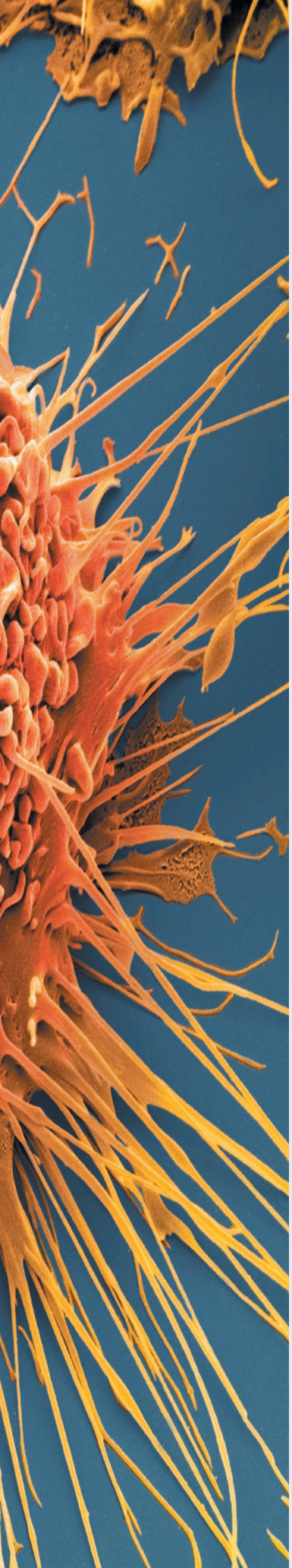


CHAPTER 1

Innate immunity

Key topics

- Knowing when to make an immune response 4
- Pattern recognition receptors detect nonself 7
- Immune responses are tailored towards particular types of infection 8
- Innate versus adaptive immunity 10
- External barriers against infection 12
- Cells of the immune system 12
- The beginnings of an immune response 18
- There are several classes of pattern recognition receptors 22
- Phagocytic cells engulf and kill microorganisms 29
- Phagocytes employ an array of killing mechanisms 30
- Complement facilitates phagocytosis and bacterial lysis 34
- Humoral mechanisms provide an additional defensive strategy 38
- Natural killer cells kill virally infected cells 42
- Dealing with large parasites 45
- The innate immune system instigates adaptive immunity 45

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

Companion website: www.roitt.com

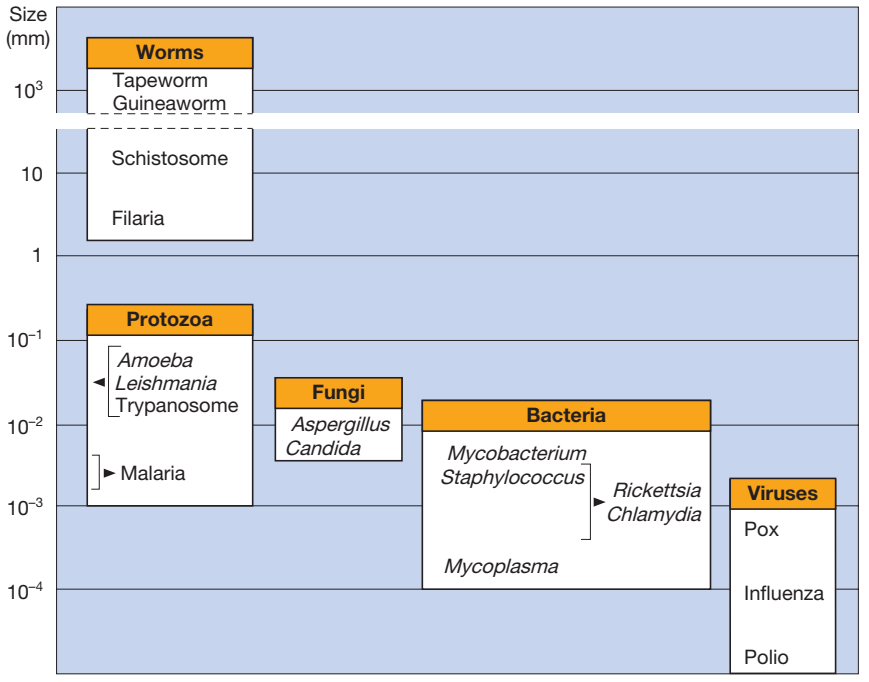


Figure 1.1 The formidable range of infectious agents that confront the immune system. Although not normally classified as such because of their lack of a cell wall, the mycoplasmas are included under bacteria for convenience. Fungi adopt many forms and approximate values for some of the smallest forms are given. Square brackets with right arrowheads indicate where a range of sizes is observed for the organism(s); square brackets with left arrowheads indicate list of organisms with a definite size.

Introduction

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

Aside from ill-understood constitutional factors that make one species innately susceptible and another resistant to certain infections, a number of relatively nonspecific but nonetheless highly effective antimicrobial systems (e.g., phagocytosis, production of antimicrobial peptides and reactive oxygen species) have been recognized that are ***innate*** in the sense that they are not affected by prior contact with the infectious agent and take immediate effect upon encounter with anything that our immune systems deem to be an unwelcome guest. We shall discuss these systems and examine how, in the state of ***adaptive immunity***, their effectiveness can be greatly increased though custom tailoring of the response towards microbial intruders.

Knowing when to make an immune response

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

The vertebrate immune system is a conglomeration of cells and molecules that cooperate to protect us from infectious agents and also provides us with a surveillance system to monitor the integrity of host tissues. Although the immune system is quite elaborate, as we shall see, its function can be boiled down to two basic roles: ***recognition*** of foreign substances and organisms that have penetrated our outer defences (i.e., the skin epithelium and the mucosal surfaces of the gut and reproductive and respiratory tracts) and ***elimination*** of such agents by a diverse repertoire of cells and molecules that act in concert to neutralize the potential threat. Thus, a critical role of the immune system is to determine what is foreign (what immunologists often call "nonself") from what is normally present in the body (i.e., self). As a consequence, the cells and molecules that comprise the innate immune system are preoccupied with detecting the presence of particular ***molecular patterns*** that are typically associated with infectious agents (Figure 1.2). Charlie Janeway dubbed such molecules ***pathogen-associated molecular patterns (PAMPs)*** and it is these structures that trigger activation of the innate immune system.

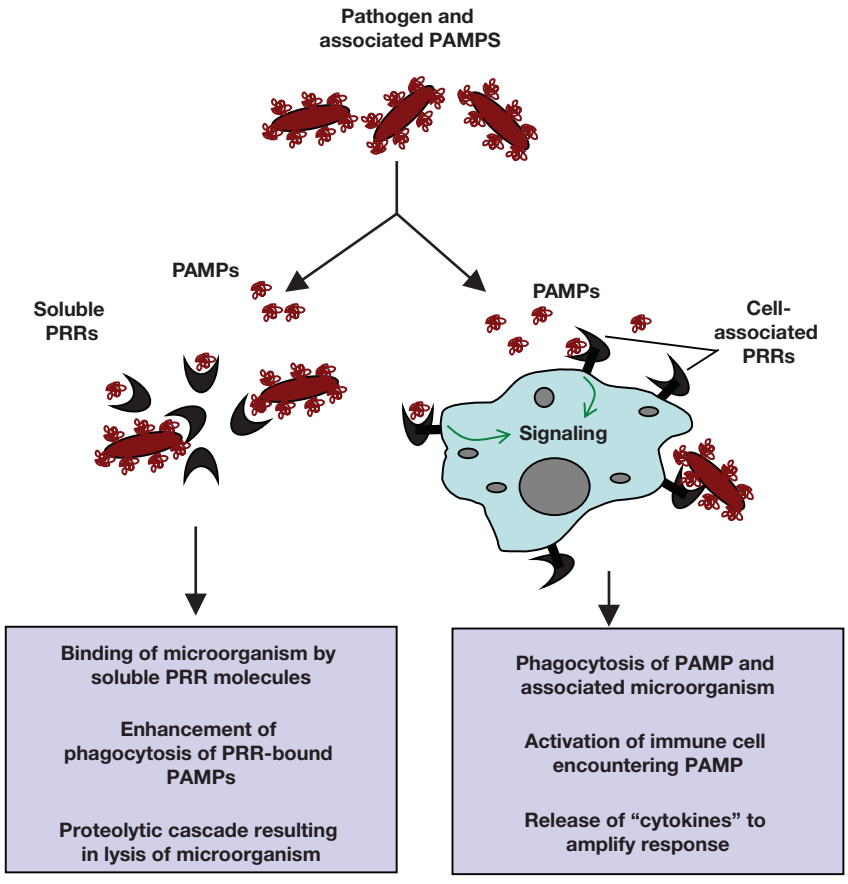


Figure 1.2 Pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs) and initiate immune responses. PRRs can be either soluble or cell-associated and can instigate a range of responses upon encountering their appropriate ligands.

In addition to the fundamental roles of recognition and elimination of infectious agents, it is also very useful to be able to learn from encounters with pathogens and to maintain a reserve of cells that are able to respond swiftly to a new infection with a previously encountered microbe. Forewarned is forearmed, and in this situation it may be possible to deliver a decisive blow that ends a nascent infection before it has begun. Fortunately, our immune systems have also acquired this ability, which is what our ***adaptive immune system*** excels in, and this property is termed ***immunological memory***.

Immune responses need to be proportional to the infectious threat

Having established that recognition, elimination and memory of infectious agents are fundamental to the operation of an effective immune system, there is another important factor, ***proportionality***, which is key to ensuring that everything runs smoothly and that our immune systems do not lose sight of their purpose. This is because, as we shall see, the immune system can deploy a variety of weapons, each with their own risk of collateral damage, which can sometimes cause as much trouble as the infection itself. In extreme cases, the immune response can be much more destructive than the agent that triggered it (which is what underpins allergy) and in some situations this can lead to a sustained state of ***chronic immune activation*** where the immune system becomes confused between what is self and nonself and mounts sustained responses against its own tissues (called autoimmunity). Thus, there is a cost–benefit analysis that must be conducted during the initial stages of an infection to ascertain the nature of the infection, the level of infection, and whether the infectious agent is perturbing tissue function (by triggering cell death for example).

For these reasons, a number of ***immune regulatory*** mechanisms exist to ensure that immune responses are proportional to the level of threat that a particular infectious agent poses, as well as to ensure that immune responses are not directed against self and that responses directed against nonself are terminated when the infectious agent has been successfully eliminated from the body. Immune regulatory mechanisms (or ***immune checkpoints***) set thresholds for the deployment of immune responses and are vital to the proper operation of the immune system. As we shall see in later chapters, many diseases are caused by the failure of immune

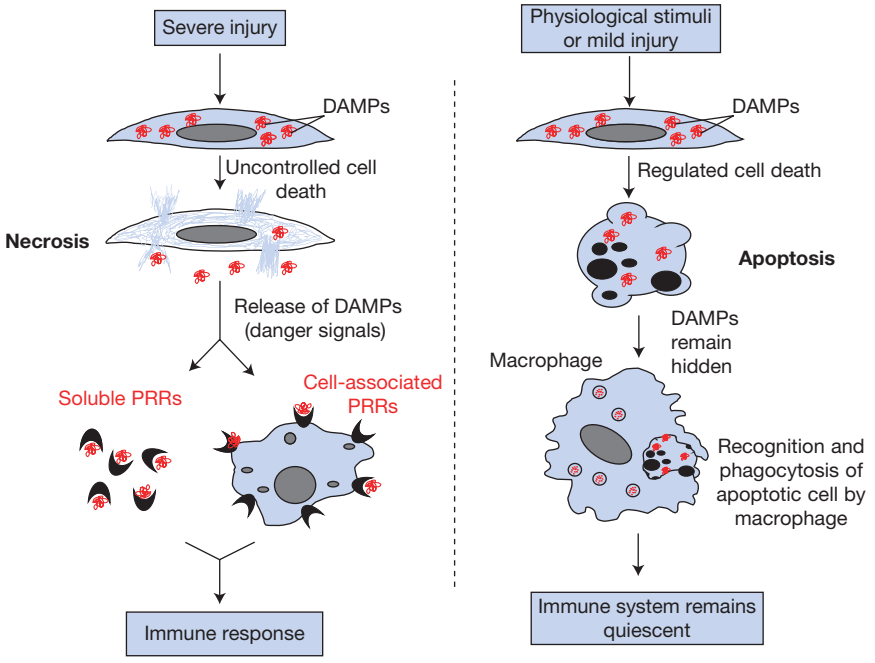


Figure 1.3 Necrotic cells release danger-associated molecular patterns (DAMPs), whereas apoptotic cells typically do not. Stimuli that induce necrosis frequently cause severe cellular damage, which leads to rapid cell rupture with consequent release of intracellular DAMPs. DAMPs can then engage cells of the immune system and can promote inflammation. On the other hand, because stimuli that initiate apoptosis are typically physiological and relatively mild, apoptotic cells do not rupture and their removal is coordinated by macrophages and other cells of the innate immune system, before release of DAMPs can occur. For this reason, apoptosis is not typically associated with activation of the immune system.

checkpoints, leading to conditions such as rheumatoid arthritis, Crohn's disease, and even cancer.

Tissue damage can also instigate an immune response

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

The precise identity of the molecules that act as danger signals – now more commonly called ***danger-associated molecular patterns (DAMPs)*** or alarmins – is an area of active investigation at present, but molecules such as HMGB1, a chromatin-binding protein, as well as the immunological messenger proteins interleukin-1α (IL-1α) and IL-33, represent good candidates. It might seem surprising that the immune system can also be activated by self-derived molecules, however, this makes good sense when one considers that events leading to necrotic cell death are often rapidly followed or accompanied by infection. Furthermore, if a pathogen manages to evade direct detection by the immune system, its presence will be betrayed if it provokes necrosis within the tissue it has invaded.

Before moving on, we should also note that there is another mode of cell death that frequently occurs in the body that is both natural and highly controlled and is not associated with plasma membrane rupture and release of intracellular contents. This mode of cell death, called ***apoptosis*** (see Videoclip 2), is under complex molecular control and is used to eliminate cells that have reached the end of their natural lifespans. Apoptotic cells do not activate the immune system because cells dying in this manner display molecules on their plasma membranes (e.g., phosphatidylserine) that mark these cells out for removal through phagocytosis before they can rupture and release their intracellular contents. In this way, DAMPs remain hidden during apoptosis and such cells do not activate the immune system (Figure 1.3).

Pattern recognition receptors detect nonself

Pattern recognition receptors (PRRs) raise the alarm

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

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

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

A variety of responses can occur downstream of pattern recognition

One way of dealing with unwelcome intruders involves the binding of soluble (humoral) pattern recognition molecules, such as ***complement*** (an enzyme cascade we will deal with later in this chapter), ***mannose-binding lectin***, ***C-reactive protein***, or ***lysozyme***, to the infectious agent. The binding of soluble pattern recognition molecules to a pathogen has a number of outcomes (Figure 1.2).

First, this can lead directly to ***killing of the pathogen*** through destruction of microbial cell wall constituents and breaching of the plasma membrane because of the actions of such proteins. Second, humoral factors are also adept at coating microorganisms (a process called ***opsonization***) and this greatly enhances their uptake through ***phagocytosis*** and subsequent destruction by phagocytic cells.

Other PRRs are cell associated and engagement of such receptors can also lead to ***phagocytosis*** of the microorganism followed by its destruction within phagocytic vesicles. Just as importantly, cellular PRR engagement also results in the activation of signal transduction pathways that greatly enhance the ***effector functions*** of cells bearing these receptors (such as increasing their propensity for phagocytosis or the production of antimicrobial proteins) and also culminate in the release of soluble messenger proteins (***cytokines***, ***chemokines***, and other molecules) that mobilize other components of the immune system. PRR engagement on effector cells can also result in ***differentiation*** of such cells to a more mature state that endows specialized functions on such cells. Later we will deal with a very important example of this when we discuss the issue of dendritic cell maturation, which is initiated as a consequence of engagement of PRR receptors on these cells by microbial PAMPs. Therefore, pattern recognition of a pathogen by soluble or cell-associated PRRs can lead to:

- direct lysis of the pathogen
- opsonization followed by phagocytosis
- direct phagocytosis via a cell-associated PRR
- enhancement of phagocytic cell functions
- production of antimicrobial proteins
- production of cytokines and chemokines
- differentiation of effector cells to a more active state.

There are several classes of pattern recognition receptor

As we shall see later in this chapter, there are a number of different classes of cell-associated PRRs (Toll-like receptors [TLRs], C-type lectin receptors [CTLRs], NOD-like receptors [NLRs], RIG-I-like receptors [RLRs], among others) and it is the engagement of one or more of these different categories of receptors that not only enables the ***detection of infection***, but also conveys information concerning the ***type*** of infection (whether yeast, bacterial, or viral in origin) and its ***location*** (whether extracellular, endosomal, or cytoplasmic). In practise, most pathogens are likely to engage several of these receptors simultaneously, which adds another level of complexity to the signaling outputs that can be generated through engagement of these receptors. This, in turn, enables the ***tailoring of the subsequent immune response*** towards the particular vulnerabilities of the pathogen that raised the alarm.

