

Innate immunity I: physical and humoral protection

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described above as well as organs transplanted from unrelated donors. Protection from these is afforded by a variety of cognitive and destructive processes, the understanding of which forms the basis of immunology.

Immunity from infection is the result of a complex process, as we shall see, but there are some general principles about immunity that we can deduce from our own everyday experience. For example, some features of protection from infection are obvious: if you have an open wound or burn, it is important to maintain cleanliness and protect the exposed tissues from becoming infected. Thus, loss of physical barriers lowers immunity. We also know that some infections can arise at any age and be dealt with by the immune system without necessarily needing antibiotics. On the other hand, we consider the newborn to be at greater overall risk of infection: they have less immunity. Other well-recognised truths relate to the infections of childhood. If you have chicken pox as a child, you are extremely unlikely to suffer the same illness again. However, having had chicken pox would not stop a child catching measles. We can conclude that we are born with some immunity and that the rest may be acquired during life; immune responses can be highly specific for a microbe: they may be learned and retained in an 'immunological memory'.

Freedom from the burden of disease

The Latin *immunis*, meaning *free from burden*, has provided the English term immunity; it is often used in non-scientific contexts such as diplomatic immunity, crown immunity and so on. In biology, the burden is disease — caused by a variety of viruses, fungi, bacteria, protozoa, worms and toxins — and the physiological role of the immune system is to keep it at bay.

A broad definition of the immune system would be that it evolved to be able to identify **self**, and thus recognise **non-self**. The ability to make such a distinction is relatively primordial: sea anemones also have the capacity to recognise and react to non-self. The immune system in humans is often challenged by non-self, including pathogens such as those

Types of immunity

Innate immunity

Immunity present at birth is termed innate. The innate immune system is the main, first-line defence against invading organisms. Its characteristics are that it is present for life, has no specificity and no memory. (An exception, to be discussed later, is the protective antibodies that babies acquire from their mothers.) Innate responses are most useful in protection against:

- pyogenic ('pus-forming') organisms, e.g. *Staphylococcus aureus*, *Haemophilus influenzae*
- fungi, e.g. *Candida albicans*
- multicellular parasites, e.g. worms such as *Ascaris*, the roundworm.

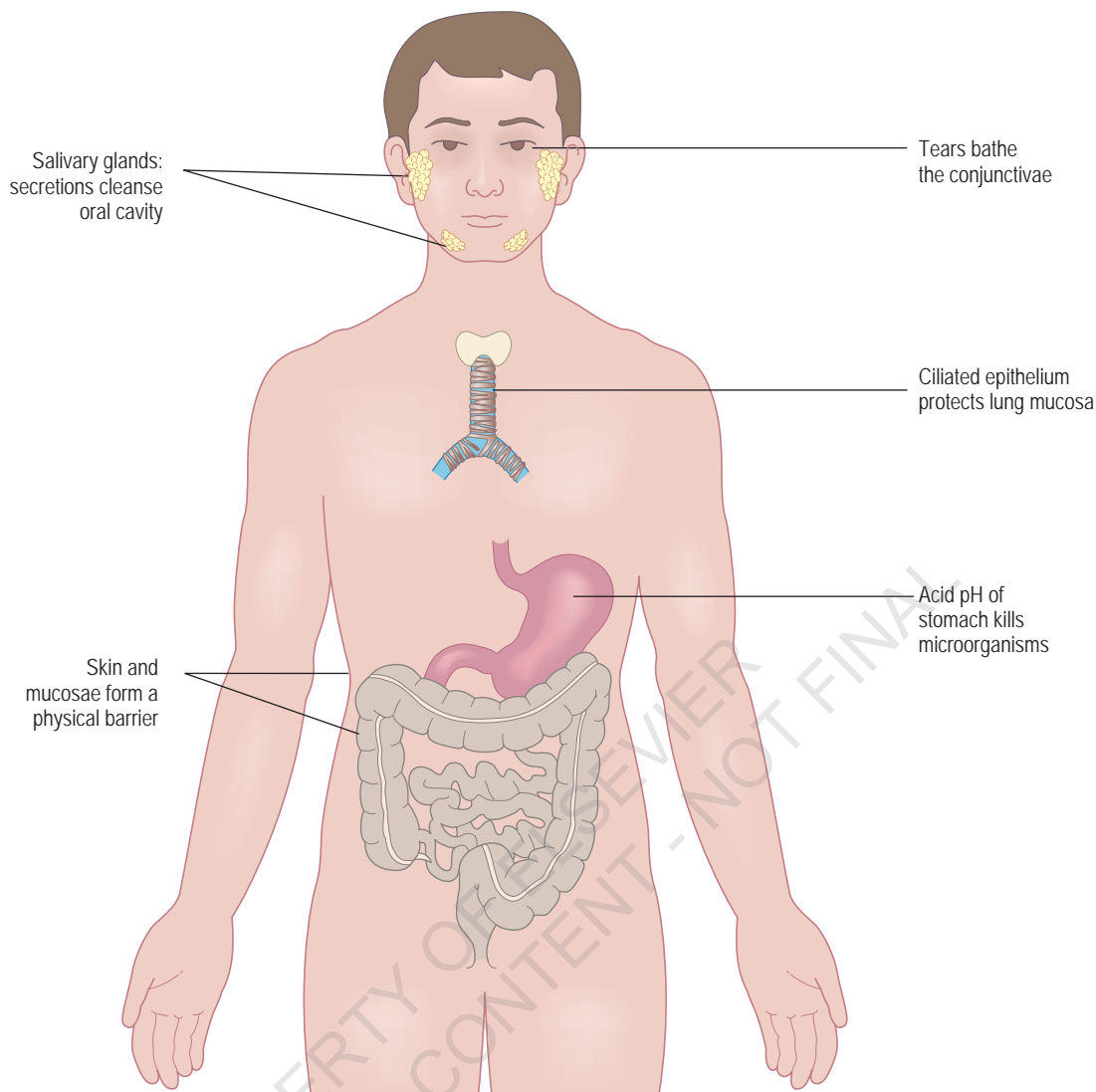


Fig. 2.1 **Physicochemical barriers to infection which form the first line of innate defence against infection.** Secretions such as saliva and tears also contain antibacterial enzymes such as lysozyme.

