Innate immunity I: physical and humoral protection

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’.

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.

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 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’.

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- fungi, e.g. Candida albicans
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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.
**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.
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.

### Table 2.1 Terminology of the complement system

<table>
<thead>
<tr>
<th>State of component</th>
<th>Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precursor molecules</td>
<td>Capital C followed by a number for the classical and common pathways, e.g. C1, C2</td>
</tr>
<tr>
<td></td>
<td>Capital letter followed by number for the alternative pathway, e.g. B1</td>
</tr>
<tr>
<td>Fragments</td>
<td>Small letter suffix, e.g. C4a, C2b (‘a’ fragments are smaller than ‘b’ fragments)</td>
</tr>
<tr>
<td>Inactivated components</td>
<td>Letter i prefix, e.g. iC3b</td>
</tr>
<tr>
<td>Active state</td>
<td>Bar over symbols, e.g. C4bC2b</td>
</tr>
</tbody>
</table>

**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.
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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.

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.

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 \( Clr \), 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.

**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**
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 polymerises 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.

**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.

**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.

**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
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...
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...