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

An important feature of the immune system is the ability of its constituent cells to communicate with each other upon encountering a pathogen to initiate the most appropriate response. As we shall see shortly, there are quite a number of different "ranks" among our immune forces, each with their own particular arsenal of weapons, and it is critical that a measured and appropriate response is deployed in response to a specific threat. This is because, as we have already alluded to, many of the weapons that are brought into play during an immune response are destructive and have the potential to cause collateral damage. Furthermore, initiation and escalation of an immune response carries a significant metabolic cost to

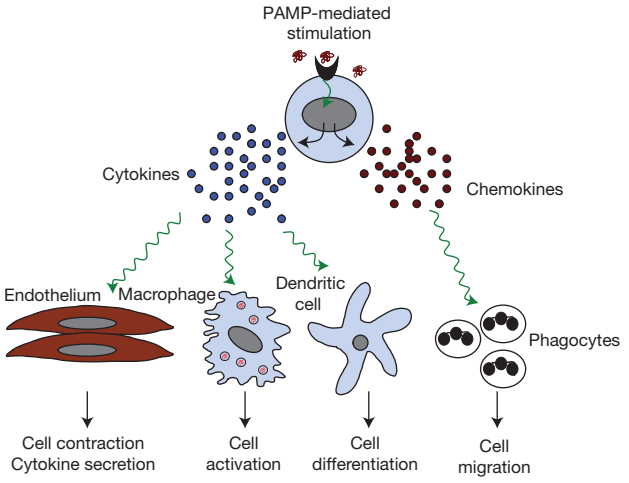


Figure 1.4 Cytokines and chemokines can have pleiotrophic effects. Stimulation of cells of the innate immune system frequently leads to the production of inflammatory cytokines and chemokines that trigger responses from other cell types, as depicted. Note that the effects of chemokines and cytokines shown are not exhaustive.

the organism (due to the necessity to make numerous new proteins and cells). Thus, communication among the different immune battalions is essential for the initiation of the correct and proportional response to the particular agent that triggered it. Although cells of the immune system are capable of releasing numerous biologically active molecules with diverse functions, two major categories of proteins – ***cytokines*** and ***chemokines*** – have particularly important roles in shaping and escalating immune responses.

Cytokines are a diverse group of proteins that have ***pleiotropic effects***, including the ability to activate other cells, ***induce differentiation*** to particular effector cell subsets and enhance microbicidal activity (Figure 1.4). Cytokines are commonly released by cells of the immune system in response to PAMPs and DAMPs, and this has the effect of altering the ***activation state*** and ***behavior*** of other cells to galvanize them into joining the fight. Chemokines are also released upon encountering PAMPs/DAMPs and typically serve as ***chemotactic factors***, helping to lay a trail that guides other cells of the immune system to the site of infection or tissue damage. Both types of messenger proteins act by diffusing away from the cells secreting them and binding to cells equipped with the appropriate plasma membrane receptors to receive such signals.

The interleukins are an important class of cytokines

A particularly important group of cytokines in the context of immune signaling is the interleukin (IL) family, which has over 40 members at present, numbered in the order of their discovery. Thus, we have IL-1, IL-2, IL-3, IL-4, etc. Interleukins, by definition, are cytokines that signal between members of the leukocyte (i.e., white blood cell) family. However, these molecules often have effects on other tissues that the immune system needs to engage in the course of initiating immune responses. So, although ***interleukins are heavily involved in communication between immune cells***, these cytokines also have profound effects on endothelial cells lining blood capillaries, hepatocytes in the liver, epithelial cells, bone marrow stem cells, fibroblasts, and even neurons within the central nervous system. It is also important to note that ***the same interleukin can trigger different functional outcomes*** depending on the cell type that it makes contact with; these are simply "switch" molecules that can turn different functions on or off in the cells they encounter. The function that is switched on, or off, will depend on the target cell and the other cytokine signals that this cell is receiving in tandem. Thus, just as we integrate lots of different sources of information (e.g., from colleagues, friends, family, newspapers, TV, radio, books, websites, social media, etc.) in our daily lives that can all influence the decisions we make, ***cells also integrate multiple sources of cytokine information to make decisions*** on whether to divide, initiate phagocytosis, express new gene products, differentiate, migrate, and even die. We will discuss cytokines, chemokines, and their respective receptors at length in Chapter 8.

Immune responses are tailored towards particular types of infection

Not all pathogens are equal

We will shortly get into the specifics of the immune system, but before doing so it is useful to consider the diversity of infectious agents that our immune systems may encounter (Figure 1.1), and to contemplate whether a "one size fits all" immune response is likely to suffice in all of these situations. One of the frustrations expressed by many students of immunology is that the immune system appears to be almost byzantine in its complexity. Although this is indeed partly true, the reasons for this are two-fold. First, because there are different types of infection, ***immune responses need to be tailored towards the particular class of infection*** (whether viral, extracellular bacterial, intracellular bacterial, worm, fungal, etc.) in order to mount the most effective immune response towards a particular infectious agent. Second, although there is indeed complexity in the immune system, there is also a great deal of order and repeated use of the same basic approach when recognizing pathogens and initiating an immune response. Therefore, although many of molecules used in the pursuit of pathogen recognition belong to different classes, many of these plug into the same effector mechanisms as soon as the pathogen is successfully identified. So, dear reader, please bear with us while we try to make sense of the apparent chaos. But meanwhile, let us get back to pathogens to consider why our immune systems need to be fairly elaborate and multi-layered.

Infectious agents are a broad church and have evolved different strategies to invade and colonize our bodies, as well as to evade immune detection. Some, such as yeasts and extracellular

bacteria, are happy to live in the extracellular space, stealing nutrients that would otherwise nourish our own tissues. Others, such as intracellular bacteria and viruses, invade the cytoplasm and even our genomes and may lurk for months or years within our bodies. Then there are the large worms (helminths) and unicellular eukaryotic protozoa that live parasitic lifestyles with their own particular adaptations.

Because of the diversity of infectious agents, all of which have their own strategies to evade and neutralize the best efforts of our immune systems, we have responded by evolving multiple ways of dealing with intruders, depending on the nature of the infectious agent and how this type of infection is best dealt with. Indeed, it is the constant threat of infection (rather than environmental change) that is the major driver of natural selection over the short term, as viruses and bacteria can mutate with frightening speed to acquire adaptations that can leave their hosts highly vulnerable to infection. For this reason, genes that are involved in the functioning and regulation of the immune system are among the most diverse among human and animal populations (i.e., undergoing the fastest rates of mutation) and are frequently duplicated into large gene families (which are typically variations on a very useful theme) that permits us to hedge our bets and stay ahead in the ongoing battle against those organisms that would have us for lunch.

Because of the diverse nature of the infectious agents that we are confronted with, ***immune responses come in a number of different flavors*** and are ***tailored towards the nature of the pathogen that provoked the response*** in the first place. As the book progresses, we will elaborate on this concept in much more detail, but do keep this in mind when trying to understand the underlying simplicity among the apparent complexity of the immune responses that we will encounter.

There are different types of immune response

So, what do we mean by different types of immune response? We are not going to be exhaustive at this stage, but let us consider the difference between how our immune system might deal with a virus versus an extracellular bacterium. For both pathogen classes, a system that enables us to recognize these agents and to remove them, either by destroying them (through membrane lysis) or by eating them up (through phagocytosis) followed by degradation within endosomes, would likely be very effective. And indeed, our immune systems have evolved a number of ways of doing both of these things; as we have mentioned earlier, there are multiple classes of proteins that ***recognize and lyse bacteria and viruses*** in the extracellular space (complement, acute phase proteins, antimicrobial peptides) and the same proteins are frequently involved in ***decorating infectious agents for recognition and phagocytosis*** by phagocytic cells (e.g., macrophages and neutrophils) that are specialized in doing just that. Molecules that are involved in the decoration of infectious agents to prepare them for removal are called ***opsonins*** (from the Greek, to prepare for eating) in immunological parlance. So far, so good.

However, once the virus enters a cell, the proteins and phagocytic cells mentioned above will no longer be of any use in dealing with this type of infection as proteins cannot freely diffuse across the plasma membrane to either lyse or tag the infectious agent for phagocytosis. So, it is here that the immune response to an extracellular bacterial infection versus an intracellular viral infection must diverge, as now we need a way of looking inside cells to see whether they are infected or not. Consequently, we have evolved a number of ***intracellular PRRs that can detect pathogens that have entered cells***, and this results in the production of signals (e.g., cytokines and chemokines) that alert the immune system to the presence of an infectious agent. Just as importantly, we have also evolved a fiendishly clever way of displaying the breakdown products of pathogens to cells of the adaptive immune system (***major histocompatibility complex*** [MHC] molecules are centrally involved in this process) irrespective of whether the infectious agent lives inside or outside the cell. We will deal with MHC molecules extensively in Chapters 4 and 5. The latter process enables a cell that has been infected by a virus to display fragments of viral proteins on its plasma membrane, within grooves present in MHC molecules that have evolved for this purpose, thereby alerting cells of the immune system to the nature of its predicament. Ingenious!

So, how does our immune system deal with a virus or other pathogen that has invaded a host cell? Although some specialized phagocytic cells (i.e., macrophages) can kill intracellular bacteria that have invaded them, most cells cannot do this very effectively and so another solution is required. For most other cell types, this is achieved through ***killing the infected cell*** (typically by apoptosis) and removing it through ***phagocytosis***, which is easy to write, but involves a series of steps that permit the recognition of infected host cells, the delivery of the "kiss of death" and the engulfment of the infected corpse in a manner that minimizes the escape of the pathogen lurking within. Our immune systems have solved the intracellular infection problem by evolving cells (called ***cytotoxic T-cells*** and ***natural killer cells***) that have the ability to detect infected cells and to kill them; we will deal with natural killer (NK) cells in detail later in this chapter.

Obviously, such powers of life or death carry with them the heavy responsibility of ensuring that uninfected cells are not accidently killed, as it is a basic tenet of multicellularity that one does not go around randomly killing good cellular citizens. Thus, a number of checks and balances have been incorporated into this killing system to ensure that only errant cells are dispatched in this way. We will deal with the detailed mechanisms of cytotoxic T-cell-mediated killing in Chapter 8.

However, some pathogens require a different approach, which involves sending in large numbers of highly phagocytic cells (such as neutrophils) into a tissue that can also deploy destructive proteases, carbohydrases (such as lysozyme), and other nasty molecules into the extracellular space in order to quickly overwhelm and destroy a rapidly dividing pathogen, or a worm parasite. This type of response comes with a certain

degree of collateral damage (due to the use of enzymes that do not discriminate between friend and foe) and is typically only mounted when this is warranted.

From the preceding discussion, we hope that it will be evident that ***different types and severities of immune responses are necessary to fight different types of infection*** and it is for this reason that the immune system has a variety of cells and weapons at its disposal. Thus, there are different types of immune response, broadly dictated by whether a pathogen lives ***intracellularly or extracellularly***.

The PRRs of the innate immune system generate a molecular fingerprint of pathogens

As we have already alluded to, the PRRs not only help to identify the ***presence*** of infectious agents through detection of their associated PAMPs, but they also convey information as to the ***nature*** of the infectious agent (whether of fungal, bacterial or viral origin) and the ***location*** of the infectious agent (whether extracellular, intracellular, endosomal, cytoplasmic, or nuclear). This is because, as we shall see later, the various classes of PRRs (e.g., Toll-like receptors, C-type lectin receptors, NOD-like receptors, cytoplasmic DNA sensors) are specific for different types of pathogen components (i.e., PAMPs), and reside in different cellular compartments. Thus, we have an ingenious system where the combination of PRRs that is engaged by an infectious agent conveys important information about the precise nature and location of infection and generates a ***molecular fingerprint of the pathogen***. In turn, ***this information is then used to shape the most effective immune response*** towards the particular pathogen class that provoked it.

Cytokines help to shape the type of immune response that is mounted in response to a particular pathogen

We have already mentioned that cytokines are involved in communication between cells of the immune system and help to alert the correct cell types that are appropriate for dealing with different classes (i.e., whether viral, bacterial, yeast, etc.) of infectious agents. Cytokines are also capable of triggering the ***maturation and differentiation*** of immune cell subsets into more specialized ***effector cell*** classes that possess unique capabilities to enable them to fight particular types of infection. In this way, detection of an infection (i.e., PAMPs) by a particular class of PRR is translated into the most appropriate immune response ***through the production of particular patterns of cytokines and chemokines***. These cytokine patterns then call into play the correct cell types and trigger maturation of these cells into even more specific effector cell subtypes. Later, in Chapter 8, we will see how this process is used to produce specialized subsets of T-cells that are central to the process of adaptive immunity. Let us now look at how the different layers of our immune defenses are organized.

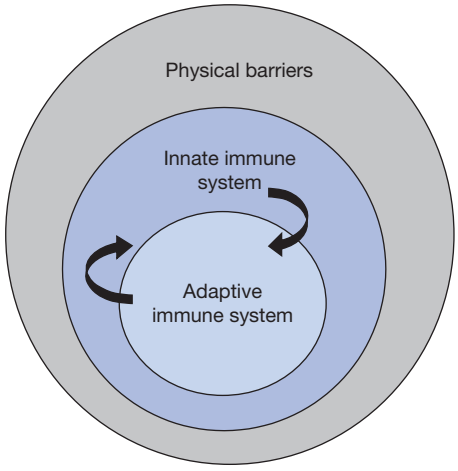


Figure 1.5 The vertebrate immune system comprises three levels of defense. The physical barriers of the skin and mucosal surfaces comprise the first level of defense. Infectious agents that successfully penetrate the physical barriers are then engaged by the cells and soluble factors of the innate immune system. The innate immune system is also responsible for triggering activation of the adaptive immune system, as we will discuss later in this chapter. The cells and products of the adaptive immune system reinforce the defense mounted by the innate immune system.

Innate versus adaptive immunity

Three levels of immune defense

Before we get into the details, we will first summarize how the immune system works in broad brushstrokes. The vertebrate immune system comprises three levels of defense (Figure 1.5). First, there is a ***physical barrier*** to infection that is provided by the skin on the outer surfaces of the body, along with the mucous secretions covering the epidermal layers of the inner surfaces of the respiratory, digestive, and reproductive tracts. Any infectious agent attempting to gain entry to the body must first breach these surfaces that are largely impermeable to microorganisms; this is why cuts and scrapes that breach these physical barriers are often followed by infection. The second level of defense is provided by the ***innate immune system***, a relatively broad-acting but highly effective defense layer that is largely preoccupied with trying to kill infectious agents from the moment they enter the body. The actions of the innate immune system are also responsible for alerting the cells that operate the third level of defense, the ***adaptive (or acquired) immune system***. The latter cells represent the elite troops of the immune system and can launch an attack that has been specifically adapted to the nature of the infectious agent using sophisticated weapons such as antibodies. As we shall see, the innate and adaptive immune systems each have their own particular advantages and disadvantages and therefore act cooperatively to achieve much more effective immune protection than either could achieve in isolation.

Innate immune responses are immediate and relatively broad acting

Upon entry of a foreign entity into the body, the innate immune response occurs almost immediately. Innate immune responses do not improve (at least to a dramatic degree) upon frequent encounter with the same infectious agent. The innate immune system recognizes broadly conserved components of infectious agents, the aforementioned PAMPs, which are not normally present in the body. The molecules and receptors (i.e., PRRs) used by the innate immune system to detect PAMPs are hard-wired (i.e., germline encoded, which means that such genes are passed in essentially identical form from parent to offspring) and respond to ***broad categories*** of foreign molecules that are commonly expressed on microorganisms. The relatively invariant nature of PRRs is a strength, as well as a weakness, of the innate immune system. It is a strength in terms of discriminating self from nonself very reliably (as PRRs have evolved over millions of years to be able to detect nonself, while ignoring self), but is a weakness in that the ***specificity of a given PRR towards an individual pathogen is poor*** as these receptors do not mutate at any appreciable rate. Thus, innate immune responses cannot be uniquely tailored towards a specific pathogen, at least beyond the number of individual PRRs that our innate immune systems possess.

Because the receptors of the innate immune system are encoded by the germline, innate immune responses are therefore quite similar between individuals of the same species. Upon detecting a PAMP, the innate immune system mounts an immediate attack on anything displaying such molecules by either engulfing such entities or through attacking them with destructive enzymes, such as proteases or membrane-attacking proteins (Figure 1.2). The clear intent is to bludgeon the unwanted intruder into submission as quickly as possible. This makes sense when one considers the prodigious rates of proliferation that bacteria can achieve (many bacterial species are capable of dividing every 20 minutes or so), particularly in the nutrient-rich environment our bodies provide. Key players in the innate immune response include ***macrophages***, ***neutrophils***, and soluble bactericidal (i.e., bacteria killing) proteins such as ***complement*** and ***lysozyme***. Although highly effective, innate immune responses are not always sufficient to completely deal with the threat, particularly if the infectious agent is well adapted to avoid the initial attack. In this situation, a more specific immune response is required, tailored towards particular determinants that are present on individual pathogens. This is where the adaptive immune response comes into play.

Adaptive immune responses are delayed but highly specific

Because of the way in which adaptive immune responses are initiated, such responses take longer to achieve functional significance, typically 4–5 days after the innate immune response, but are ***exquisitely tailored*** to the nature of the infectious agent. How the pathogen-detecting receptors of the adaptive immune system (such as antibody) achieve their high specificity will be discussed at length in later chapters, but in brief this involves the shuffling of a relatively small number of receptor precursors that, through the power of random genetic recombination, can produce a truly spectacular number of specific antigen receptors (numbering in the tens of millions). The major downside to this genetic recombination process is that it is ***prone to producing receptors that recognize self***. However, the adaptive immune system has evolved ways of dealing with this problem, as will be discussed in Chapter 10.

Importantly, because the antigen receptors of the adaptive immune system are custom-built to recognize specific pathogens, such responses improve upon each encounter with a particular infectious agent, a feature called ***immunological memory***, which underpins the concept of vaccination. The adaptive immune response is mediated primarily by ***T- and B-lymphocytes*** and these cells display specific receptors on their plasma membranes that can be tailored to recognize an almost limitless range of structures. By definition, molecules that are recognized by T- and B-lymphocytes are called ***antigens***. Recognition of antigen by a lymphocyte triggers proliferation and differentiation of such cells and this has the effect of greatly increasing the numbers of lymphocytes capable of recognizing the particular antigen that triggered the response in the first place. This rapidly swells the ranks of lymphocytes (through a process called ***clonal expansion***, which enables the rapid division of cells carrying a particular antigen receptor) capable of dealing with the infectious agent bearing the specific antigen and results in a ***memory response*** if the same antigen is encountered at some time in the future. We will look in detail at the receptors used by T- and B-cells to see antigen in Chapter 4.