Innate immunity has three components: physicochemical, humoral and cellular.

Physical barriers (Fig. 2.1) are the **skin** and **mucosae, secretions**, which continually wash and cleanse mucosal surfaces, and **cilia**, which help the removal of debris and foreign matter. Immunologically active factors present in mucosal secretions, in blood and in the cerebrospinal fluid (the *humors*) are termed humoral. The most important of these is **complement** and the **mannan-binding lectin**, as well as additional **opsonins** (an opsonin aids digestion of bacteria by neutrophils), such as **C-reactive protein**, and proteolytic **enzymes** (e.g. **lysozyme**). Cellular components are the **neutrophil**, the **eosinophil** and the **mast cell**, as well as the **NK cell**.

Acquired immunity

In contrast, some types of immune response are not present at birth but are gained as part of our development. The acquired or specific immune response is the antithesis of

innate immunity. It is absent at birth, increases with age and has specificity and memory; hence it may also be termed **adaptive**.

Paralysis of one component of either of these two forms of immunity can have a profound effect on the host defence against infection.

SUMMARY BOX 2.1

Contrasting characteristics of innate and acquired immunity



Innate immunity

- Characteristics: non-specific, is present at birth and does not change in intensity with exposure.
- Components: mechanical barriers, secreted products (complement) and cells (granulocytes, dendritic cells, NK cells).
- Protects from: bacteria, fungi, worms.

SUMMARY BOX 2.1

Contrasting characteristics of innate and acquired immunity—cont'd



Acquired immunity

- Characteristics: specific responses, acquired from exposure and increases in intensity with exposure.
- Components: secreted products (antibodies) and cells (lymphocytes).
- Protects from: bacteria, including intracellular infection, viruses and protozoa.

Complement

Complement was described at the turn of the century during studies on the nature of immune reactions to bacteria in serum. Serum removed from animals that have been infected with a microorganism can subsequently agglutinate (clump together) and then lyse the same bacteria in a test tube (Fig. 2.2). Lysis, but not agglutination, is inhibited by pre-heating the serum at 56°C for 30 minutes. The lysing activity can be reconstituted using fresh serum from an animal not previously exposed to the bacteria. Therefore, a heat-labile factor without specificity for an organism is essential for its lysis.

