another class of lymphocyte, the T-cell, is concerned with control of intracellular infections. Like the B-cell, each T-cell has its individual antigen receptor (although it differs structurally from antibody) which recognizes antigen and undergoes clonal expansion to form effector and memory cells providing specific acquired immunity. The T-cell recognizes

cell surface antigens in association with molecules of the major histocompatibility complex (MHC).

T-helper cells which see antigen with class II MHC on the surface of macrophages, release γ -interferon to activate the macrophage and enable it to kill intracellular parasites. Cytotoxic T-cells recognize antigen plus class I MHC on the surface of virally infected cells which are killed before the virus replicates. They also release γ -interferon which can make surrounding cells resistant to viral spread (figure 2.18).

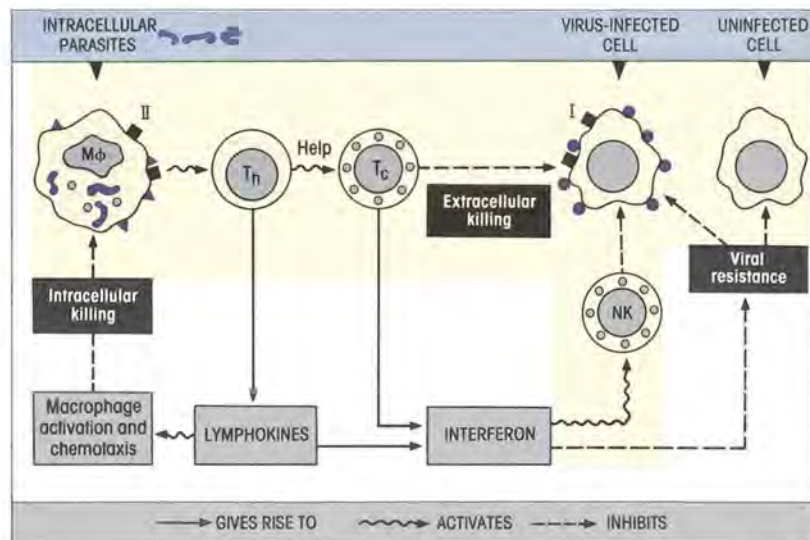


Figure 2.18. T-cells link with the innate immune system to resist intracellular infection. Class I and class II major histocompatibility molecules (■) are important for T-cell recognition of surface antigen. The T-helper cells (T_h) cooperate in the development of cytotoxic T-cells (T_c) from precursors. The macrophage ($M\phi$) microbicidal mechanisms are switched on by macrophage activating lymphokines. Interferon inhibits viral replication and stimulates NK cells which together with T_c kill virus-infected cells.

Although the innate mechanisms do not improve with repeated exposure to infection as do the acquired, they play a vital role since they are intimately linked to the acquired systems in two different 'trinities' which all but encapsulate the whole of immunology. Antibody, complement and polymorphs give protection against most extracellular organisms while T-cells, soluble cytokines and

macrophages deal with intracellular infections (figure 2.19).

Immunopathologically-mediated tissue damage can occur as a result of inappropriate hypersensitivity reactions to exogenous antigens, loss of tolerance to self-giving autoimmune disease and reaction to foreign grafts. Immunodeficiency leaves the individual susceptible to infection.

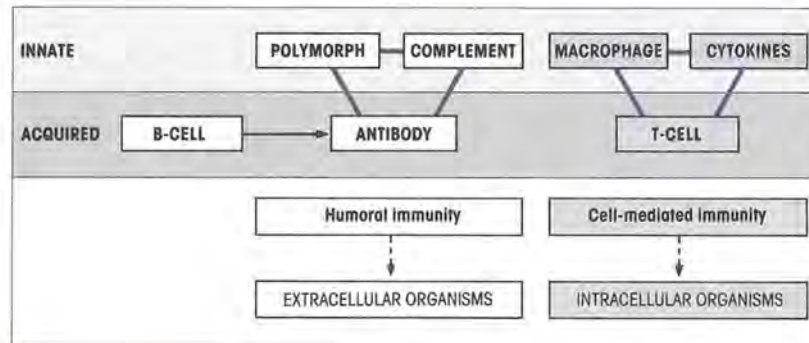


Figure 2.19. The two trinities linking innate and acquired immunity.