Innate and adaptive immune responses are interdependent

The innate and adaptive immune systems work in tandem to identify and kill infectious agents (Figure 1.5). As we shall see in later chapters, while the innate and adaptive immune systems have their own individual strengths, there are multiple points at which the innate immune system feeds into the adaptive immune system and visa versa. In this way, both systems synergize to deal with infectious agents. Thus, when an infection occurs, ***the innate immune system serves as a rapid reaction force*** that deploys a range of relatively nonspecific (but nonetheless highly effective) weapons to eradicate the infectious agent, or at the very least to keep the infection contained. This gives time for the initially sluggish adaptive immune system to select and clonally expand cells with receptors that are capable of making a much more specific response that is uniquely tailored to the infectious agent. ***The adaptive immune response to an infectious agent reinforces and adds new weapons to the attack*** mounted by the innate immune system.

Although it was once fashionable to view the innate immune system as somewhat crude and clumsy when compared

to the relative sophistication of the adaptive immune system, an explosion of new discoveries over the past 10–15 years has revealed that the innate immune system is just as highly adapted and sophisticated as the adaptive immune system. Moreover, it has also become abundantly clear that ***the adaptive immune system is highly dependent on cells of the innate immune system for the purposes of knowing when to respond, how to respond, and for how long***.

The main reason for this, as we discussed earlier, is that the innate immune system uses hard-wired receptors (PRRs) that are highly reliable in terms of discriminating self from nonself. In contrast, because the adaptive immune system uses receptors that are ***generated* de novo *through random genetic recombination in response to each infectious agent that is encountered, these receptors can easily end up recognizing self, a situation that is highly undesirable.*** Therefore, ***cells of the adaptive immune system require instruction (or permission) by cells of the innate system*** as to whether an immune response should be mounted towards a particular antigen. Furthermore, the precise nature of the PRRs that are engaged on cells of the innate immune system in the initial stages of an infection dictate the type of adaptive immune response that is required (through the production of specific cytokines and chemokines). We will return to these important issues later in this chapter, but for now let us consider the external barriers to infection in a little more detail.

External barriers against infection

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

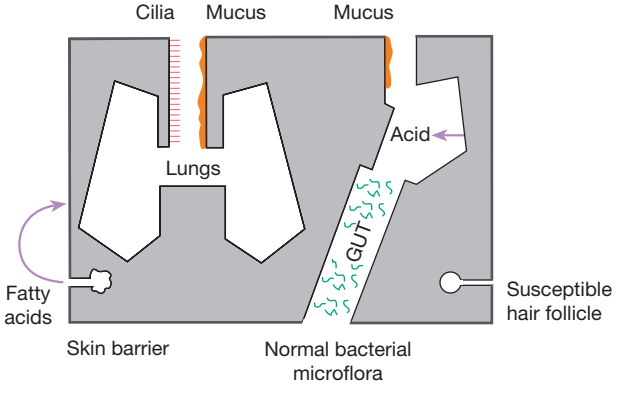


Figure 1.6 The first lines of defense against infection: protection at the external body surfaces.

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

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

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

Cells of the immune system

The cells of the immune system can be divided broadly into two main classes – myeloid and lymphoid cells

Immune cells, which are collectively called leukocytes (white blood cells), can be divided broadly into myeloid and lymphoid subsets (Figure 1.7).

Myeloid cells, which comprise the majority of the cells of the innate immune system, include ***macrophages*** (and their ***monocyte*** precursors), ***mast cells***, ***dendritic cells***, ***neutrophils***, ***basophils***, and ***eosinophils***. All myeloid cells have some degree of phagocytic capacity (although basophils are very poorly phagocytic compared to other myeloid cell types) and specialize in the detection of pathogens via membrane or endosomal PRRs, followed by engulfment and killing of infectious agents

by means of a battery of destructive enzymes contained within their intracellular granules.

Neutrophils are by far the most abundant leukocyte circulating in the bloodstream, comprising well over 50% of leukocytes, and these cells are ***particularly adept at phagocytosing and killing microbes.*** However, because of their destructive potential, neutrophils are not permitted to exit the blood and enter tissues until the necessity of their presence has been confirmed through the actions of other cells of the innate immune system (especially macrophages and mast cells), as well as soluble PRRs such as complement. As we shall see, certain myeloid cells, such as macrophages and dendritic cells, have particularly important roles in ***detecting and instigating immune responses***, as well as presenting the components of phagocytosed microbes to cells of the lymphoid system. Broadly speaking, activated myeloid cells also have an important function in ***escalating immune responses*** through the secretion of multiple cytokines and chemokines as well as additional factors that have powerful effects on local blood vessels.

The other major class of immune cells, the ***lymphoid cells***, comprise three main cell types, ***T-lymphocytes***, ***B-lymphocytes***, and ***natural killer (NK) cells***. T- and B-lymphocytes are the central players in the adaptive immune system and have the ability to generate ***highly specific cell surface receptors*** (T- and B-cell receptors), through genetic recombination of a relatively limited number of precursors for these receptors (discussed in detail in Chapters 4 and 5). T-cell receptors (TCRs) and B-cell receptors (also called antibodies) can be generated that are exquisitely specific for particular molecular structures, called antigens, and can fail to recognize related antigens that differ by only a single amino acid. NK cells, although lymphocytes, play a major role within the innate immune system, but these cells also police the presence of special antigen-presenting molecules (the aforementioned MHC molecules) that are expressed on virtually all cells in the body and play a key role in the operation of the adaptive immune system. NK cells use germline-encoded receptors (called NK receptors) that are distinct from the receptors of T- and B-cells and are endowed with the ability to kill cells that express abnormal MHC receptor profiles, as well as other signs of infection.

Milestone 1.1 Phagocytosis

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

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

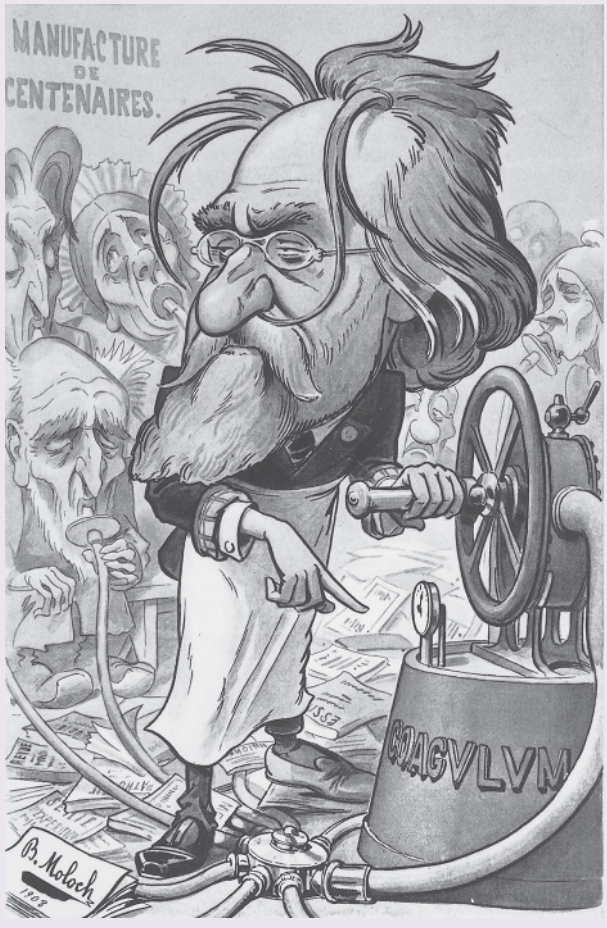


Figure M1.1.1 Caricature of Professor Metchnikoff. (Source: From *Chanteclair*, 1908, No. 4, p. 7. Reproduced with permission of The Wellcome Institute Library, London, UK.)

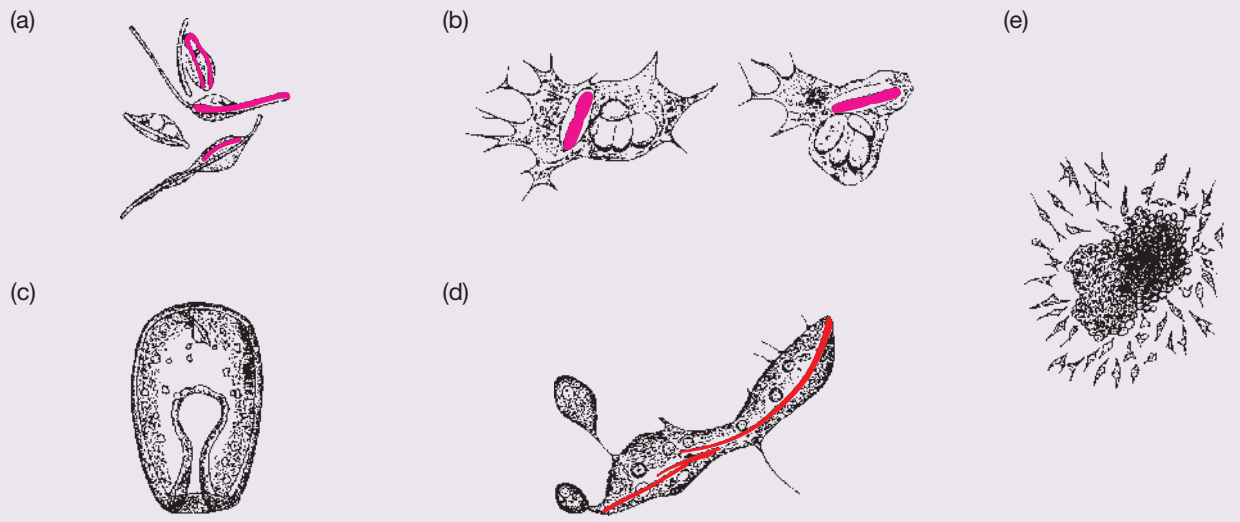


Figure M1.1.2 Reproductions of some of the illustrations in Metchnikoff's book, *Comparative Pathology of Inflammation* (1893). (a) Four leukocytes from the frog, enclosing anthrax bacilli; some are alive and unstained, others, which have been killed, have taken up the vesuvine dye and have been colored.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

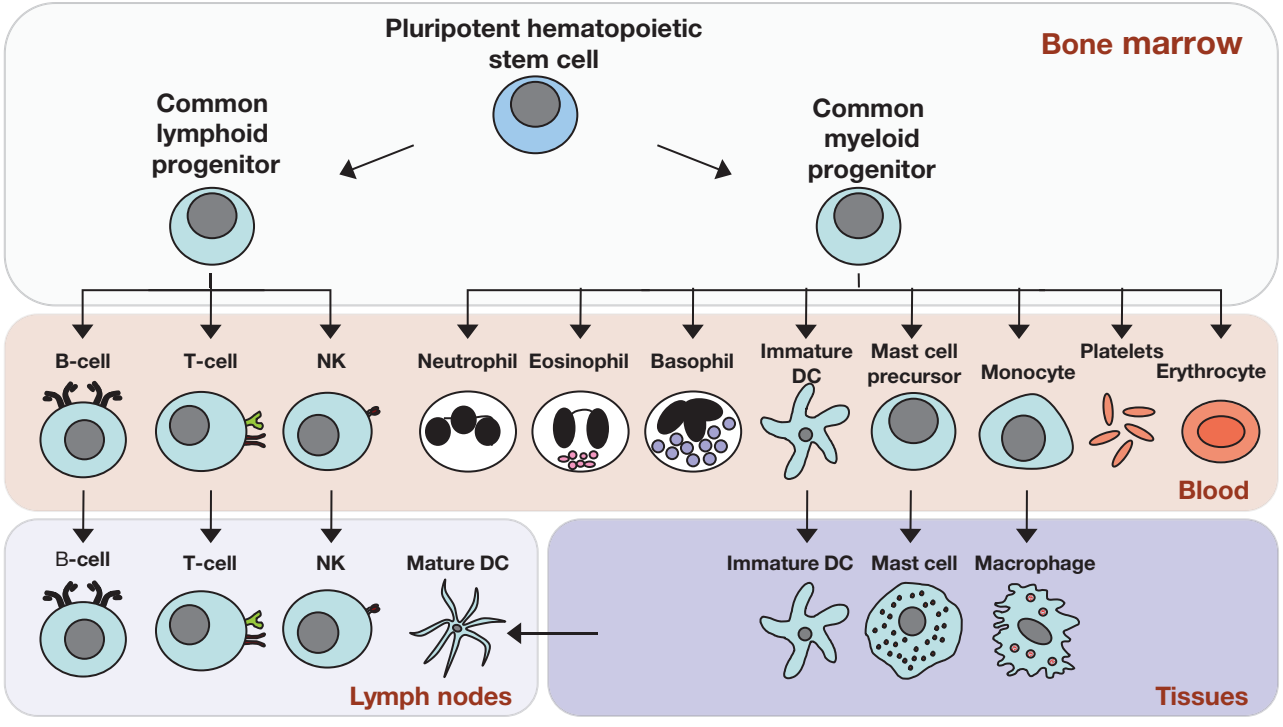


Figure 1.7 The cells of the immune system originate in the bone marrow from pluripotent hematopoietic stem cells. Pluripotent hematopoietic stem cells give rise to a common lymphoid progenitor, which gives rise to all of the major lymphoid cell types (T-cells, B-cells, and NK cells) or a common myeloid progenitor, which gives rise to all of the major myeloid cell types (neutrophils, eosinophils, basophils, dendritic cells [DCs], mast cells, and monocytes/macrophages) as well as the erythrocytes and megakaryocytes (which generate platelets). See further details of individual cell types in Figure 1.8, Figure 1.9, and Figure 1.11.

Cells of the immune system originate in the bone marrow

All cells of the lymphoid and myeloid lineages are derived from a ***common hematopoietic stem cell progenitor*** in the bone marrow (Figure 1.7). These stem cells, which are self-renewing, give rise to a ***common lymphoid progenitor*** as well as a ***common myeloid progenitor***, from which the various types of lymphoid and myeloid cells differentiate (Figure 1.7). This process, called ***hematopoiesis***, is complex and takes place under the guidance of multiple factors within the bone marrow, including stromal cells, the factors they produce and the influence of the extracellular matrix. Indeed, the study of this process is a whole research discipline in itself (hematology) and it has taken many years to unravel the multiplicity of cues that dictate the production of the formed elements of the blood. However, the basic scheme is that the various soluble and membrane-bound hematopoietic factors influence the differentiation of the various myeloid and lymphoid cell types in a stepwise series of events that involve the switching on of different ***transcriptional programs*** at each stage of the hierarchy, such that immature precursor cells are guided towards a variety of specific terminally differentiated cellular phenotypes (monocytes, neutrophils, mast cells, etc.). This process can also be influenced by factors external to the bone marrow (such as cytokines that are produced in the context of immune responses), to ramp up the production of specific cell types according to demand. Make no mistake, this is a large-scale operation with the average human requiring the production of almost 4×10^{11} leukocytes (400 billion) per day. One of the reasons for this prodigious rate of cell production is that many of the cells of the immune system, particularly the granulocytes (neutrophils, basophils, and eosinophils), have half-lives of only a day or so. Thus, these cells require practically continuous replacement.

Upon differentiation to specific mature lymphoid and myeloid cell types, the various leukocytes exit the bone marrow and either circulate in the bloodstream until required or until they die (granulocytes), or migrate to the peripheral tissues where they differentiate further under the influence of tissue-specific factors (monocytes, mast cells, dendritic cells), or undergo further selection and differentiation in specialized compartments (e.g., T-cells undergo further maturation and quality control assessment in the thymus, see Chapter 10).

Myeloid cells comprise the majority of cells of the innate immune system

Macrophages and mast cells

Macrophages and ***mast cells*** are tissue-resident cells and are frequently the first dedicated immune cells to detect the presence of a pathogen (Figure 1.8). Both of these cell types have an ***important role in sensing infection*** and in amplifying immune responses, through the production of cytokines, chemokines, and other soluble mediators (such as vasoactive amines and lipids) that have effects on the local endothelium and facilitate the migration of other immune cells (such as neutrophils) to the site of an infection through recruitment of the latter from the blood. Mast cells in particular have an important role in promoting vasodilation through production of histamine, which has profound effects on the local vasculature. Macrophages are derived from ***monocyte*** precursors that circulate in the bloodstream for a number of hours before exiting the circulation to take up residence in the tissues, where they undergo differentiation into specialized tissue macrophages.

Tissue macrophages have historically been given a variety of names based on their discovery through histological analysis of different tissues. Thus we have Kupffer cells in the liver, microglial cells in the brain, mesangial cells in the kidney, alveolar cells in the lung, osteoclasts in the bone, as well as a number of other macrophage types. Although macrophages do have tissue-specific functions, all tissue-resident macrophages are highly phagocytic, can kill ingested microbes, and can generate cytokines and chemokines upon engagement of their PRRs. We will discuss the specific functions of macrophages and mast cells later in this chapter.