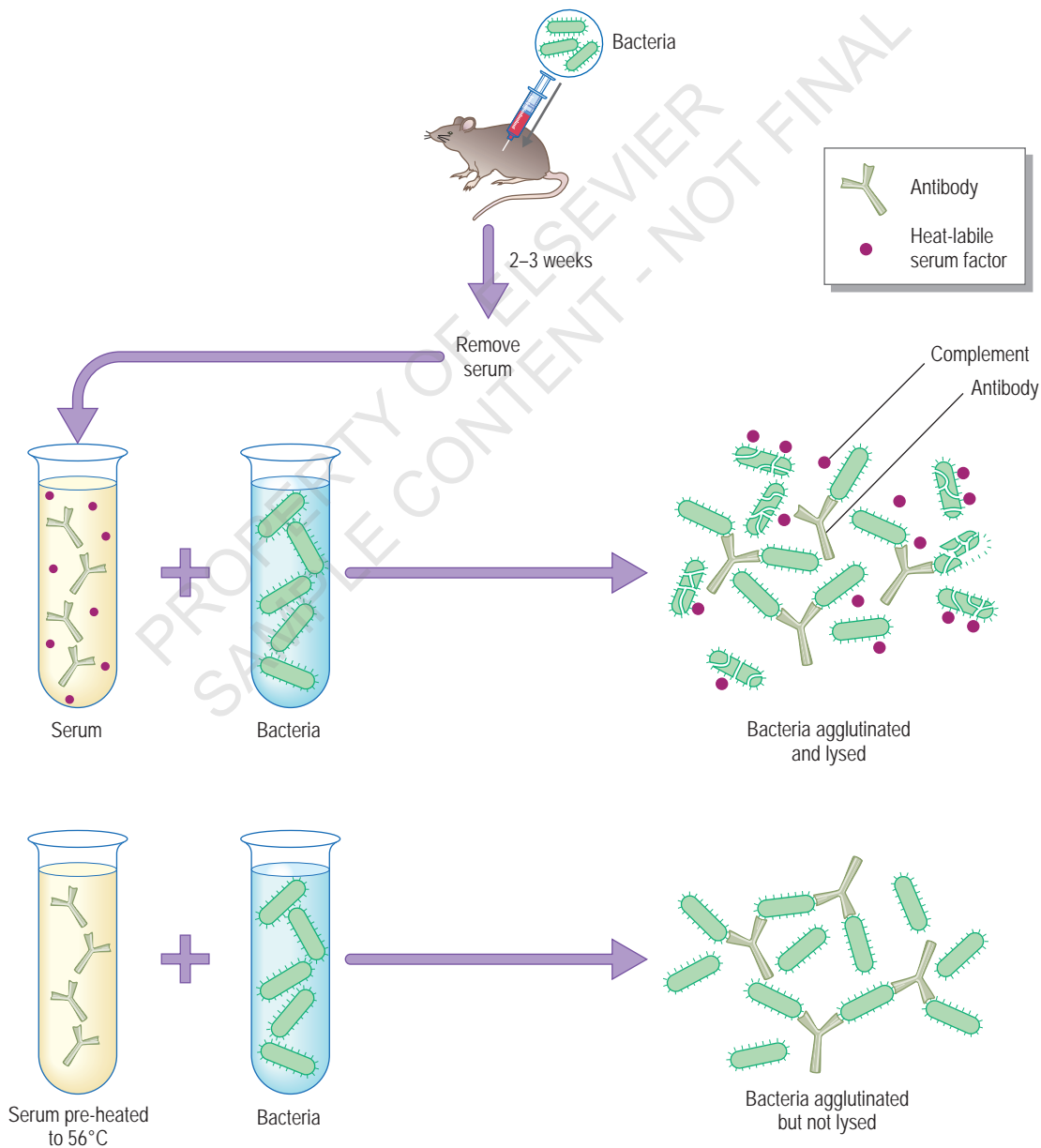


Fig. 2.2 Animals exposed to bacteria develop antibody, which specifically agglutinates (“binds together”) the same organism. Lysis is achieved through the action of a heat-labile serum factor which complements the action of antibody.



SCIENCE BOX 2.1

Antibody



Antibody is a term we have all become used to. They are glycoproteins produced by lymphocytes following stimulation with a macromolecule (usually termed the antigen). An example of an antigen would be a protein coating the surface of a bacterium or virus. Antibody is a sophisticated glycoprotein that occurs in several different types with differing functions, but it is sufficient for the present to view it simplistically as a molecule with a shape like the letter 'Y'. The two smaller arms are identical to each other and each carries the ability to bind antigen; the trunk of the Y has specialised sites for interaction with complement proteins or specific receptors on cells. Granulocytes and mast cells, for example, bear receptors for antibody. Through interaction with complement and cells, antibody can provide the innate immune system with a specificity that, on its own, it does not possess. This serves as a reminder that the innate and acquired immune systems work best in concert.

These studies indicated the presence of two antibacterial agents in serum. One was fairly heat stable, inducible by the organism and capable of agglutinating but not killing it. This first factor was originally termed **antibody** (Science Box 2.1) and is specific for the target that induces it (the **antigen**), in this case a foreign organism. Since it is capable of specific reactions, antibody is part of the acquired/specific/adaptive immune system and will be discussed later. The second serum factor is heat labile and helps destruction of the organism by antibody. This factor was termed **complement**, now the name of a group of serum proteins that *complement* antibody in the destruction of organisms. It later became established that complement could, under many circumstances, be activated directly by pathogenic organisms without the need for antibody (therefore termed the alternative pathway), and is therefore a component of the innate immune system.

Complement is a protein cascade (cf. the kinin and clotting cascades) composed of more than 40 proteins including regulatory factors. The components are made in the liver, though some local production at sites of inflammation may be undertaken by macrophages. Complement has four pathways: the **alternative**, **classical** and **mannan-binding lectin** pathways, which are all capable of igniting the third pathway, known as the **common** or **membrane attack pathway**.

Complement proteins

The majority of complement proteins are soluble, although some are membrane bound. The soluble proteins circulate in an inactive state, and each must be activated sequentially for the reaction to proceed. Each activated molecule can catalyse

the conversion of several molecules of the next component in the sequence; this gives the cascade the key attribute of amplification. The overall serum concentration of complement proteins is 3–4 g/l (i.e. around 10% of serum proteins).

Several biological activities appear as a consequence of complement activation, the main ones being cell or bacterial **lysis**, the production of **pro-inflammatory mediators**, which amplify and perpetuate the process, and **solubilisation** of antigen–antibody complexes.

The confused and ever-changing terminology of the complement system was partly to blame for its past unpopularity with students and clinicians. In recent years the World Health Organization has proposed a standard nomenclature to obviate this (see Table 2.1). The precursor molecules, the fragments derived from enzymatic cleavage of the parent molecule, the inactivated component and the active state of isolated or integrated complement components are all clearly defined in the same way for every component of the pathways.

Complement activation

Activation of the complement system occurs through three distinct pathways, **alternative**, **classical** and **mannan-binding lectin**. These converge for the final common pathway, which provides most of the biological activity (Fig. 2.3). The alternative, classical and mannan-binding lectin pathways are composed of three distinct enzyme cascades that culminate in the cleavage of C3 and C5. Cleavage of C3 produces important biological consequences, while breakdown of C5 achieves the same and, in addition, provides the

Table 2.1 Terminology of the complement system

State of component	Nomenclature
Precursor molecules	Capital C followed by a number for the classical and common pathways, e.g. C1, C2 Capital letter followed by number for the alternative pathway, e.g. B1
Fragments	Small letter suffix, e.g. C4a, C2b ('a' fragments are smaller than 'b' fragments)
Inactivated components	Letter i prefix, e.g. iC3b
Active state	Bar over symbols, e.g. $\overline{\text{C4bC2b}}$

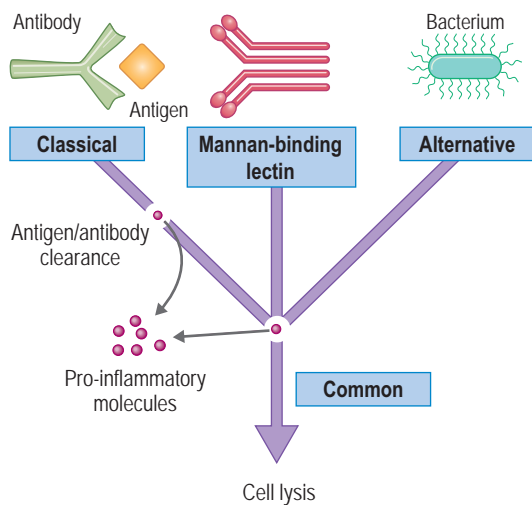


Fig. 2.3 Overview of complement activation pathways. The final common pathway may be activated via the classical or alternative routes, which are initiated by antigen–antibody complexes and bacteria, respectively, or by mannan-binding lectin. Three main results of activation are clearance of complexes, release of biologically active mediators and direct cell lysis.