Further reading

- Golub E.S. (1981) *The Cellular Basis of the Immune Response*. Sinauer Assoc., Mass., USA.
- Herberman R.B., Reynolds C.W. & Ortaldo J. (1986) Mechanisms of cytotoxicity by NK cells. *Ann. Rev. Immunol.*, 4, 651.
- Joiner K.A., Brown E.J. & Frank M.M. (1984) Complement and bacteria. *Ann. Rev. Immunol.*, 2, 461.
- Lewis G.P. (1986) *Mediators of Inflammation*. Wright, Bristol.
- Mims C.A. & White D.O. (1984) *Viral Pathogenesis and Immunology*. Blackwell Scientific Publications, Oxford.
- Porter R.R., Lachmann P.J. & Reid K.B.M. (1984) A discussion on the biochemistry and genetics of complement. *Phil. Trans. Roy. Soc.(B)*, 306, no. 1129.

General reading

- Herbert W.J., Wilkinson P.C. & Stott D.I. (eds) (1985) *Dictionary of Immunology*, 3rd edn. Blackwell Scientific Publications, Oxford.
- Hood L.E., Weissman I.L., Wood W.B. & Wilson J.H. (1984) *Immunology*, 2nd edn. Benjamin/Cummings, California, USA. (A very good, in-depth textbook of fundamental immunology in which concepts are extended by numerous mind-bending problems, many of which are based on original data. Thin on clinical side.)
- Klein J. (1982) *Immunology: the Science of Self-Nonself Discrimination*. Wiley, N.Y. (In-depth textbook of basic immunology on the grand scale.)
- McConnell I., Munro A. & Waldmann H. (1981) *The Immune System: a Course on the Molecular and Cellular Basis of Immunity*, 2nd edn. Blackwell Scientific Publications, Oxford. (An excellent text for further study of fundamental immunological principles, but a little dated.)

- Playfair J.H.L. (1987) *Immunology at a Glance*, 4th edn. Blackwell Scientific Publications, Oxford. (Very useful for revision.)
- Roitt I.M., Brostoff J. & Male D.K. (1985) *Immunology*. Gower Medical Publishing, London. (An extensively and colourfully illustrated textbook.)
- Stites D.P., Stobo J.D., Fudenberg H.H. & Wells J.V. (1984) *Basic and Clinical Immunology*, 5th edn. Lange Medical Publications, California. (I find this of most use for reference purposes.)

Historical

- Humphrey J.H. & White R.G. (1970) *Immunology for Students of Medicine*, 3rd edn. Blackwell Scientific Publications, Oxford. (Dated but scholarly.)
- Landsteiner K. (1946) *The Specificity of Serological Reactions*. Harvard University Press (reprinted 1962 by Dover Publications, New York).
- Metchnikoff E. (1893) *Comparative Pathology of Inflammation*. Transl. F.A. & E.H. Starling. Kegan Paul, Trench, Trübner & Co., London.
- Parish H.J. (1968) *Victory with Vaccines*. Churchill Livingstone, Edinburgh.

Series for the advanced reader

- * *Advances in Immunology* (Annual). Academic Press, London.
- Ann. Rev. Immunology*. Ann Reviews Inc., California.
- Contemporary Topics in Molecular Immunology*. Plenum Press, New York.
- Contemporary Topics in Immunobiology*. Plenum Press, New York.
- * *Immunological Reviews* (ed. G. Møller). Munksgaard, Copenhagen.
- Modern Trends in Immunology*. Butterworth, London.

Progress in Allergy. S. Karger, Basle.

* *Progress in Immunology*. Academic Press, Orlando, USA. (Triennial proceedings of the international congresses in immunology with an up-to-date account of a wide range of subjects.)

Protides of the Biological Fluids. Pergamon Press, Oxford.

* In depth treatment.

Current information

Current Titles and Abstracts in Immunology, Transplantation & Allergy. Elsevier, Amsterdam. (Quick coverage of current literature.)

Immunology Today. Elsevier Science Publications, Amsterdam. (The immunologist's 'newspaper'.)

Major journals

Nature, *Lancet*, *Science*, *J. Exp. Med.*, *Immunology*, *J. Immunology*, *Clin. Exp. Immunology*, *Mol. Immunol.*, *Immunopharmacology*, *Infect & Immunity*, *Int. Arch. Allergy*, *Cell. Immunology*, *European J. Immunology*, *Scand. J. Immunol.*, *Clin. Immunol. & Immunopath.*, *J. Clin. Immunol.*, *J. Immunogenetics*, *J. Immunol. Methods*, *J. Reticuloendoth. Soc.*, *Tissue Antigens*, *Immunogenetics*, *Human Immunol.*, *Transplantation*, *Ann. d'Immunologie*, *Cancer Immunol. Immunotherapy*, *J. Allergy Clin. Immunol.*, *Clin. Allergy*, *Ann. Allergy*, *J. Clin. Lab. Immunology*, *Parasitic Immunol.*