Granulocytes

Neutrophils and their close relatives, ***basophils*** and ***eosinophils***, which are collectively called ***granulocytes*** (Figure 1.9), are not tissue-resident but instead circulate in the bloodstream awaiting signals that permit their entry into the peripheral tissues. Neutrophils, which are also sometimes called polymorphonuclear neutrophils (PMNs), are by far the most numerous of the three cell types, making up almost 97% of the granulocyte population, and are highly phagocytic cells that are adept at hunting down and capturing extracellular bacteria and yeast. Neutrophils arrive very rapidly at the site of an infection, within a matter of a couple of hours after the first signs of infection are detected. Indeed, very impressive swarms of these cells migrate into infected tissues like a shoal of voracious piranha that can boast neutrophil concentrations up to 100-fold higher than are seen in the blood circulation (Figure 1.10).

Basophils and eosinophils have more specialized roles, coming into their own in response to large parasites such as helminth worms, where they use the constituents of their specialized granules (which contain histamine, DNAases, lipases, peroxidase, proteases, and other cytotoxic proteins, such as major basic protein) to attack and breach the tough outer cuticle of such worms. Because worm parasites are multicellular, they cannot be phagocytosed by macrophages or neutrophils but instead must be attacked with a ***bombardment of destructive enzymes***. This is achieved through release of the granule contents of eosinophils and basophils (a process called ***degranulation***) directly onto the parasite, a process that carries a high risk of collateral damage to host tissues. Basophils and eosinophils are also important sources of cytokines, such as IL-4, that have very important roles in shaping the nature of adaptive immune responses (discussed later in Chapter 8).

Granulocytes have relatively short half-lives (amounting to a day or two), most likely related to the powerful destructive

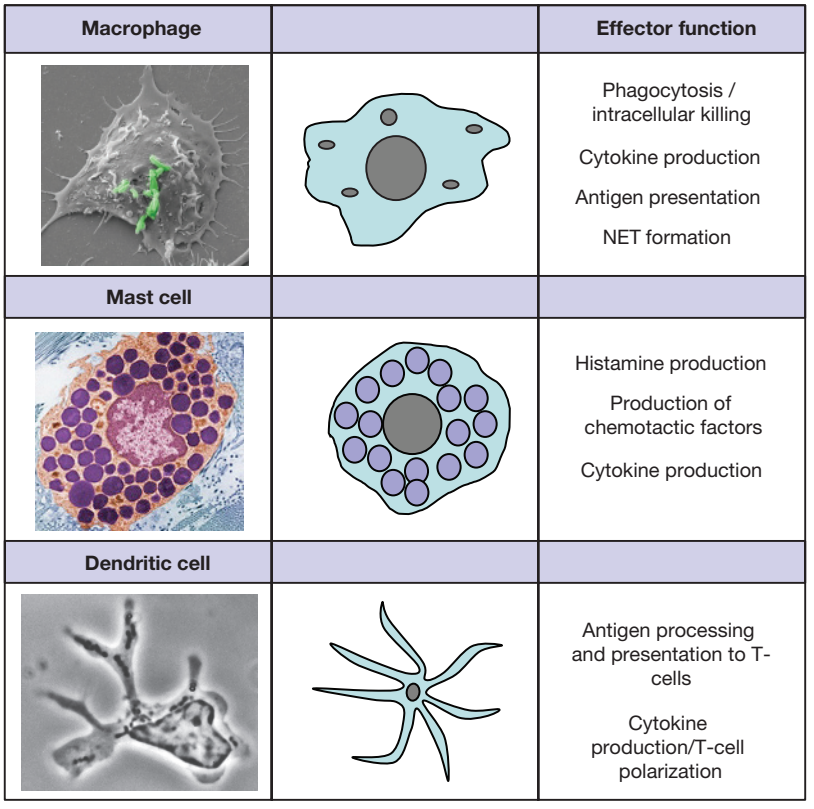


Figure 1.8 Macrophages, mast cells, and dendritic cells act as the sentinels of the innate immune system. Macrophages and mast cells play an important role in the initiation of innate immune responses through the liberation of inflammatory mediators and recruitment of additional cells (particularly neutrophils) to the site of infection. Macrophages also serve an important role as phagocytes in engulfing and killing microbes. Dendritic cells act as an important conduit between the innate and adaptive immune systems. Some of the major functions of these cells are shown (see main text for further details). NET, neutrophil extracellular trap. (Source: Macrophage image: Dr. Jean Pieters, University of Basel, Switzerland.)

enzymes that are contained within their cytoplasmic granules. These are the riot police of the immune system and, being relatively heavy-handed, are only called into play when there is clear evidence of an infection. Thus, the presence of granulocytes in a tissue is clear evidence that an immune response is underway. Egress of granulocytes from the circulation into tissues is facilitated by changes in the local endothelium lining blood vessels, instigated by vasoactive factors and cytokines/chemokines released by activated tissue macrophages and mast cells, which alter the adhesive properties of the lining of blood vessels closest to the site of infection. The latter changes, which include the upregulation of adhesion molecules on the surface of the local blood vessels, as well as the dilation of these vessels to permit the passage of cells and other blood-borne molecules more freely into the underlying tissue, facilitate the ***extravasation*** of granulocytes from the blood into the tissues.

Dendritic cells

Dendritic cells (DCs), which were among the first immune cell types to be recognized, are a major conduit between the innate and adaptive arms of the immune system. DCs have characteristic highly elaborated morphology (Figure 1.8), with multiple long cellular processes (dendrites) that enable them to maximize contact with their surroundings. Although most DCs are tissue-resident cells with phagocytic capacity similar to macrophages, their primary role is not the destruction of microbes, but rather the ***sampling of the tissue environment*** through continuous ***macropinocytosis*** and ***phagocytosis*** of extracellular material. Upon detection and internalization of a PAMP (and its associated microbe) through phagocytosis, DCs undergo an important transition (called ***DC maturation***) from a highly phagocytic but inefficient antigen-presenting cell into a lowly phagocytic but highly migratory DC that is now equipped to present antigen efficiently to T-cells within local lymph nodes. We will return to this subject later in this chapter, but the importance of the dendritic cell in the induction of adaptive immunity cannot be overstated.

Lymphoid cells comprise the majority of the cells of the adaptive immune system

T- and B-lymphocytes

Lymphocytes constitute ~20–30% of the leukocyte population and have a rather nondescript appearance (Figure 1.11), which belies their importance within the adaptive immune system.

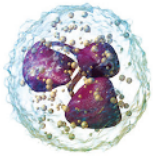
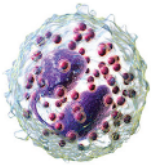
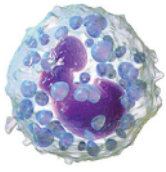
Neutrophil		Effector function
		Phagocytosis / intracellular killing Degranulation/ extracellular killing NET formation Cytokine production
Eosinophil		
		Parasite attack Degranulation/ extracellular killing Histamine production Cytokine production
Basophil		
		Parasite attack Degranulation/ extracellular killing Histamine production Cytokine production

Figure 1.9 Granulocytes form an important part of the innate immune system. Schematic representations of neutrophil, eosinophil, and basophil granulocytes are depicted along with their major functions. NET, neutrophil extracellular trap. (Source: Artistic impressions in the left panels © Blausen.com, with permission.)

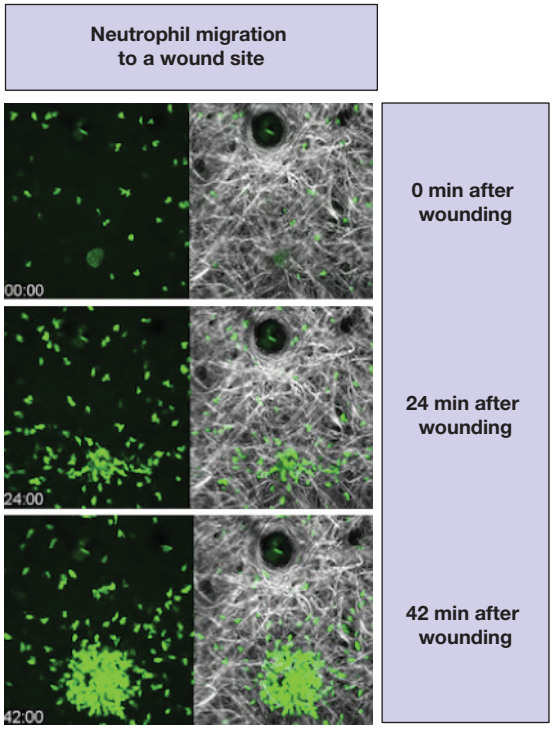


Figure 1.10 Neutrophils migrate in large numbers to sites of infection. Timelapse microscopy of neutrophils (green) migrating to a wound site. (Source: Dr. Tim Lämmermann, Max Planck Institute, Freiburg, Germany and Dr. Ron Germain, National Institute of Allergy and Infectious Disease, USA.)

As mentioned earlier, T- and B-lymphocytes are the central players in the adaptive immune system and have the ability to generate ***highly specific cell surface receptors***, through genetic recombination of a relatively limited number of receptor precursors that are exquisitely specific for particular molecular structures, called antigens. In principle, T-cell receptors (TCRs) and B-cell receptors (BCRs, more commonly known as antibodies) can be generated to recognize practically any molecular stucture (i.e., antigen), whether self- or nonself-derived. However, as we shall see in Chapters 4 and 10, lymphocyte receptors undergo a process of careful inspection after they have been generated to make sure that those that recognize self antigens (or indeed fail to recognize anything useful at all) are weeded out to ensure that the immune response does not become targeted against self (a state called autoimmunity). T- and B-lymphocytes also have the ability to undergo clonal expansion, which enables those lymphocytes that have generated useful (i.e., pathogen-specific) TCRs and BCRs to undergo rapid amplification, permitting the generation of large numbers of pathogen-specific T- and B-cells within 5–7 days of the initiation of an immune response. Specific T- and B-cells can also persist in the body for many years (called ***memory cells***), which endows upon them the ability to "remember" previous encounters with particular pathogens and to rapidly mount a highly specific immune response upon a subsequent encounter with the same pathogen.

T-cells can be further subdivided into three broad subsets: helper (Th), cytotoxic (Tc), and regulatory (Treg) subsets that have roles in helping B-cells to make antibody (Th), killing

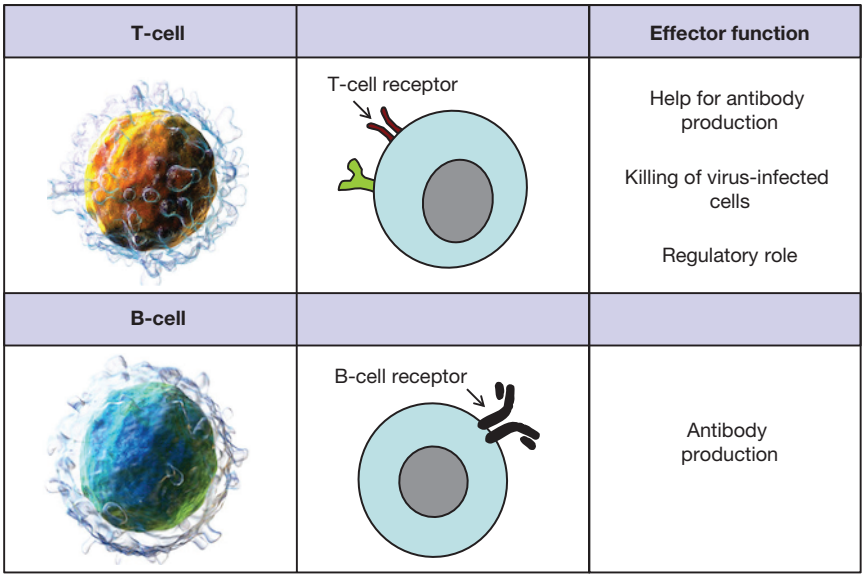


Figure 1.11 T- and B-lymphocytes comprise the major lymphocytes of the adaptive immune system. Schematic representations of T- and B-lymphocytes are depicted along with their major functions. (Source: Artistic impressions in the left panels © Blausen.com, with permission.)

virally-infected cells (Tc,) or policing the actions of other T-cells (Treg). We will discuss T-cells and their different subsets extensively in Chapter 8.

Natural killer (NK) cells

NK cells, while also lymphocytes, play a major role within the innate immune system, although these cells also police the presence of special antigen-presenting molecules (called MHC molecules) that are expressed on virtually all cells in the body and play a key role in the operation of the adaptive immune system. NK cells use germline-encoded receptors (NK receptors) that are distinct from the receptors of T- and B-cells and are endowed with the ability to kill cells that express abnormal MHC receptor profiles. Viruses often interfere with MHC molecule expression as a strategy to attempt to evade the adaptive immune response, which solicits the attentions of NK cells and can lead to rapid killing of virally infected cells. NK cells also have receptors for a particular antibody class (IgG) and can use this receptor (CD16) to display antibody on their surface and in this way can seek out and kill infected cells, a process called ***antibody-dependent cellular cytotoxicity***. We will discuss NK cells more extensively later in this chapter.

The beginnings of an immune response

Macrophages play an important role in instigating innate immune responses

As noted above, a major player in the initiation of immune responses is the ***macrophage***. These cells are relatively abundant in most tissues (approaching 10–15% of the total cell number in some areas of the body) and act as sentinels for infectious agent through an array of pathogen recognition receptors (PRRs) borne on their plasma membranes as well as other cellular compartments such as endosomes. Tissue macrophages are relatively quiescent cells, biding their time sampling the environment around them through continuous phagocytosis. However, upon entry of a microorganism that engages one or more of their PRRs (such as a Toll-like receptor or a NOD-like receptor), a startling transition occurs. Engagement of the PRR on the macrophage switches on a battery of genes that equip it to carry out a number of new functions (Figure 1.12).

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

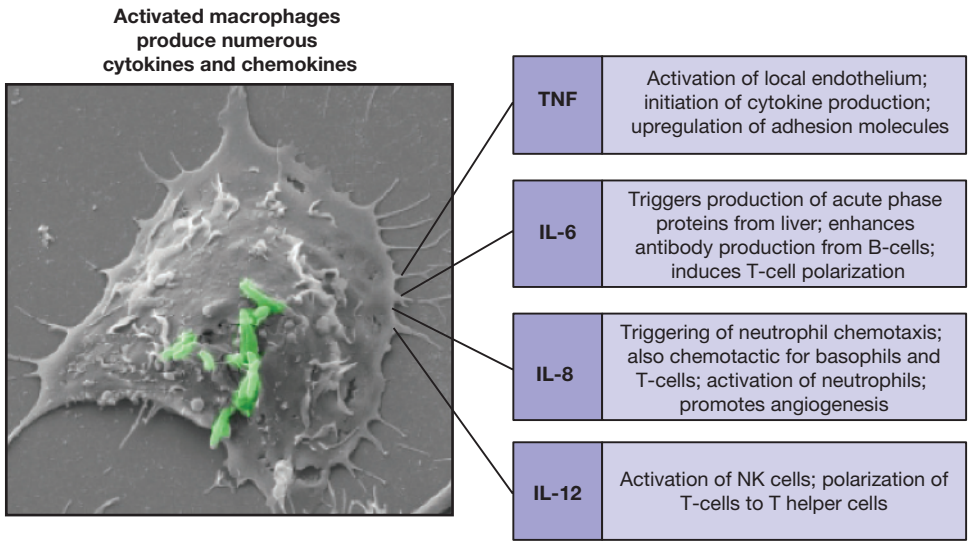


Figure 1.12 Activated macrophages secrete a diverse array of cytokines and chemokines. Electron micrograph of an activated macrophage with several bacteria (*Mycobacterium bovis*) attached to its cell surface. Only a small fraction of the numerous soluble mediators that are liberated from PAMP-activated macrophages are shown. (Source: Macrophage image courtesy of Dr. Jean Pieters, University of Basel, Switzerland.)

microorganisms they encounter with gusto. We will deal with the specific mechanisms that neutrophils employ to attack and kill microbes later in this chapter.

The inflammatory response

Inflammation is the term given to the series of events that surround an immune response and display a number of characteristic features, including local swelling (edema), redness (due to capillary dilation), pain, and heat. These features are the collective consequence of the release of cytokines, chemokines, and vasoactive amines from macrophages and mast cells upon the initial encounter with a pathogen. Byproducts of complement activation (i.e., C3a and C5a), which will be discussed later, also contribute to the inflammatory response through promoting neutrophil chemotaxis, as well as activation of mast cells (Figure 1.13). All of these inflammatory mediators help to recruit neutrophils as well as plasma proteins to the site of infection by inducing vasodilation of the blood vessels close to the site of infection and by acting as chemotactic factors for neutrophils circulating in blood. The extra cells and fluid that gather at the site of an infection (which contribute to the swelling seen), the increased redness of skin tone in the area, and associated tenderness constitute the classic inflammatory reaction.

Mast cells collaborate with macrophages to promote vascular permeability

As we have already alluded to above, the macrophage plays a key role in the initiation of an inflammatory response through the secretion of cytokines and chemokines in response to engagement of its PRRs and through encounter with opsonized microbes (Figure 1.12). However, another innate immune cell, the ***mast cell***, is instrumental in provoking increased permeability of blood vessels due to release of the contents of the numerous cytoplasmic granules that such cells possess (Figure 1.8). Mast cell granules contain, among other factors, copious amounts of the vasoactive amino acid histamine (Figure 1.14). Mast cell degranulation can be provoked by direct injury, in response to complement components (C3a and C5a), encounter with PAMPs and through binding of specific antigen to a class of antibody (IgE) that binds avidly to mast cells via surface receptors (we will discuss antibody classes at length in Chapter 3). Histamine provokes dilation of postcapillary venules, activates the local endothelium, and increases blood vessel permeability. Irritation of nerve endings is another consequence of histamine release and is responsible for the pain often associated with inflammation, an evolutionary adaptation that most likely encourages the host to protect the infected or injured area to minimize further damage.