SUMMARY BOX 2.2

Complement



- Complement comprises a large number of serum proteins that are mainly made in the liver.
- Complement forms protein cascades, each activated component catalysing the activation of several molecules of the next component, causing amplification of the response.
- The consequences of complement activation are cell lysis, production of pro-inflammatory mediators and solubilisation of antigen–antibody complexes.
- There are four pathways.

Complement pathways

The three parallel initial pathways of complement each activate the final common pathway. In evolutionary terms, the alternative pathway is relatively primitive and a part of the innate immune system; the classical pathway, which is relatively recent, combines with antibody to initiate activation and is, therefore, an adjunct to the acquired immune system. The mannan-binding lectin pathway is probably somewhere in between — it interacts directly with pathogens and is therefore part of the innate immune system, but structurally it resembles early components of the classical pathway.

SCIENCE BOX 2.2

Complement genes



Gene duplication is thought to account for the striking similarities in the structure of proteins in the classical and alternative pathways. A further example of gene duplication is found in the region on human chromosome 6 that encodes some of the complement proteins, where there are two adjacent loci encoding C4. Approximately 35 different forms (alleles) of the C4 gene have been described, each encoding slightly different proteins. This is termed gene polymorphism and, although it is not a feature of all complement genes, it was used to settle the question of the relative contributions of macrophages and liver cells to the synthesis of complement proteins in the circulation. This was elegantly clarified by studies on C4 in the circulation of patients before and after liver transplantation. In post-transplant patients, the complement components have totally converted to the type of the liver donor, indicating the liver as the site of synthesis of circulating complement factors.

triggering stimulus to the final common pathway. The alternative, classical and mannan-binding lectin pathways bear striking resemblances, particularly in terms of protein structure (e.g. C2 and B, C1q and mannan-binding lectin), which is thought to arise from gene duplications occurring during the evolution of the cascade (Science Box 2.2). The pathways are, however, triggered by different substances and through different initiation mechanisms.

The classical pathway

The classical pathway is activated by an interaction between antigen and antibody, forming a so-called **immune complex**. Antibodies can bind to, or ‘fix’, complement only after reacting with their antigen. The formation of the complex provokes a conformational change in the antibody molecule that discloses a site for binding of the first complement component **C1**. C1 is a multimeric compound composed of six molecules termed **C1q**, and two each of **C1s** and **C1r**. C1q is an elongated protein with a rod-like stem composed of a triple helical structure and a globular head resembling a tulip (Fig. 2.4). It is the globular head that binds antibody. Six C1q molecules arrange themselves in a ‘bunch’ and the four C1r and C1s molecules attach in a calcium-dependent interaction. When antibody binds to two or more heads of C1q, C1r is cleaved to give an active molecule **C1r̄**, which cleaves C1s. C1s extends the activation process by cleaving the next complement component **C4** to C4b, which continues the reaction process, and C4a, which has other biological properties (see below).

Cleavage of C4 to C4b reveals an internal thioester bond, which is swiftly inactivated by binding water molecules unless it can form covalent bonds with cell surface proteins or carbohydrates. Should this happen, C4b becomes relatively stable and binds to **C2** in a magnesium-dependent



reaction (Fig. 2.5). This illustrates one of the important forms of control over the complement cascade, namely that enzymatically active molecules are unstable and tend to degrade rapidly unless a solid surface, usually that of a target such as a bacterium, is available.

The C2 is itself then cleaved by C1s to form the complex C4b2b, known as the **classical pathway C3 convertase**. C3

is a similar molecule to C4, having an internal thioester bond. Two fragments derive from C3 cleavage. The smaller of these, C3a, has powerful biological properties; the larger, C3b, displays the labile binding site that allows the molecule to bind to membranes close to, but distinct from, C4b2b. The proximity of C3b to C4b2b leads to the generation of the last enzyme of the classical pathway, C4b2b3b (the **classical pathway C5 convertase**), which cleaves C5, a component of the membrane attack pathway.

In addition to antigen-antibody complexes, the activation of the classical pathway can be initiated by aggregated immunoglobulins and by non-immunological stimuli such as C-reactive protein.

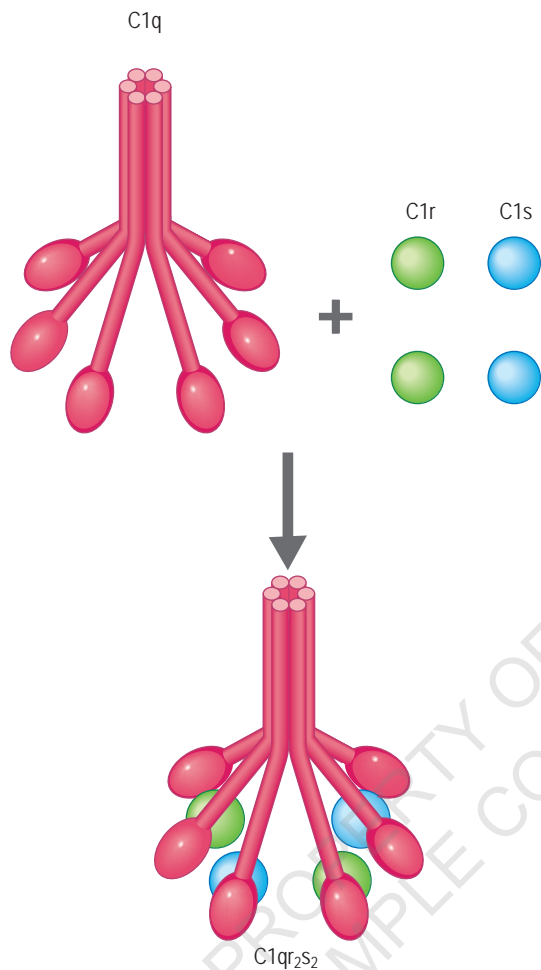


Fig. 2.4 'Tulip' structure of the C1q hexamer with dimers of C1r and C1s in place.