The relaxation induced in arteriolar walls causes increased blood flow and dilatation of the small vessels, whereas contraction of capillary endothelial cells allows exudation of plasma proteins. Under the influence of the chemotaxins, neutrophils slow down and the surface adhesion molecules they are stimulated to express cause them to 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 ***complement-opsonized*** microbes (the details of complement-mediated opsonization will be discussed a little later in this

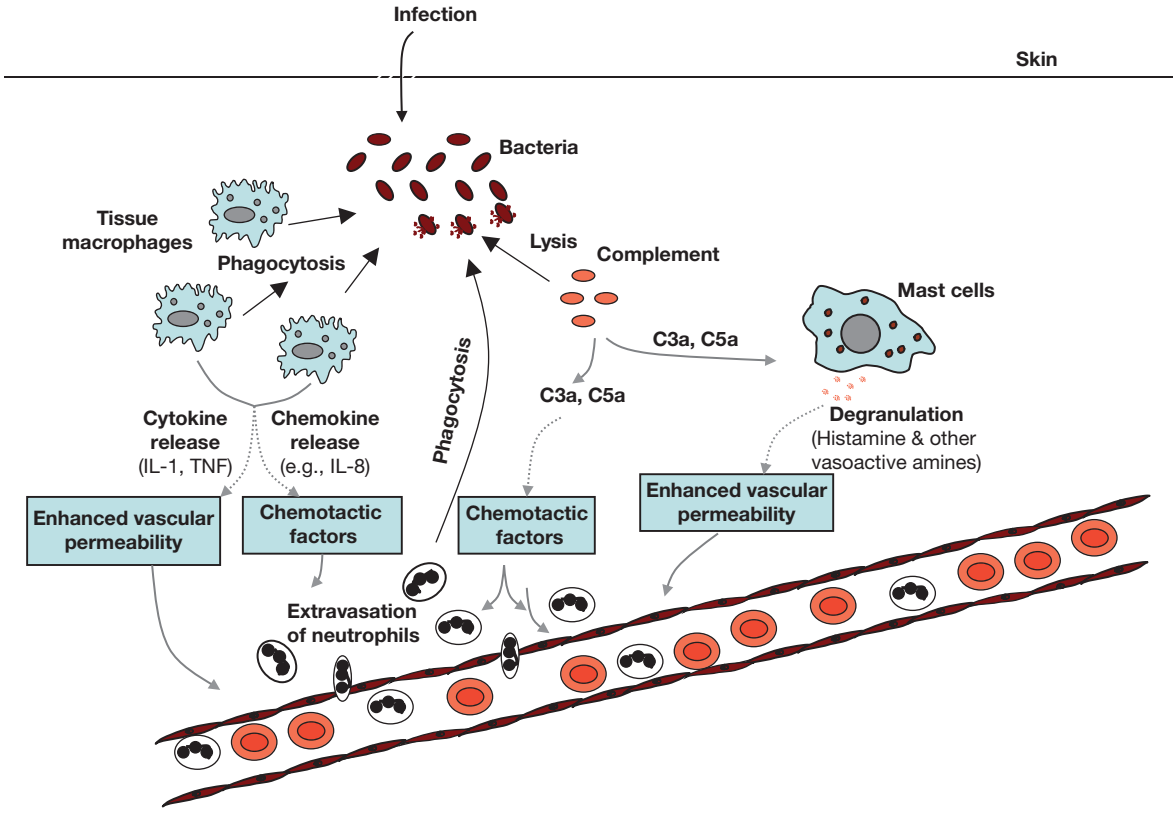
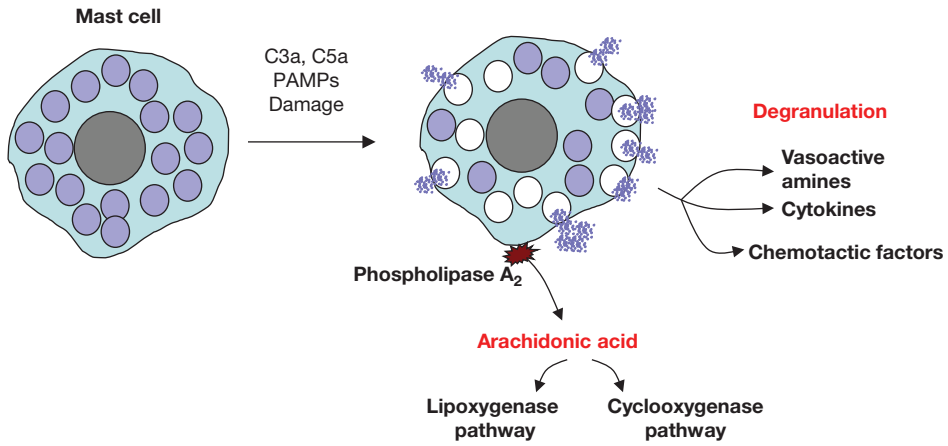


Figure 1.13 The acute inflammatory reaction. Bacterial infection initiates a series of responses through activation of the alternative complement pathway, producing C3a and C5a, as well as through stimulation of tissue-resident macrophages that detect bacterial-derived PAMPs. The C3b component of complement binds to bacteria, opsonizing the latter for more effective phagocytosis by macrophages and neutrophils. Complement activation can also lead to direct lysis of bacteria through assembly of membrane attack complexes. Activation of macrophages by PAMPs and complement components induces secretion of mediators (i.e., cytokines and chemokines) of the acute inflammatory response that increase vascular permeability and induce neutrophils to migrate from the blood into the tissue. C3a and C5a trigger mast cell activation and secretion of mediators that provoke capillary dilatation and exudation of plasma proteins. Attracted by C3a and C5a, as well as other factors, blood neutrophils stick to the adhesion molecules on the endothelial cell and use this to provide traction as they force their way between the cells, through the basement membrane (with the help of secreted elastase) and up the chemotactic gradient.

chapter). Adherence to the neutrophil complement (C3b) receptors then takes place, C3a and C5a (byproducts of complement activation which will be discussed later) at relatively high concentrations in the chemotactic gradient activate neutrophil killing mechanisms and, hey presto, the slaughter of the last act can begin!

Neutrophils are rapidly recruited to sites of infection

We have mentioned that the cytokines, chemokines, and vasoactive factors (such as histamine) that are released by activated macrophages and mast cells are instrumental in triggering neutrophil recruitment from the circulation into the site of infection, a process called ***extravasation*** (Figure 1.15). Because neutrophils are so numerous and so adept at phagocytosis, their recruitment to an inflammatory site is a critical step in innate immunity. So, let us take a look at this process in a little more detail. Normally, neutrophils circulate in the bloodstream and are prevented from adhering to blood vessel walls owing to the rapid rate of movement of the blood within the vessels. To exit the bloodstream, neutrophils must first lightly adhere to and roll along the vessel wall until they gain a firm foothold that allows them to come to a stop, whereupon they initiate the process of squeezing between the endothelium. The cytokines secreted by activated macrophages, especially TNFα and IL-1β, have particular roles in this regard, as the latter cytokines increase the adhesiveness of the endothelial cells lining the blood capilleries closest to the site of infection through triggering the exposure of ***P-and E-selectins*** on these cells. The selectins present on the activated endothelium permit neutrophils to initiate the stopping process and to start rolling along the endothelial wall through binding interactions with carbohydrate ligands (e.g., sialyl-Lewis^X) present on the neutrophil cell surface (Figure 1.15).



	Preformed	Effect
Granule release	Histamine	Vasodilatation, Incr. capillary permeability, chemokinesis, bronchaconstriction
	Proteoglycan	Binds granule proteases
	Neutral proteases β-Glucosaminidase	Activates C3 Splits off glucosamine
	ECF NCF	Eosinophil chemotaxis Neutrophil chemotaxis
	Platelet-activating factor	Mediator release
	Interleukins 3, 4, 5 & 6 GM-CSF, TNF	Multiple, Including macrophage activation, trigger acute phase proteins, etc.

	Newly synthesized	Effect
Lipoxygenase pathway	Leukotrienes C_4, D_4 (SRS-A), B_4	Vasoactive, branchoconstriction, chemotaxis
Cyclooxygenase pathway	Prostaglandins Thromboxanes	Affect bronchial muscle, platelet aggregation and vasodilatation

Figure 1.14 Mast cell triggering leading to release of mediators by two major pathways. (i) Release of preformed mediators present in the granules; and (ii) the metabolism of arachidonic acid produced through activation of a phospholipase. Intracellular Ca^{2+} and cyclic AMP are central to the initiation of these events but details are still unclear. Mast cell triggering may occur through C3a, C5a, and even by some microorganisms that can act directly on cell surface receptors. ECF, eosinophil chemotactic factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; NCF, neutrophil chemotactic factor; TNF, tumor necrosis factor. Chemotaxis refers to directed migration of granulocytes up the pathway concentration gradient of the mediator.

At this stage, the neutrophil will also experience the chemotactic factors, such as IL-8 and complement products, that are emanating from the site of infection. These factors initiate the process of neutrophil activation, which triggers conformational changes in adhesion molecules called ***integrins*** (e.g., LFA-1, CR3) on the neutrophil surface that permits stronger interactions with their cognate ***intercellular cell adhesion molecules*** (ICAMs) receptors on the activated endothelium that arrests the neutrophil. Finally, neutrophils squeeze between the slightly wider gaps in the activated endothelium than normal (due to the relaxation effects of histamine) and start to follow the gradient of chemotactic factors (IL-8, complement factors C3a and C5a) to its source. As we have seen earlier (Figure 1.10), this process is very efficient indeed and results in huge increases in neutrophil numbers at the site of infection within a matter of hours.

A similar process is also used to recruit ***monocytes*** from the bloodstream to reinforce their macrophage counterparts within the tissues, but this wave of recruitment usually takes place 6–8 hours after the peak of neutrophil extravasation and under the influence of a different chemokine, ***monocyte chemotactic***

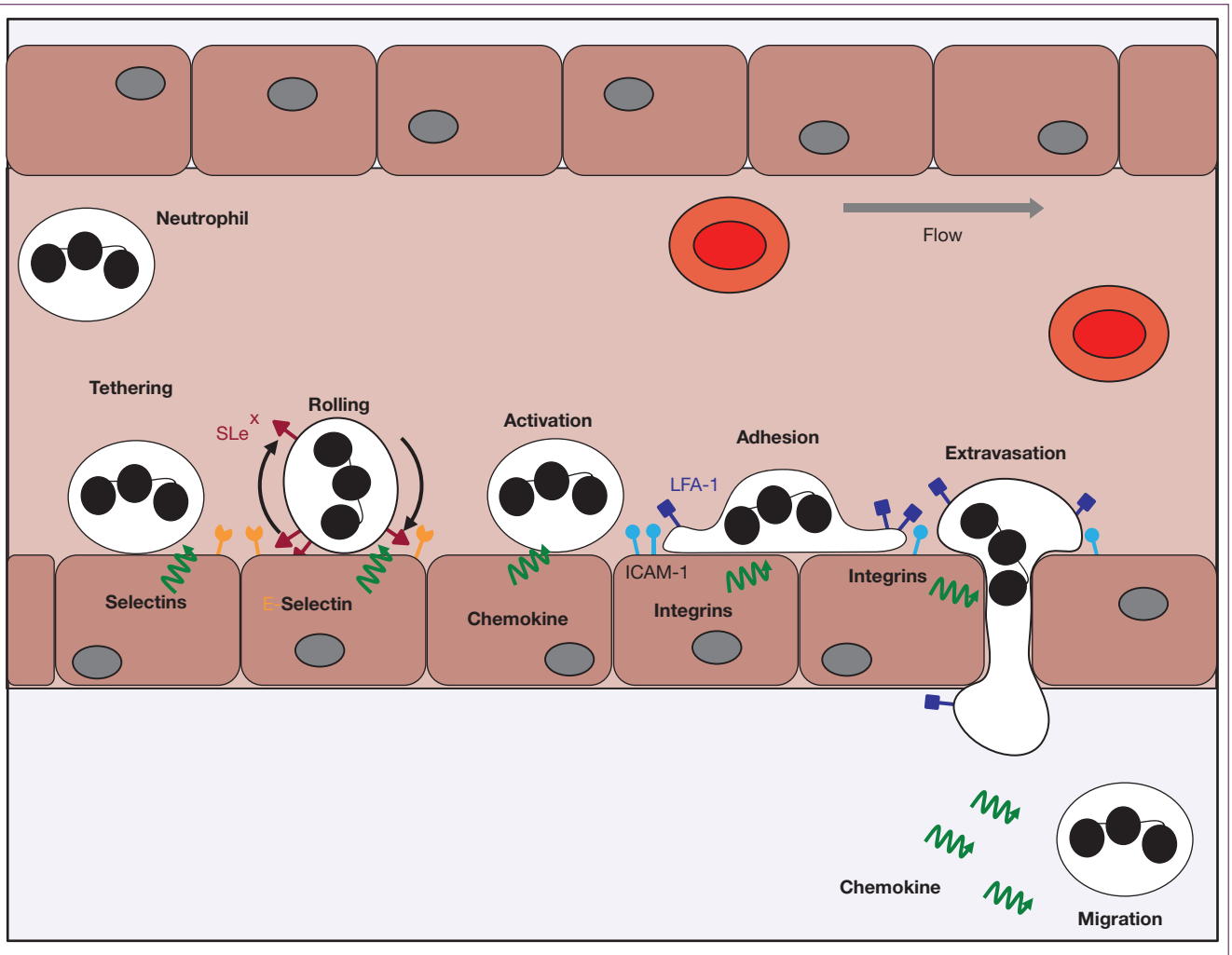


Figure 1.15 Neutrophil extravasation. Neutrophils are induced to migrate from blood vessels adjoining sites of infection through changes to the endothelial cells lining the blood vessels that are induced by the products of activated macrophages and mast cells, such as IL-1, TNF, IL-8, and histamine. Neutrophils initially loosely attach and roll along the endothelium mediated via sialyl-Lewis^x-mediated interactions with P- and E-selectins that are upregulated on the activated endothelium. Under the influence of chemokines, such as IL-8, neutrophils become activated, leading to activation of cell surface integrins (LFA-1, CR3) that provide firmer attachment to their cognate receptors (ICAMs) on the endothelium. The latter interactions enable neutrophils to arrest on the endothelial wall and to extravasate through the basement membrane of the endothelium and migrate into the tissue towards the source of chemotactic factors (IL-8, C3a, C5a).

protein-1 (MCP-1). Indeed, one of the reasons for the recruitment of extra monocytes (which differentiate into macrophages upon entering the tissues) is to help remove all of the battle-weary neutrophils, many of which will be stuffed to the gills with microbes, as well as other debris from the tissue and to initiate the process of ***wound healing***.

There are several classes of pattern recognition receptors

PRRs on phagocytic cells recognize and are activated by PAMPs

Because the ability to discriminate friend from foe is of paramount importance for any self-respecting phagocyte, macrophages are fairly bristling with receptors capable of recognizing diverse PAMPs. Many of the PRRs are also expressed on DCs, NK cells, neutrophils and mast cells, as well as cells of the adaptive immune system. Several of these PRRs are lectin-like and bind multivalently with considerable specificity to exposed microbial surface sugars with their characteristic rigid three-dimensional geometric configurations. They do not bind appreciably to the array of galactose or sialic acid groups that are commonly the penultimate and ultimate sugars that decorate mammalian surface polysaccharides, so providing the molecular basis for discriminating between self and nonself microbial cells. Other PRRs detect nucleic acids derived from bacterial and viral genomes by virtue of modifications not commonly found within vertebrate nucleic acids or conformations not normally found in the cytoplasm (e.g., double-stranded RNA).

PRRs are a diverse group of receptors that can be subdivided into at least five distinct families (TLRs, CTLRs, NLRs, RLRs, and scavenger receptors) based upon structural features. Another class of sensors has also emerged in recent years, the cytosolic DNA sensors (CDSs), which contains a structurally diverse set of cytosolic DNA-sensing receptors that are predominantly involved in detecting intracellular bacteria and viruses. Multiple receptors also exist in each class, with the result that in excess of 50 distinct PRRs may be expressed by a phagocyte at any given time.

Cell-associated PRRs decode the nature of infection

As noted earlier, there are several classes of cell-associated PRRs, some of which are plasma membrane-associated (e.g., many of the Toll-like receptors as well as the C-type lectin receptors and scavenger receptors), some of which face the luminal space of endosomes (TLR3, 7, 8, 9) and some of which are cytoplasmic (NOD-like receptors, RIG-I-like receptors, cytoplasmic DNA sensors). In general terms, each PRR is specific for a distinct PAMP and, combined with the different cellular compartments that PRRs reside in, this conveys considerable information concerning the nature of the pathogen and whether it is extracellular, has been captured through phagocytosis (i.e., is within endosomes) or has invaded the cytoplasm. This information helps to tailor the response towards what will be most effective for the particular class of pathogen by influencing the nature of the cytokines that are produced by the responding cell.

Engagement of several categories of PRR simultaneously may be required for effective immune responses

Although this is an area of ongoing research, ***combinatorial PRR signaling*** is probably very important for the initiation of effective immune responses. Thus, the triggering of a single type of PRR, in a DC for example, may not be fully effective for the initiation of a robust adaptive immune response, as this could indicate either a low level of infection, or that the DC is at a considerable distance from the site of infection (and has simply encountered a few stray PAMPs that have been released owing to lysis of the infectious agent). However, ***phagocytosis of a single bacterium by a DC is likely to stimulate multiple categories of PRR simultaneously***, leading to synergistic activation of several signal transduction pathways, thereby signifying that a robust response is warranted. Furthermore, it is likely that engagement of different combinations of PRRs underpins the different types of immune response that are required to successfully contain different types of infection: intracellular, extracellular, large parasite, yeast, bacterial, viral, etc.

As we shall see throughout this book, delivery of two (or more) different signals in tandem is a common theme in immune reactions and can lead to very different outcomes compared with delivery of either signal on its own. We will now look at the various PRR families in more detail.

Toll-like receptors (TLRs)

A major subset of the PRRs belong to the ***Toll-like receptor (TLR) family***, named on the basis of their similarity to the Toll receptor in the fruit fly, *Drosophila*. The history of the discovery of the TLR family is interesting, as it perfectly illustrates the serendipitous nature of scientific discovery and illustrates how very important findings can originate in the most unlikely places. Lipopolysaccharide (LPS, also called endotoxin), a major component of the cell walls of Gram-negative bacteria, was long known to provoke strong immune responses in animals and is a good example of a classical PAMP. Indeed, LPS is one of the major contributors to septic shock, the severe immune reaction that results when a bacterial infection reaches the bloodstream, and which is often fatal. For these reasons, immunologists tried to identify the LPS receptor in human and mouse for many years, largely without success. However, a major breakthrough came when the Toll receptor was found to be involved in sensing microbial infection in adult fruit flies. This in itself was quite a surprise because the Toll receptor had already been identified, many years before, as a major regulator of dorsal–ventral patterning (i.e., specifying which surface of the fly is the back and which is the underside) during early embryonic development of *Drosophila*. A curious fact that emerged was that the intracellular domain of *Drosophila* Toll contained a motif, now known as the Toll/IL-1 receptor (TIR) signaling motif, that was very similar to the cytoplasmic signaling domain identified in the IL-1 receptor, a molecule that was already well known to be involved in immune signaling in mammals. Putting two and two together, this led to the identification of the whole TLR family in mammals, as these receptors all possess a TIR domain within their cytoplasmic regions.

A series of TLRs have now been identified (there are 10 distinct TLRs in humans), all of which act as sensors for PAMPs (Figure 1.16). TLR ligands include peptidoglycan, lipoproteins, mycobacterial lipoarabinomannan, yeast zymosan, flagellin, microbial DNA, microbial RNAs, as well as other pathogen-derived ligands (Table 1.1). Although many TLRs are displayed on the cell surface, some, such as TLR3 and TLR7/8/9 that are responsive to intracellular viral RNA and unmethylated bacterial DNA, are located in endosomes and become engaged upon encounter with phagocytosed material (Figure 1.16). Engagement of TLRs with their respective ligands drives activation of nuclear factor kB (NFkB) and several members of the interferon-regulated factor (IRF) family of transcription factors, depending on the specific TLR. Combinatorial activation of TLRs is also possible, for example TLR2 is capable of responding to a wide diversity of PAMPs and typically functions within heterodimeric TLR2/TLR1 or TLR2/TLR6 complexes (Table 1.1).

All TLRs have the same basic structural features, with multiple N-terminal leucine-rich repeats (LRRs) arranged in a horseshoe- or crescent-shaped solenoid structure that acts as the PAMP-binding domain (Figure 1.17). Upon binding of a

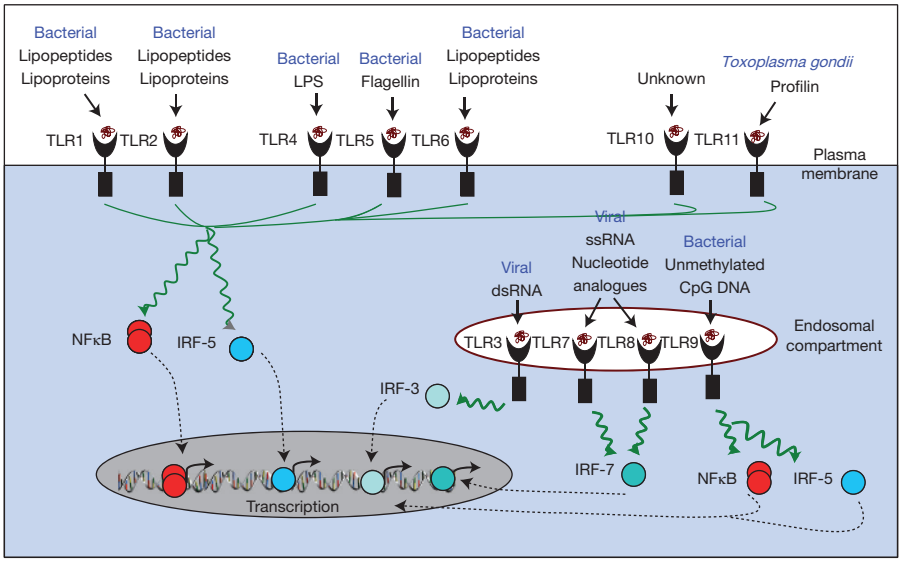


Figure 1.16 A family of Toll-like receptors (TLRs) act as sensors for pathogen-associated molecular patterns (PAMPs). TLRs reside within plasma membrane or endosomal membrane compartments, as shown. Upon engagement of the TLR ectodomain with an appropriate PAMP (some examples are shown), signals are propagated into the cell that activate the nuclear factor κB (NFκB) and/or interferon-regulated factor (IRF) transcription factors, as shown. NFκB and IRF transcription factors then direct the expression of numerous antimicrobial gene products, such as cytokines and chemokines, as well as proteins that are involved in altering the activation state of the cell.

Table 1.1 Ligands for Toll-like receptors (TLRs).

TLR	Ligand	Location
TLR1/ TLR2 heterodimer	Bacterial lipopeptides	Plasma membrane
TLR2/TLR6 heterodimer	Lipoteichoic acid (Gram-positive bacteria), zymosan (fungi)	Plasma membrane
TLR3	dsRNA	Endosomal
TLR4	LPS	Plasma membrane
TLR5	Flagellin (motile bacteria)	Plasma membrane
TLR7	Viral ssRNA	Endosomal
TLR8	Viral ssRNA	Endosomal
TLR9	Unmethylated CpG DNA (bacterial)	Endosomal
TLR10	Unknown	Plasma membrane
TLR11 (mouse only)	Profilin and profilin-like proteins	Plasma membrane

PAMP, TLRs transduce signals into the cell via their TIR domains, which recruit adaptor proteins within the cytoplasm (such as MyD88) that possess similar TIR motifs. These adaptors propagate the signal downstream, culminating in activation of NFκB and interferon regulatory family (IRF) transcription factors, which regulate the transcription of a whole battery of inflammatory cytokines and chemokines (Figure 1.16 and Figure 1.18). As we will discuss later in this chapter, the IRF transcription factors control the expression of, among other things, type I interferons. The latter cytokines are especially important in defense against viral infections as they can induce the expression of a series of proteins that can interfere with viral mRNA translation and viral replication, as well as induce the degradation of viral RNA genomes.

C-type lectin receptors (CTLRs)

Phagocytes also display another set of PRRs, the cell-bound ***C-type (calcium-dependent) lectins***, of which the macrophage mannose receptor is an example. Other members of this diverse and large family include Dectin-1, Dectin-2, Mincle, DC-SIGN, Clec9a, and numerous others. These transmembrane proteins possess multiple carbohydrate recognition domains whose engagement with their cognate microbial PAMPs generates intracellular activation signals through a variety of signaling pathways. However, some C-type lectin receptors (CTLRs) do not trigger robust transcriptional responses and function primarily as phagocytic receptors. The CTLR family is highly diverse and the ligands for many receptors in this category are the subject of ongoing research. But it can be said that members of the CTLR family broadly serve as sensors for extracellular fungal species. Some examples of ligands for CTLRs include β-glucans (which binds Dectin-1), mannose (which binds Dectin-2), and α-mannose (which binds Mincle).

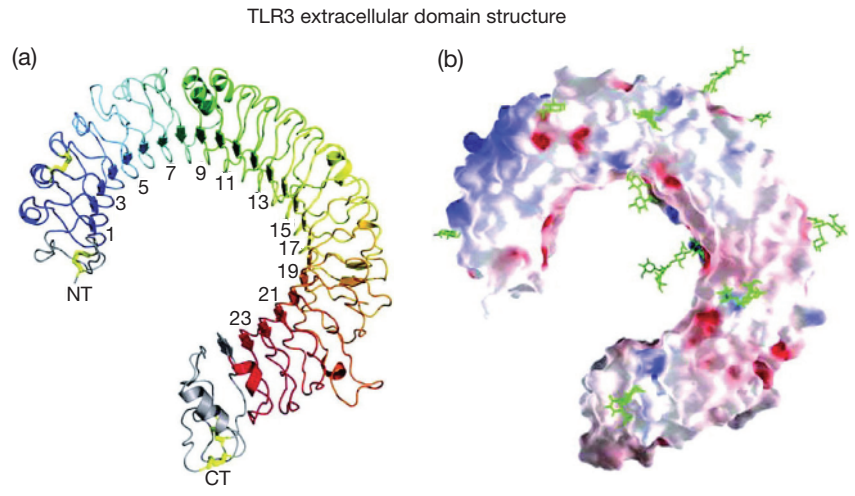


Figure 1.17 Toll-like receptor (TLR) structure. TLR3 ectodomain structure. (a) Ribbon diagram. Leucine-rich repeats (LRRs) are colored from blue to red, beginning at LRR1 and proceeding to LRR23, as indicated. NT, N-terminus; CT, C-terminus. (b) Electrostatic potential surface shows positive (blue) and negative (red) charges at neutral pH. The *N*-linked glycans are shown as green ball-and-stick. (Source: Bell J.K. *et al.* (2005) *Proceedings of the National Academy of Sciences USA* **102**, 10976–10980. Reproduced with permission.)

NOD-like receptors (NLRs)

Turning now to the sensing of infectious agents that have succeeded in gaining access to the interior of a cell, microbial products can be recognized by the so-called NOD-like receptors (NLRs). Unlike TLRs and CTLRs that reside within the plasma membrane or intracellular membrane compartments, NLRs are soluble proteins that reside in the cytoplasm, where they also act as receptors for PAMPs. Although a diverse family of receptors (Figure 1.19), NLRs typically contain an N-terminal protein–protein interaction motif that enables these proteins to recruit proteases or kinases upon activation, followed by a central oligomerization domain and multiple C-terminal leucine-rich repeats (LRRs) that act as the sensor for pathogen products (Figure 1.19). The NLRs can be subdivided into four subfamilies on the basis of the motifs present at their N-termini. NLRs are thought to exist in an autoinhibited state with their N-terminal domains folded back upon their C-terminal LRRs, a conformation that prevents the N-terminal region from interacting with its binding partners in the cytoplasm. Activation of these receptors is most likely triggered through direct binding of a PAMP to the C-terminal LRRs which has the effect of disrupting the interaction between the N- and C-termini of the NLR and permits oligomerization into a complex that is now capable of recruiting either an NFkB-activating kinase (such as RIP-2) or members of the caspase family of proteases that can proteolytically process and activate the IL-1β precursor into the mature, biologically active cytokine.

A very well-studied NLR complex, called ***the inflammasome***, is assembled from NLRP3 in response to LPS in combination with bacterial virulence factors, and is important for the production of IL-1β as well as IL-18. However, for full activation of the inflammasome and liberation of IL-1β, a second signal in the form of a membrane-damaging bacterial toxin (which can also be mimicked by a variety of noxious agents) is required. This second signal appears to permit the efflux of K^+ ions from the cytosol, which permits full assembly of the inflammasome, caspase-1 activation, and processing of IL-1β and IL-18 downstream (Figure 1.20).

RIG-I-like helicase receptors (RLRs)

The RIG-I-like helicases are a relatively recently discovered family that act as intracellular sensors for viral-derived RNA (Figure 1.21). Similar to the NLRs, RIG-I-like helicase receptors (RLRs) are found in the cytoplasm and are activated in response to double-stranded RNA and are capable of directing the activation of NFkB and IRF3/4 that cooperatively induce antiviral type I interferons (IFNα and β). RIG-I (retinoic acid-inducible gene I) and the related MDA-5 (also called Helicard) protein can directly bind to different forms of viral RNA (either unmodified 5′-triphosphate ssRNA or dsRNA, respectively) in the cytoplasm, followed by propagation of their signals via MAVS (mitochondrial-associated viral sensor), again leading to activation of IRFs and NFkB (Figure 1.22).

Cytosolic DNA sensors

A number of proteins belonging to different families are capable of sensing cytosolic DNA or cyclic dinucleotides. Host cell DNA is normally sequestered safely in the nuclear or mitochondrial compartments and cannot trigger these sensors, except under pathological conditions that involve release of mitochondrial DNA into the cytosol, for example. However, bacterial or viral DNA can trigger the activation of the ***AIM2*** or ***IFI16*** DNA sensors and this can lead to assembly of a complex involving the Pyrin-domain-containing adaptor (ASC), leading to activation of caspase-1 and IL-1β processing.

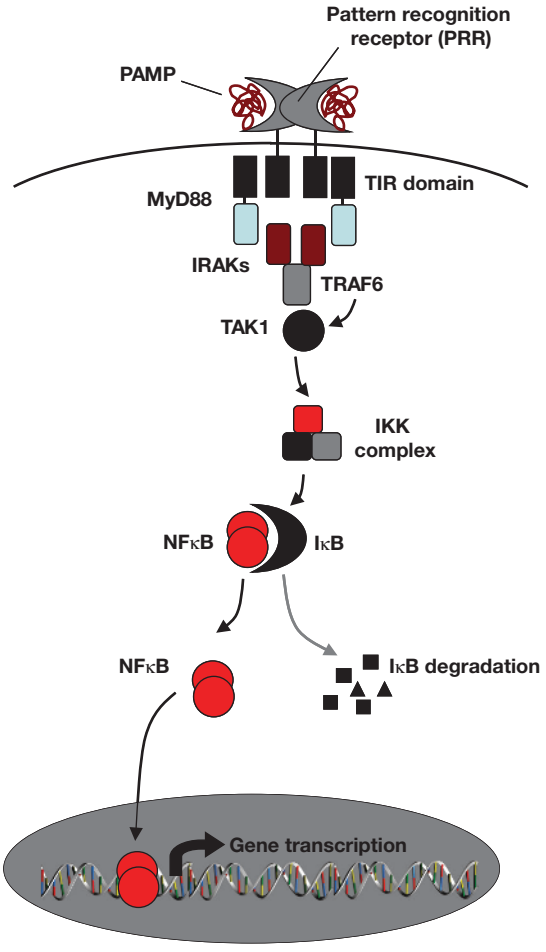


Figure 1.18 Toll-like receptors promote NFκB-dependent transcription through activation of the IkB kinase (IKK) complex. Upon engagement of a TLR dimer (or heterodimer) with its appropriate ligand, a series of adaptor proteins (as shown) are recruited to the TLR receptor Toll and IL-1 receptor-like (TIR) domain. Collectively, these proteins activate the IKK complex, which in turn phosphorylates the inhibitor of NFκB (IκB), a protein that binds and tethers NFκB in the cytosol. IκB phosphorylation targets the latter for degradation, liberating NFκB which can then translocate into the nucleus and initiate transcription of multiple genes.

Activation of the ***AIM2 inflammasome*** can also lead to death of the cell. IFI16 can also recognize cytosolic DNA and can either propagate signaling by forming a complex with ASC and caspase-1, similar to the AIM2 inflammasone, or via STING, which is discussed below. Two additional DNA-sensing pathways have also been discovered very recently and both make use of ***STING*** (stimulator of interferon genes) a molecule that can either directly bind to cytoplasmic DNA or can respond to cyclic GAMP, a molecule that is generated by an upstream enzyme called cGAS, which detects cytoplasmic DNA and synthesizes cGAMP in response (Figure 1.23). In response to STING activation, type I IFNs are generated which have potent antiviral properties.

Scavenger receptors

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

PRR engagement results in cell activation and proinflammatory cytokine production

Upon encountering ligands of any of the aforementioned PRRs, the end result is a switch in cell behavior from a quiescent state to an activated one. Activated macrophages and neutrophils are capable of phagocytosing particles that engage their PRRs and, as we have seen from our discussion of the various classes of PRRs, upon engagement of the latter they also release a range of cytokines and chemokines that amplify the immune response further (see Figure 1.12). As the reader will no doubt have noticed, engagement of many of the above PPRs results in a signal transduction cascade culminating in activation of NFkB, a transcription factor that controls the expression of numerous immunologically important molecules such as cytokines and chemokines. In resting cells, NFκB is sequestered in the cytoplasm by its inhibitor IkB, which masks a nuclear localization signal on the former. Upon binding of a PAMP to its cognate PRR, NFκB is liberated from IκB because of the actions of a kinase that phosphorylates IκB and promotes its destruction. NFκB is now free to translocate to the nucleus, seek out its target genes, and initiate transcription (see Figure 1.18).

Some of the most important inflammatory mediators synthesized and released in response to PRR engagement include the antiviral ***interferons (also called type I interferons***), the small protein cytokines IL-1β, IL-6, IL-12, and tumor necrosis factor α (TNFα), which activate other cells through binding to specific receptors, and chemokines, such as IL-8, which represent a subset of chemoattractant cytokines. Collectively, these molecules amplify the immune response further and have effects on the local blood capillaries that permit extravasation of neutrophils, which come rushing into the tissue to assist the macrophage in dealing with the situation (see Figure 1.15).