The globular heads of the C1q molecules bind antibody.

The mannan-binding lectin pathway

This has only been uncovered relatively recently. The mannan-binding lectin, or MBL, closely resembles C1q in structure and is activated by binding to microbes. Like C1q, it forms a 'bunch of tulips' structure allowing two serine proteases to bind to the stalks (MBL-associated serine proteases 1 and 2, MASP-1 and -2). This activates the MASPs, which go on to activate C4, and the remainder of the classical pathway flows as described above.

The alternative pathway

Activation of the alternative pathway proceeds in a different manner from that of the classical pathway, since it appears to be based on a 'tickover' mechanism. The concept is analogous to an automatic car. If an engine is idling or 'ticking over', any movement of the throttle will accelerate the engine and cause the car to move. Similarly, in the alternative pathway, there is a continuous, slow reaction sequence that is insufficient to produce any measurable effect. Activators of the alternative pathway are substances that act on the throttle. Availability of C3b is the essential requirement for activation of the alternative pathway to proceed; this requirement is again fulfilled by the internal thioester bond, which undergoes continuous low-grade hydrolysis. Free C3b binds **factor**

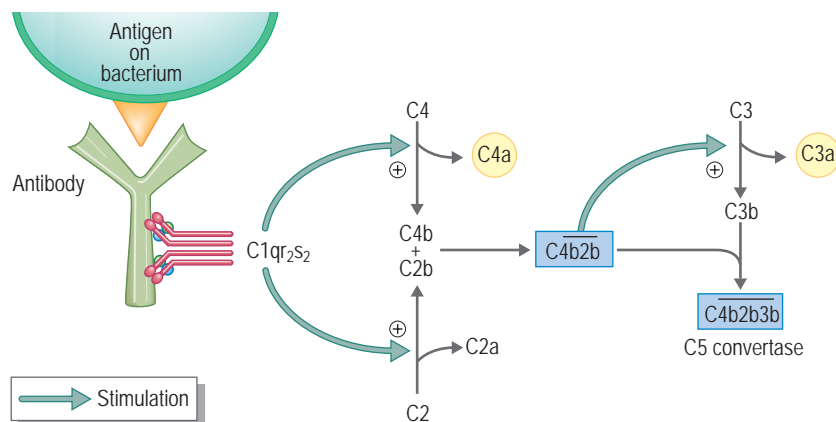


Fig. 2.5 Activation of the classical pathway.

Antigen-antibody complexes bind C1q. The C1qr₂s₂ complex cleaves C4 and C2 to form the classical pathway C3 convertase C4b2b. Following C3 cleavage, the C5 convertase is formed. Biologically active fragments C4a and C3a are generated. C3b alone has other actions and may also 'drive' forward activation of the alternative pathway.

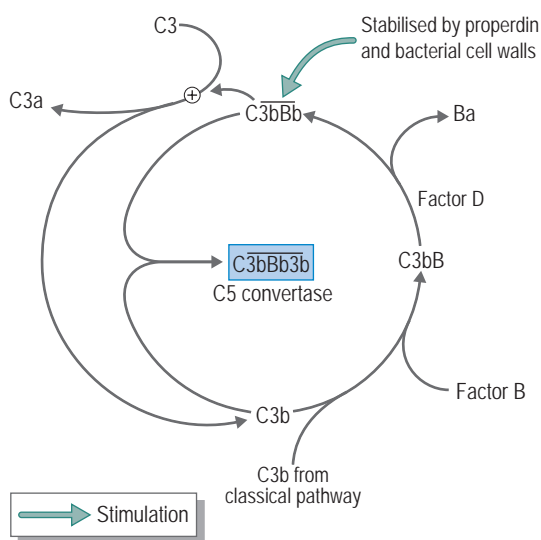


Fig. 2.6 Alternative pathway activation.

C3 'tick-over' generates C3b, C3bB and C3bBb, which in turn cleaves C3. The tick-over is accelerated if the active enzymes are stabilised on bacterial cell walls, or if more C3b is produced from the classical pathway. The alternative pathway C5 convertase C3bBb3b is generated.

B and the C3bB complex becomes the substrate of a circulating enzyme, **factor D**, which, by removing from C3bB the fragment Ba, generates C3bBb (Fig. 2.6). This complex, the **alternative pathway C3 convertase**, can cleave C3, detaching C3a from C3b, which can reinitiate the activation process.

How do the alternative pathway activators work? It is thought that bacteria provide a surface for C3b and C3bBb deposition and protection from the destructive action of circulating regulatory factors I and H, allowing the pressure on the throttle to increase. Further impetus is given by properdin, or **factor P**, which stabilises C3bBb and renders it more efficient. Positive feedback is provided here, since C3bBbP generates more C3b, which is capable of forming more enzyme. The complex C3bBb3b, analogous to C4b2b3b, is the **alternative pathway C5 convertase**, initiating the membrane attack pathway sequence.