Dying cells also release molecules capable of engaging PRRs

As we have mentioned earlier, cells undergoing necrosis (but not apoptosis) are also capable of releasing molecules (i.e., DAMPs) that are capable of engaging PRRs (see Figure 1.3). The identity of these molecules is only slowly emerging,

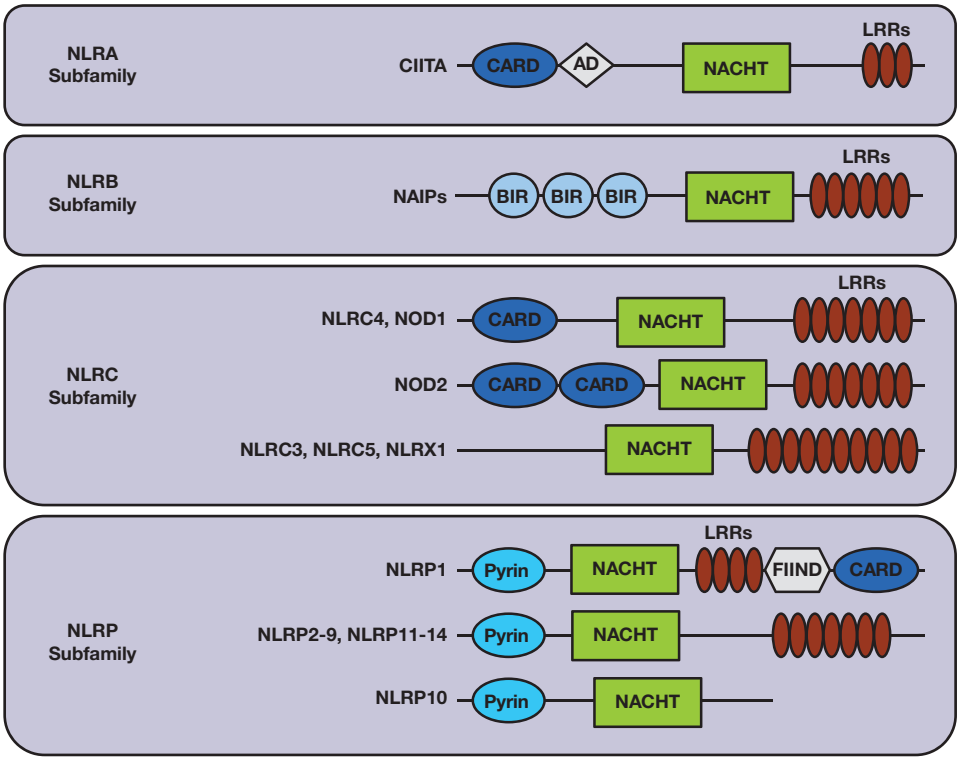


Figure 1.19 Domain organization of the NOD-like receptor (NLR) family. The four subfamilies of NLRs are depicted, separated primarily on the basis of their usage of different N-terminal domains (AD, CARD, Pyrin, BIR) that confers unique functional roles on each NLR. All of the NLRs contain a central NACHT domain, which is a motif that permits oligomerization of individual NLRs into supercomplexes. Assembly and activation of NLR complexes is induced through ligand binding to the C-terminal LRRs that serve as a sensor domain for each of the NLRs. AD, acidic transactivation domain; CARD, caspase recruitment domain; BIR, baculoviral IAP repeat; FIIND, function to find domain; LRR, leucine-rich repeat.

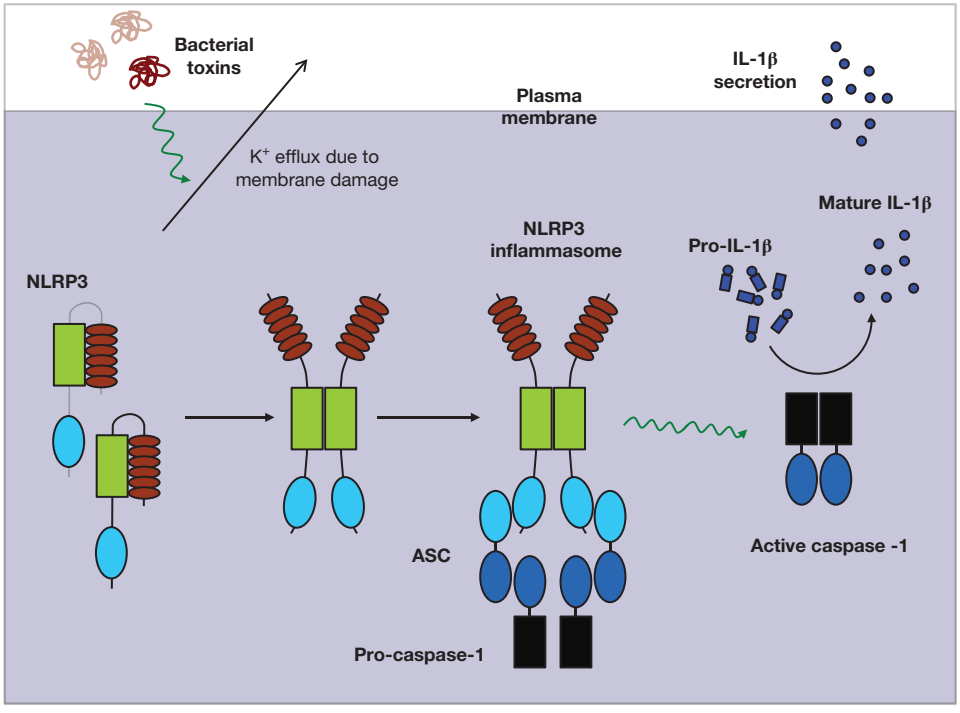


Figure 1.20 Activation of the NLRP3 inflammasome leads to caspase-1 activation and IL-1β processing and release. One example of an NLR complex is illustrated by the NLRP3 inflammasome that is assembled in response to two different signals. Signal 1 is represented by LPS, a PAMP that binds to TLR4 thereby inducing IL-1β transcriptional upregulation in an NFκB-dependent manner (not shown). However, a second signal is required for IL-1β processing and release and this is provided by the cytotoxic actions of bacterial toxins that permit K^+ efflux, through damaging the plasma membrane of an LPS-primed cell. It is the latter event (i.e., K^+ efflux) that triggers assembly of the NLRP3 inflammasome, leading to caspase-1 activation, IL-1β processing, and release of the latter cytokine through death of the injured cell. Thus, the NLRP3 inflammasome acts as a sensor for cell injury-associated K^+ efflux.

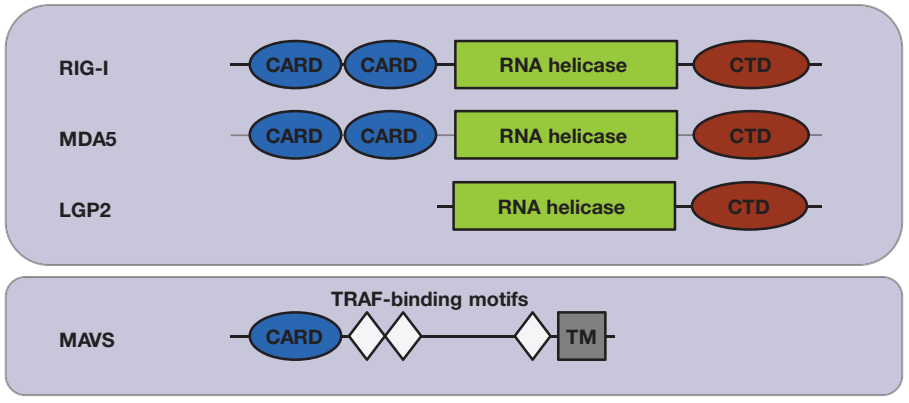


Figure 1.21 Domain organization of the RIG-I-like receptors and their common adaptor MAVS. Members of the RIG-I-like helicase family that act as cytoplasmic sensors for viral RNA are shown, along with their common adaptor protein MAVS. See also Figure 1.22.

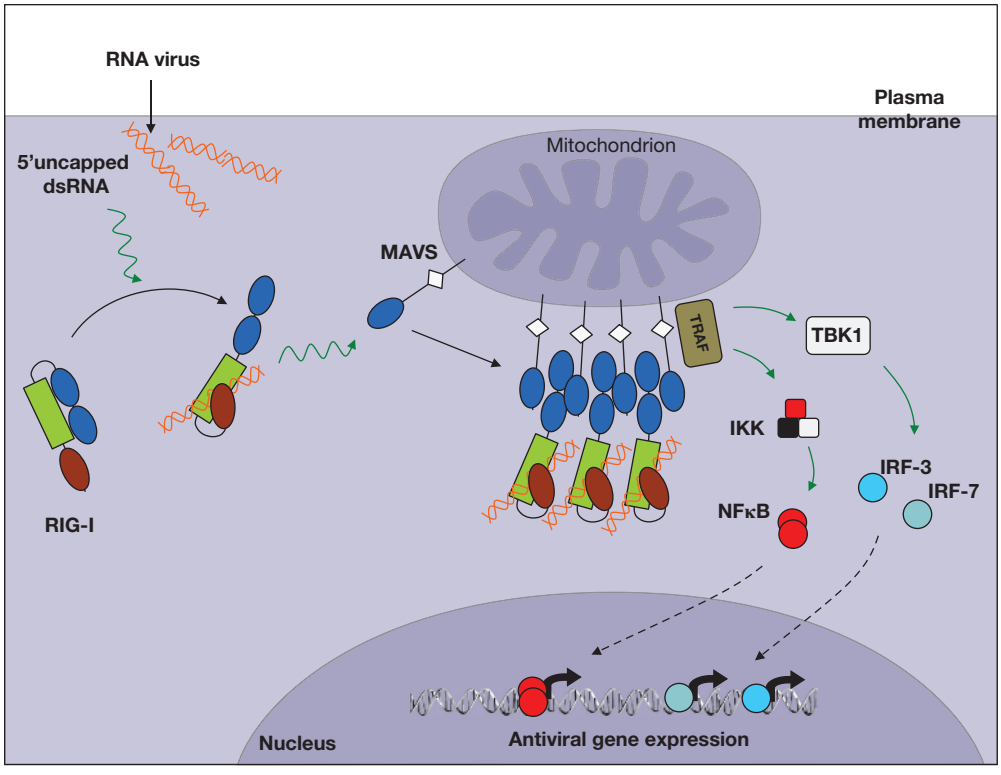


Figure 1.22 RIG-I is activated by double-stranded RNA and initiates transcription of antiviral genes via the IRF and NFκB pathways. RIG-I (retinoic acid inducible gene 1) acts as a cytoplasmic sensor for viral RNA and detects 5′-triphosphate uncapped dsRNA or ssRNA molecules. Upon binding of viral RNA, RIG-I, which is normally in an autoinhibited conformation, can then bind to MAVS (mitochondrial antiviral signaling protein) via CARD–CARD interactions with the latter to promote activation of IRF and NFκB-dependent gene transcription, as shown. CARD, caspase recruitment domain.

but includes HMGB1, members of the S100 calcium-binding protein family, HSP60 and the classical cytokines IL-1α and IL-33. Certain DAMPs appear to be able to bind to members of the TLR family (i.e., HMGB1 has been suggested to signal via TLR4), while others such as IL-1α and IL-33 bind to specific cell surface receptors that possess similar intracellular signaling motifs to the TLR receptors.

DAMPs are involved in amplifying immune responses to infectious agents that provoke cell death and also play a role in the phenomenon of ***sterile injury***, where an immune response

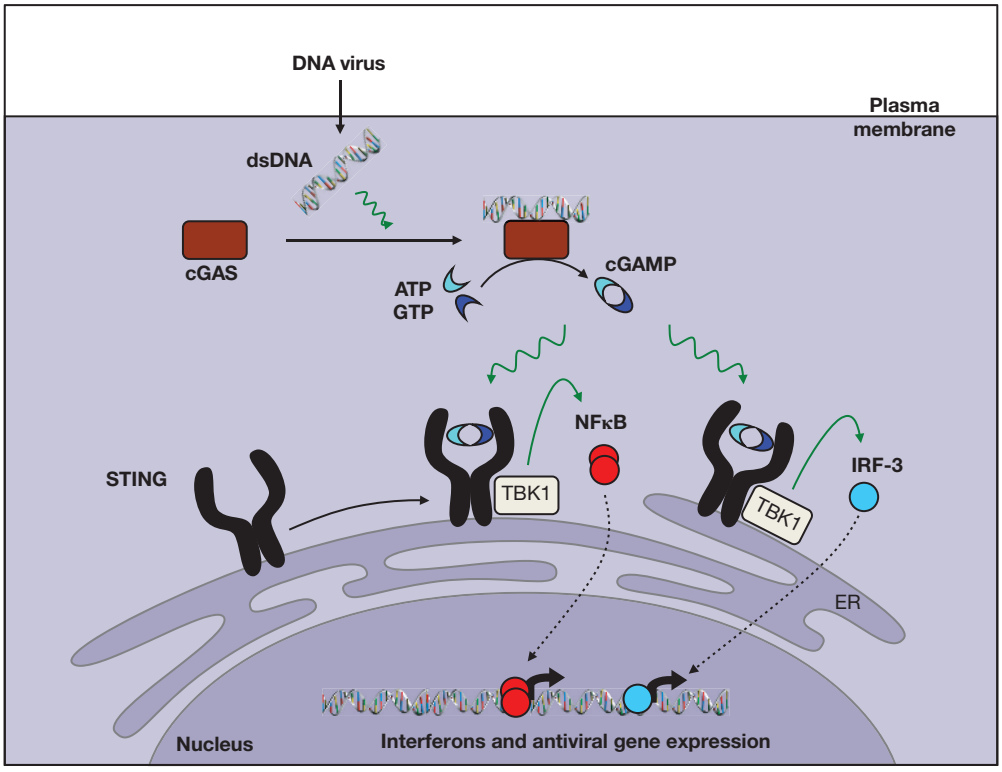


Figure 1.23 STING acts as a cytoplasmic sensor for DNA and cyclic nucleotides. STING (stimulator of interferon genes) is an endoplasmic reticulum-associated protein that can sense cytoplasmic DNA either directly, or through DNA binding to cGAS (cyclic GMP–AMP synthase) an enzyme that generates unusual cyclic dinucleotides (cGAMP) that can act as a ligand for STING to activate transcription of IRF and NFκB-dependent gene transcription. STING may also be able to sense cyclic dinucleotides that are produced by intracellular bacteria.

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

Phagocytic cells engulf and kill microorganisms

Macrophages and neutrophils are dedicated "professional" phagocytes

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

The macrophage

These cells derive from bone marrow promonocytes that, after differentiation to blood monocytes, finally settle in the tissues as mature macrophages where they constitute the ***mononuclear phagocyte system*** (Figure 1.24). 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 neutrophils, macrophages are long-lived cells with significant rough-surfaced endoplasmic reticulum and mitochondria and, whereas neutrophils provide the major defense against pyogenic (pus-forming) bacteria, as a rough generalization it may be said that macrophages are at their best in combating those bacteria, viruses, and protozoa that are capable of living within the cells of the host.

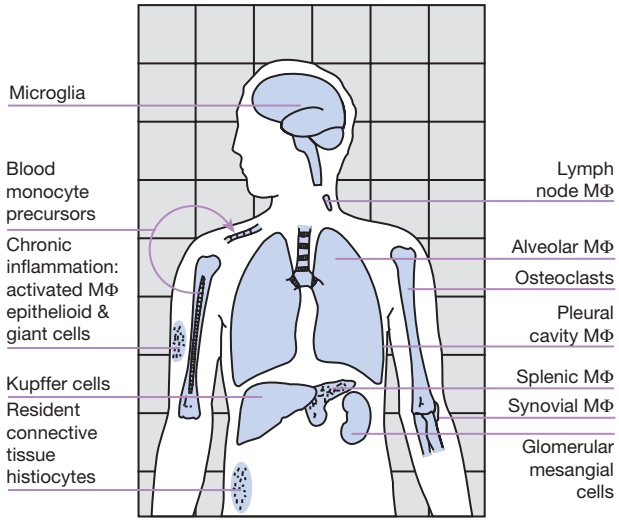


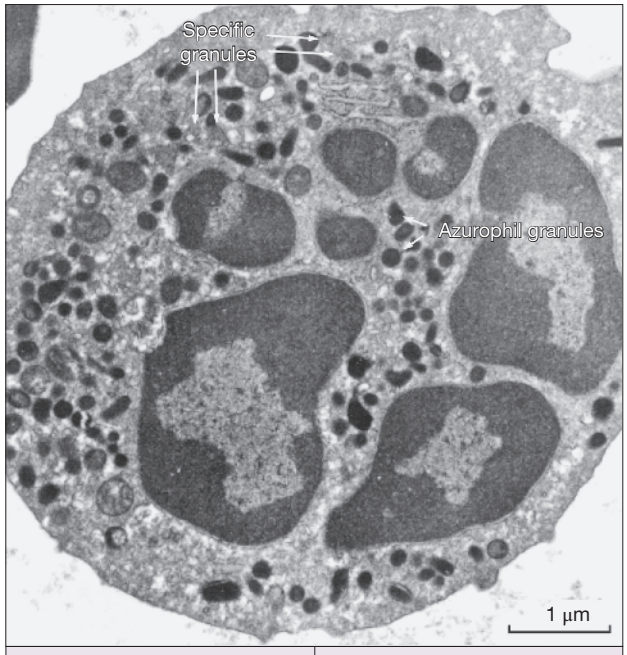
Figure 1.24 The mononuclear phagocyte system. Promonocyte precursors in the bone marrow develop into circulating blood monocytes that eventually become distributed throughout the body as mature macrophages (Mφ) as shown. The other major phagocytic cell, the polymorphonuclear neutrophil, is largely confined to the bloodstream except when recruited into sites of acute inflammation.