The membrane attack pathway

This final common complement pathway (Fig. 2.7) generates one more biologically active component, C5a, but more importantly leads to the formation of the 'killer molecule' of the system. This is known as the **membrane attack complex (MAC)**, since it provokes membrane damage. The cleavage of C5 by the classical or alternative pathway convertases gives the smaller fragment C5a and the larger C5b split product, which continues the reaction sequence by binding to **C6** and inducing it to express a labile reactive site for **C7**. The C5b67 complex is highly lipophilic and binds to membranes, where it lies as a high-affinity receptor for **C8**. C8 has three chains, one of which inserts into the membrane, anchoring the C5b678 complex. C5b678 binds and polymer-

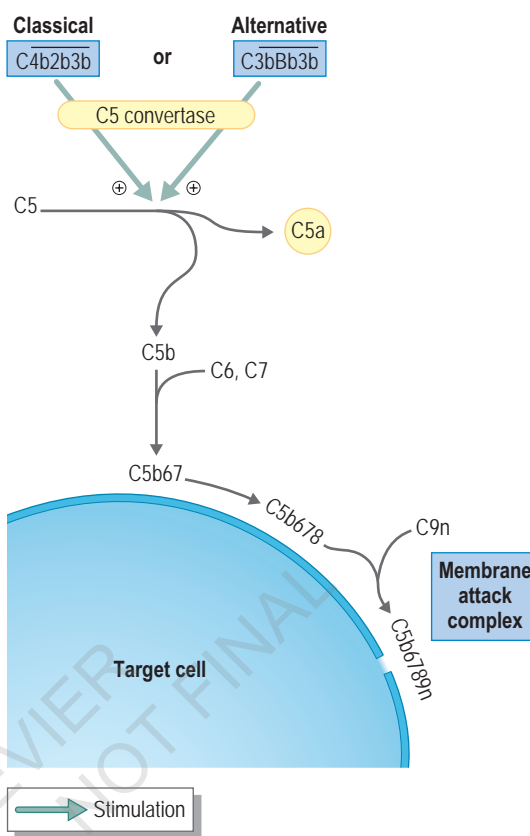


Fig. 2.7 The final common pathway.

The C5 convertases generate C5b and the pro-inflammatory C5a. C5b67 binds the target cell membrane and with addition of C8 and a C9 polymer the membrane attack complex forms.

ises **C9**, forming the MAC, the final component of the system. As many as 12 to 15 C9 molecules may cluster around one C5b678 complex, inserting into and traversing the membrane bilayer (Fig. 2.8). Holes are made in the membrane, and if a sufficient number are created death results through osmotic lysis.

SUMMARY BOX 2.3

Complement pathways

- Classical pathway is mainly activated by antigen-antibody complexes.
- Alternative pathway has a continual slow reaction that only produces effects if it is accelerated by the presence of a bacterial cell wall.
- The MBL pathway results in activation of the classical pathway.
- Each initial pathway produces a C5 convertase, which initiates the membrane attack pathway.
- The resulting membrane attack complex forms holes in cells causing lysis.
- Control is achieved by the lability of the components, by dilution and by specific regulatory proteins and receptors.

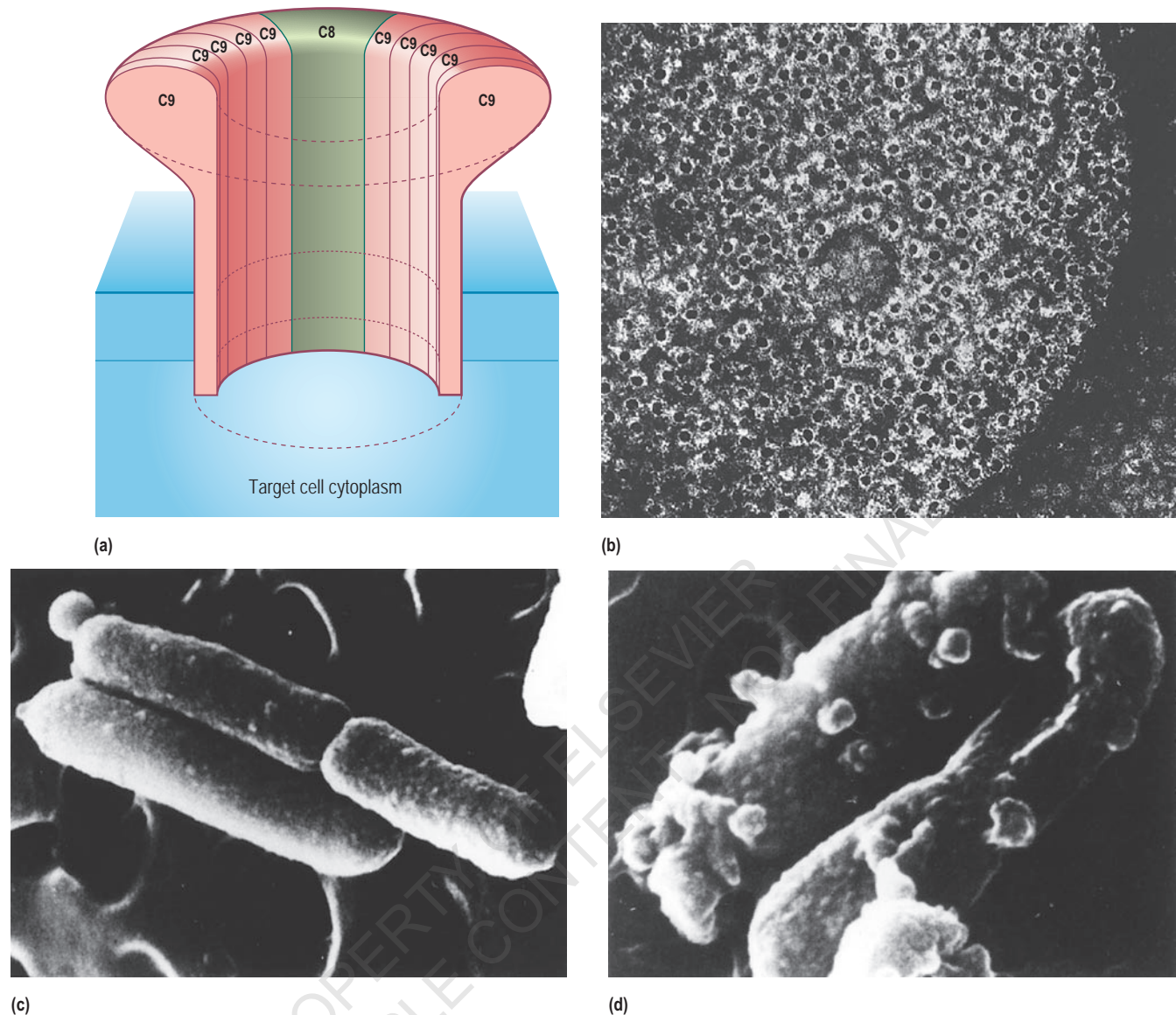


Fig. 2.8 The membrane attack complex.

(a) Schematic diagram. C5b678 is assembled and inserted into the membrane. Addition of multiple C9 molecules forms the MAC which punches holes in the membrane. (b) Electron micrograph of multiple MAC-induced holes in a red blood cell membrane. (c) Scanning electron micrograph of intact *Escherichia coli* bacterium and (d) after incubation with complement. Note dramatic expansion in size, caused by osmotic effects, and cytoplasmic blebbing (reproduced with permission from Upjohn Inc.).

Complement control mechanisms

Complement activation is kept in check by a variety of control mechanisms. The importance of such regulation is clear from the severity of the pathological states that result from congenital or acquired deficiencies of control proteins (see Ch. 19). We have already seen that lability of the active molecules is an inherent control mechanism, as is dilution into biological fluids. More specific regulation is provided by circulating or membrane-bound proteins. The classical pathway is controlled in its initial stage by **C1-inhibitor** (also known as C1 esterase), a protein in the blood that blocks the enzymatic function of activated C1 by combining with it in a virtually irreversible stoichiometric complex. In the circulation, **factor I** is an enzyme that degrades C3b while **factor**

H binds C3b and accelerates the destructive action of factor I (Fig. 2.9). Factor I is also able to restrain activation of the classical pathway by destroying C4b. This destructive process is enhanced if C4b is complexed with a protein called **C4-binding protein** (C4bp). Two circulating proteins with a similar function are **protein S** and **SP-40,40**. Both are capable of binding the C5b67 complex to form an inactive moiety, preventing membrane insertion and formation of the MAC. Finally, a circulating enzyme, **carboxy-peptidase N**, cleaves the carboxy-terminal arginine from C3a, C4a and C5a and the resulting molecules (termed, for example, C5a-des arg) are inactivated.

Other regulatory proteins are membrane bound. A membrane attack complex inhibitory factor — also known as **CD59** or **protectin** — exemplifies membrane-bound control

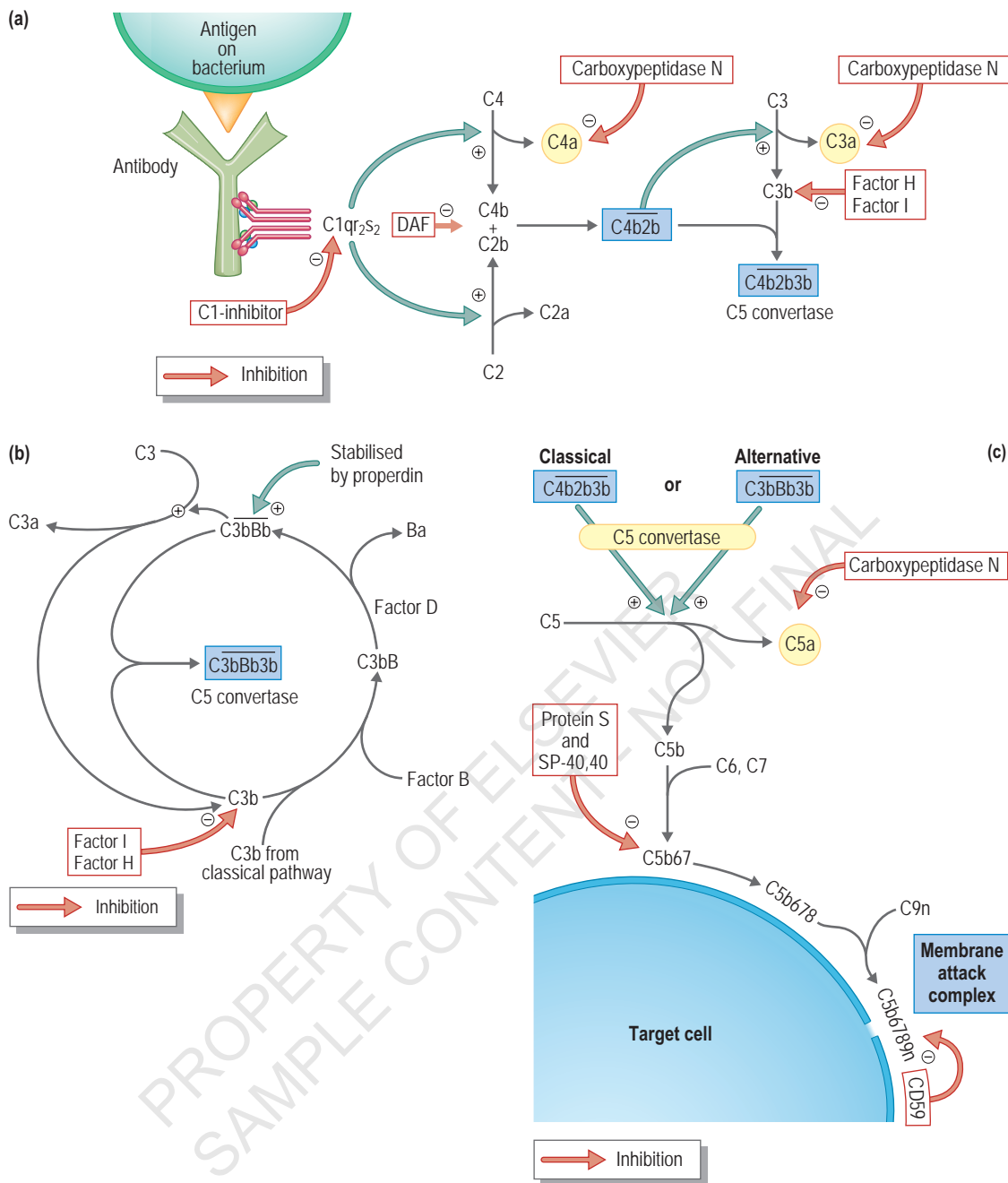


Fig. 2.9 Control mechanisms in the complement cascades. (a) The classical pathway. (b) The alternative pathway. (c) The final common pathway. See text for details.

proteins. (CD is the abbreviation for **cluster of differentiation**, and CD numbers are widely used to identify surface molecules in the immune system. An outline of the CD system is given in Science Box 2.3: ‘The CD classification’, p. 20.) CD59 is designed to avoid bystander damage: the accidental insertion of MACs destined for a bacterium into the cell wall of a lymphocyte or other host cell. CD59, constitutively expressed on mammalian cells, interferes with the MAC insertion, thus preventing cell lysis. **Decay accelerating factor (DAF)**, a transmembrane glycoprotein found on most blood cells, competes for C4b, thus inhibiting formation of the classical pathway C3 convertase.

Complement receptors

In addition to these membrane proteins, there are a group of receptors that have a more restricted distribution. The **complement receptors (CR)**, CR-1 to CR-4, bind breakdown products of C3 predominantly and are found on cells of the immune system (see below). They have a variety of functions, but CR-1 is also involved in regulation of the classical pathway, binding C4b and enhancing the action of factor I in much the same way that C4bp does.

There are two main groups of complement receptors; CR-1 to CR-4 and receptors for the biologically active molecules



SCIENCE BOX 2.3



The CD classification explained

You can be forgiven for thinking that CD has only ever meant 'compact disk', but actually the immunologists got there first. The CD classification is ultimately the new way of defining a cell.

Lymphocytes, monocytes, dendritic cells, granulocytes and a host of other cells have surface receptors and molecules that are vital in a whole range of immunological functions: cell–cell signalling, cell activation, hormone–receptor signalling and many others. The surfaces of immune cells are literally covered with such proteins. Different cells with particular functions have distinct surface proteins, whilst other molecules will be common to several cell types. A major breakthrough in defining surface molecules came in the 1970s, with the discovery of monoclonal antibodies. In a nutshell, monoclonal antibodies are proteins tailor-made to bind to a specific target. They are usually raised by injecting the target protein into mice. Researchers in the 1970s and 1980s raised monoclonal antibodies by injecting mice with crude extracts made from various types of immune cell, to try and find cell and lineage-specific markers. Some of the monoclonal antibodies generated were able to bind to structures on the surfaces of the target cells. These could be used as tools to distinguish populations of lymphocytes with different functions, as well as identifying neutrophils and monocytes.

By the mid-1980s, however, so many monoclonal antibodies were being produced around the world in so

many laboratories that confusion reigned: how did we know whether the surface molecule recognised by monoclonal antibody A produced in Atlanta was the same as that recognised by monoclonal antibody B produced in Baltimore? An international workshop was established. Monoclonal antibodies were exchanged by researchers and panels of experts sat to judge whether the antibodies recognised the same target protein. Because several different antibodies (a cluster) could recognise the same surface protein, and because surface proteins indicated the differentiation of a cell (e.g. granulocyte or lymphocyte), the monoclonal antibodies were assigned a number according to the **cluster of differentiation** to which they bound. A cluster of differentiation is, therefore, a surface molecule found on cells according to the cells' lineage and differentiation and identifiable by one or more monoclonal antibodies.

This means that cells can now be defined by the CDs they possess. For example, as you will see later on, a helper T cell could be defined as possessing the CD3 and CD4 markers, but not CD8 (i.e. 'CD3⁺CD4⁺CD8⁻'). International Workshops to define new CDs are organised periodically. The most recent was the 8th, held in 2004, at which 100 new DC designations were confirmed; up to and including CD339 (<http://www.hlda8.org/NewCDAssignments.htm>). A list of commonly used CD numbers is given in Appendix 1.

C4a, C3a and C5a. The main properties of these are shown in Table 2.2 and will be considered in the next section and Chapter 3.

Biological activities generated by complement activation

Opsonisation

The Greek word *opson* means a relish or sauce, i.e. something to make food more 'attractive'. When applied to cells such as neutrophils, which engulf microorganisms, the concept of opsonisation is that opsonins coat bacteria and thus facilitate their removal. One of the major opsonins derives from complement. The ability to bind membranes is a feature of various complement fragments, but C3b accounts for most of the complement opsonic activity. Once organisms are coated with C3b, it is simple to see how the presence of CRs 1, 3 and 4 on neutrophils can result in more efficient engulfment.

Cell recruitment and activation

The low-molecular-weight fragments C4a, C3a and C5a are known as **anaphylatoxins**. This name derives from their

putative role in a clinical syndrome **anaphylaxis** (see p. 143) in which they activate mast cells and basophils directly through specific receptors. C5a and, to a lesser extent, C3a are also **chemotactic**, a term used to describe the ability to attract cells, in this case neutrophils (see p. 28).

Cell lysis

Complete complement activation through either pathway occurring on cell surfaces leads to cell lysis. Typical targets could include bacteria and enveloped viruses, but host erythrocytes, platelets and lymphocytes may also become victims in certain pathological conditions.

Removal of immune complexes

Immune complexes of antibody and antigen are forming in the circulation continuously in small numbers, with periodic increases during infections or inflammatory episodes. These are potentially harmful, since they can become deposited in vessel walls or tissues and incite complement activation, with all the pro-inflammatory effects that that entails. Larger complexes, composed of a lattice of antibodies and antigens