The polymorphonuclear neutrophil

This cell, the smaller of the two, shares a common hematopoietic stem cell precursor with the other formed elements of the blood and is the dominant white cell in the bloodstream. It is a nondividing short-lived cell with a multilobed nucleus and an array of granules (Figure 1.9 and Figure 1.25), which are virtually unstained by histologic dyes such as hematoxylin and eosin, unlike those structures in the closely related eosinophil and basophil (Figure 1.9). Neutrophil granules are of two main types: (i) the ***primary azurophil granule*** that develops early, has the typical lysosomal morphology and contains myeloperoxidase, together with most of the nonoxidative antimicrobial effectors including defensins, bactericidal permeability increasing (BPI) protein, and cathepsin G (Figure 1.25); and (ii) the peroxidase-negative ***secondary specific granules*** containing lactoferrin, much of the lysozyme, alkaline phosphatase and membrane-bound cytochrome b_{558} (Figure 1.25). The abundant glycogen stores can be utilized by glycolysis, enabling the cells to function under anerobic conditions.

Microbes are engulfed by activated phagocytic cells

After adherence of the microbe to the surface of the neutrophil or macrophage through recognition of a PAMP (Figure 1.26b), the resulting signal (Figure 1.26c) initiates the ingestion phase by activating an actin–myosin contractile system that extends pseudopods around the particle (Figure 1.26d and Figure 1.27); 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; Figure 1.26f and Figure 1.27). Events are now moving smartly and, within 1 minute, the cytoplasmic granules fuse with the phagosome and discharge their contents around the imprisoned microorganism (Figure 1.26 g and Figure 1.28) subjecting them to a formidable battery of microbicidal mechanisms.



Azurophil granules	Specific granules
0.5 μm 1500/cell	0.2 μm 3000/cell
Lysozyme Myeloperoxidase Elastase Cathepsin G H^+ hydrolases Defensins BPI	Lysozyme Cytochrome b_{558} OH^- phosphatase Lactoferrin Vitamin B_{12} binding protein

Figure 1.25 Ultrastructure of neutrophil. The multilobed nucleus and two main types of cytoplasmic granules are well displayed. BPI, bactericidal permeability increasing protein. (Source: Dr. D. McLaren. Reproduced with permission.)

Phagocytes employ an array of killing mechanisms

Killing by reactive oxygen intermediates

Trouble starts for the invader from the moment phagocytosis is initiated. There is a dramatic increase in activity of the hexose monophosphate shunt, generating reduced nicotinamide adenine dinucleotide phosphate (NADPH). Electrons pass from the NADPH to a flavine adenine dinucleotide (FAD)-containing

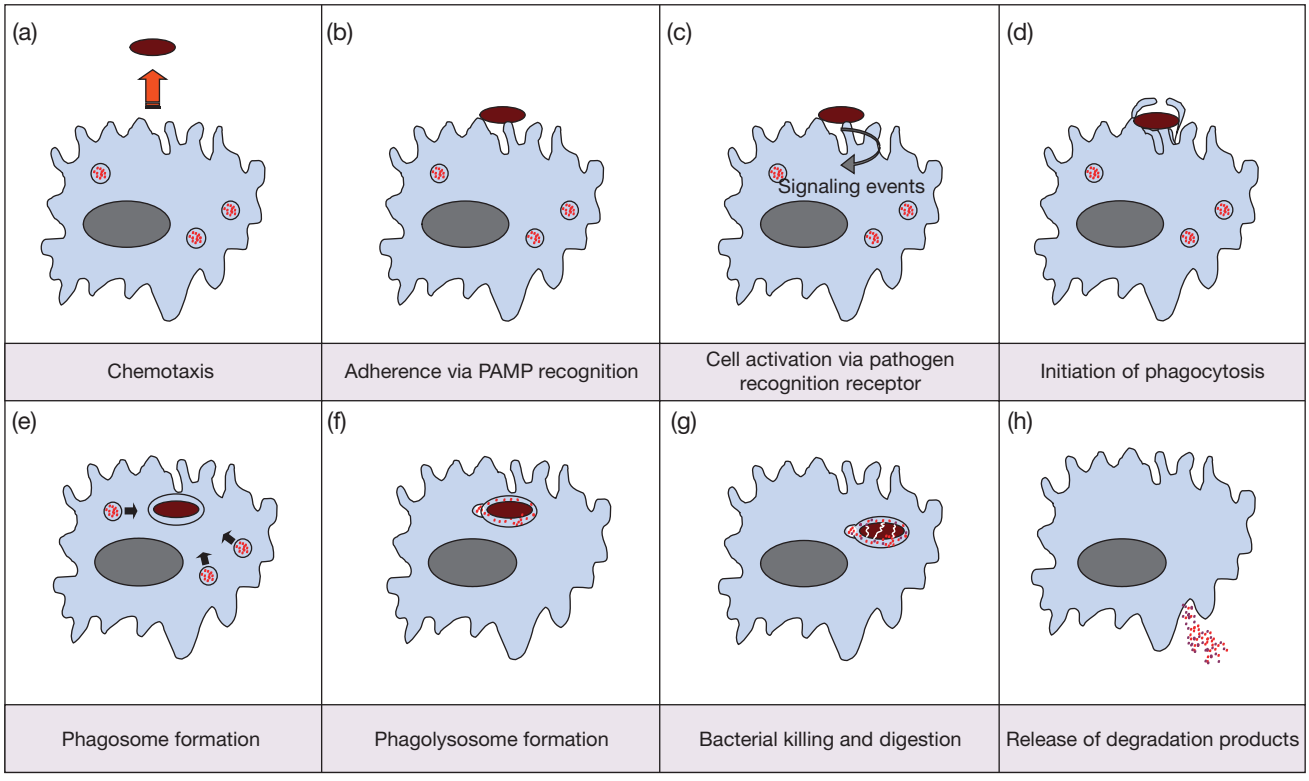


Figure 1.26 Phagocytosis and killing of a bacterium. Stage c/d, respiratory burst and activation of NADPH oxidase; stage e, damage by reactive oxygen intermediates; stage f/g, damage by peroxidase, cationic proteins, antibiotic peptide defensins, lysozyme, and lactoferrin.

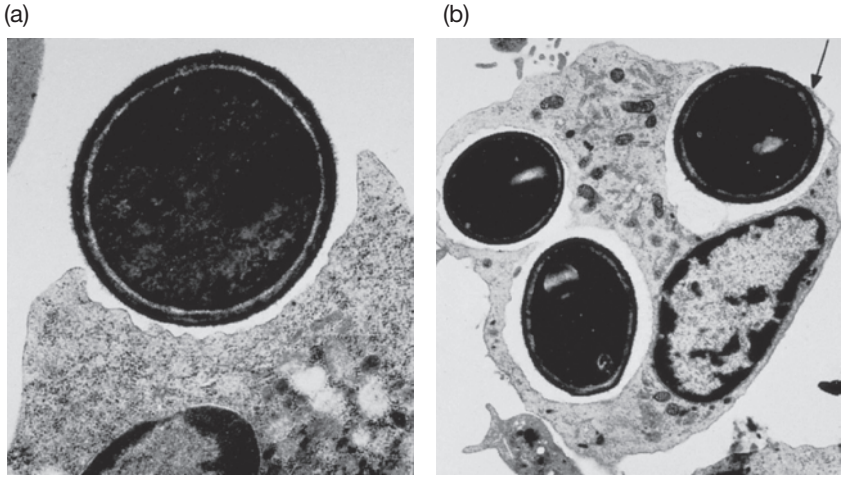


Figure 1.27 Adherence and phagocytosis. (a) Phagocytosis of *Candida albicans* by a polymorphonuclear leukocyte (neutrophil). Adherence to the yeast wall surface mannan initiates enclosure of the fungal particle within arms of cytoplasm. Lysosomal granules are abundant but mitochondria are rare (×15 000). (b) Phagocytosis of *C. albicans* by a monocyte showing near completion of phagosome formation (arrowed) around one organism and complete ingestion of two others (×5000). (Source: Dr. H. Valdimarsson. Reproduced with permission.)

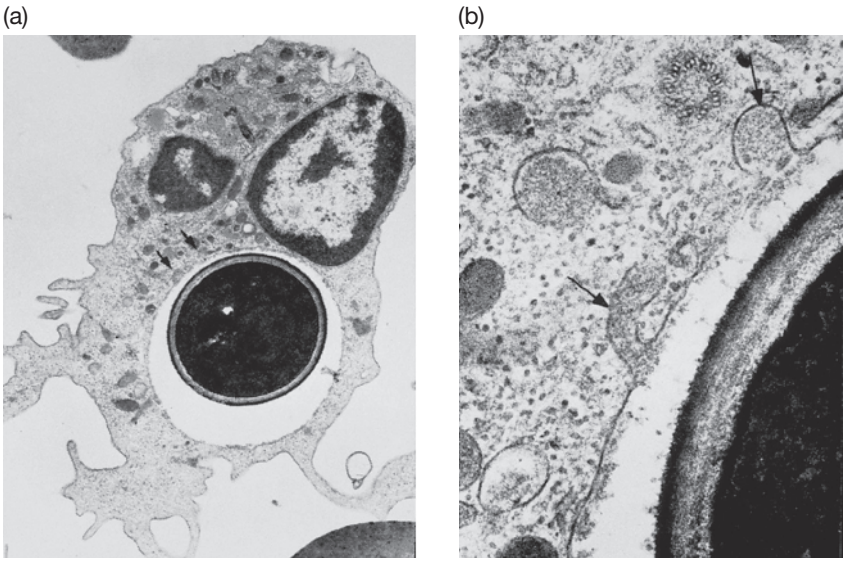


Figure 1.28 Phagolysosome formation. (a) Neutrophil 30 minutes after ingestion of *C. albicans*. The cytoplasm is already partly degranulated and two lysosomal granules (arrowed) are fusing with the phagocytic vacuole. Two lobes of the nucleus are evident (×5000). (b) Higher magnification of (a) showing fusing granules discharging their contents into the phagocytic vacuole (arrowed) (×33 000). (Source: Dr. H. Valdimarsson. Reproduced with permission.)

membrane flavoprotein and thence to a unique plasma membrane ***cytochrome (cyt b558)***. This has the very low midpoint redox potential of –245 mV that allows it to reduce molecular oxygen directly to superoxide anion (Figure 1.29a). Thus the key reaction catalyzed by this NADPH oxidase, which initiates the formation of reactive oxygen intermediates (ROI), is:

$$NADPH + O_2 \rightarrow NADP^+ + \bullet O^{2-}$$

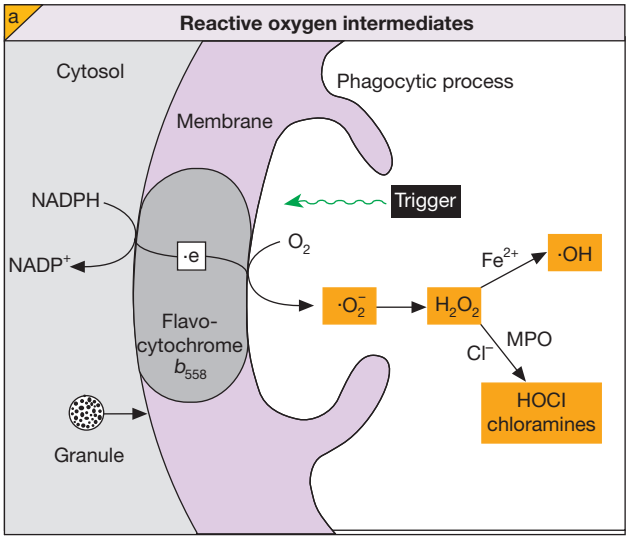
The superoxide anion undergoes conversion to hydrogen peroxide under the influence of superoxide dismutase, and subsequently to hydroxyl radicals (·OH). Each of these products has remarkable chemical reactivity with a wide range of molecular targets, making them formidable microbicidal agents; ·OH in particular is one of the most reactive free radicals known. Furthermore, the combination of peroxide, myeloperoxidase, and halide ions constitutes a potent halogenating system capable of killing both bacteria and viruses (Figure 1.29a). Although H_2O_2 and the halogenated compounds are not as active as the free radicals, they are more stable and therefore diffuse further, making them toxic to microorganisms in the extracellular vicinity.

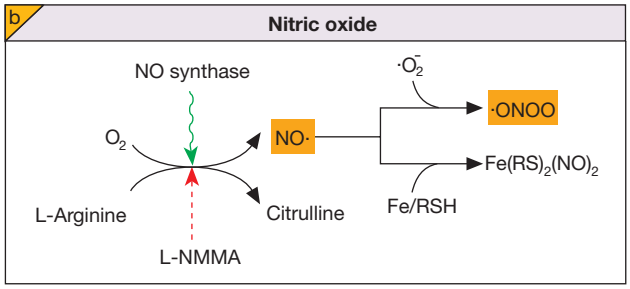
Killing by reactive nitrogen intermediates

Nitric oxide surfaced prominently as a physiologic mediator when it was shown to be identical with endothelium-derived relaxing factor. This has proved to be just one of its many roles (including the mediation of penile erection, would you believe it!), but of major interest in the present context is its formation by an inducible NO · synthase (iNOS) within most cells, but particularly macrophages and human neutrophils, thereby generating a powerful antimicrobial system (Figure 1.29b). Whereas the NADPH oxidase is dedicated to the killing of extracellular organisms taken up by phagocytosis and cornered within the phagocytic vacuole, the NO· mechanism can operate against microbes that invade the cytosol; so, it is not surprising that the majority of nonphagocytic cells that may be infected by viruses and other parasites are endowed with an iNOS capability. The mechanism of action may be through degradation of the Fe–S prosthetic groups of certain electron transport enzymes, depletion of iron, and production of toxic · ONOO radicals. The *N-ramp* gene, linked with resistance to microbes such as bacille Calmette–Guérin (BCG), *Salmonella*, and *Leishmania* that can live within an intracellular habitat, is now known to express a protein forming a transmembrane channel that may be involved in transporting NO · across lysosome membranes.

Killing by preformed antimicrobials

These molecules, contained within the neutrophil granules, contact the ingested microorganism when fusion with the phagosome occurs (Figure 1.29c). The dismutation of superoxide consumes hydrogen ions and raises the pH of the vacuole gently, so allowing the family of cationic proteins and peptides to function optimally. The latter, known as ***defensins***, are approximately 3.5–4 kDa and invariably rich in arginine, and reach incredibly high concentrations within the phagosome, of the order of 20–100 mg/mL. Like the bacterial colicins described above, they have an amphipathic structure that allows them to insert into microbial membranes to form





c Oxygen-independent mechanisms	
Cathepsin G Low mol. wt defensins High mol. wt cationic proteins Bactericidal permeability increasing protein (BPI)	Damage to microbial membranes
Lysozyme	Splits mucopeptide in bacterial cell wall
Lactoferrin	Complex with iron
Proteolytic enzymes Variety of other hydrolytic enzymes	Digestion of killed organisms

Figure 1.29 Microbicidal mechanisms of phagocytic cells. (a) Production of reactive oxygen intermediates. Electrons from NADPH are transferred by the flavocytochrome oxidase enzyme to molecular oxygen to form the microbicidal molecular species shown in the orange boxes. (For the more studious – the phagocytosis-triggering agent binds to a classic G-protein-linked seven transmembrane domain receptor that activates an intracellular guanosine triphosphate (GTP)-binding protein. This in turn activates an array of enzymes: phosphoinositol-3 kinase concerned in the cytoskeletal reorganization underlying chemotactic responses, phospholipase-Cγ2 mediating events leading to lysosome degranulation and phosphorylation of $p47^{phox}$ through activation of protein kinase C, and the MEK and MAP kinase systems (see Figure 7.10) that oversee the assembly of the NADPH oxidase. This is composed of the membrane cytochrome b_{558}, consisting of a p21 heme protein linked to gp91 with binding sites for NADPH and FAD on its intracellular aspect, to which phosphorylated p47 and p67 translocate from the cytosol on activation of the oxidase.) (b) Generation of nitric oxide. The enzyme, which structurally resembles the NADPH oxidase, can be inhibited by the arginine analog *N*-monomethyl-L-arginine (L-NMMA). The combination of NO· with superoxide anion yields the highly toxic peroxynitrite radical ·ONOO that cleaves on protonation to form reactive ·OH and NO_2 molecules. NO· can form mononuclear iron dithioldinitroso complexes leading to iron depletion and inhibition of several enzymes. (c) The basis of oxygen-independent antimicrobial systems.

destabilizing voltage-regulated ion channels (who copied whom?). These antibiotic peptides, at concentrations of 10–100 μg/mL, act as disinfectants against a wide spectrum of Gram-positive and Gram-negative bacteria, many fungi, and a number of enveloped viruses. Many exhibit remarkable selectivity for prokaryotic and eukaryotic microbes relative to host cells, partly dependent upon differential membrane lipid composition. One must be impressed by the ability of this surprisingly simple tool to discriminate large classes of nonself cells (i.e., microbes) from self.

As if this was not enough, further damage is inflicted on the bacterial membranes both by neutral protease (cathepsin G) action and by direct transfer to the microbial surface of BPI, which increases bacterial permeability. Low pH, lysozyme, and lactoferrin constitute bactericidal or bacteriostatic factors that are oxygen independent and can function under anerobic circumstances. Interestingly, lysozyme and lactoferrin are synergistic in their action. Finally, the killed organisms are digested by hydrolytic enzymes and the degradation products released to the exterior (Figure 1.26 h).

Neutrophils and macrophages can also deploy extracellular traps for microbes through releasing DNA

Recent discoveries have also revealed quite a surprising strategy that neutrophils (as well as their close granulocyte relatives) engage in for the purpose of immobilizing and killing extracellular bacteria and yeast: ***the formation of NETs*** (***neutrophil extracellular traps***). It appears that activated neutrophils can activate a self-destruction pathway, the details of which are only emerging, that results in the release of the intracellular contents of the activated neutrophil into the extracellular space to act as a spider's web-like structure that can enmesh microbes and kill them *in situ* (Figure 1.30). The NETs themselves

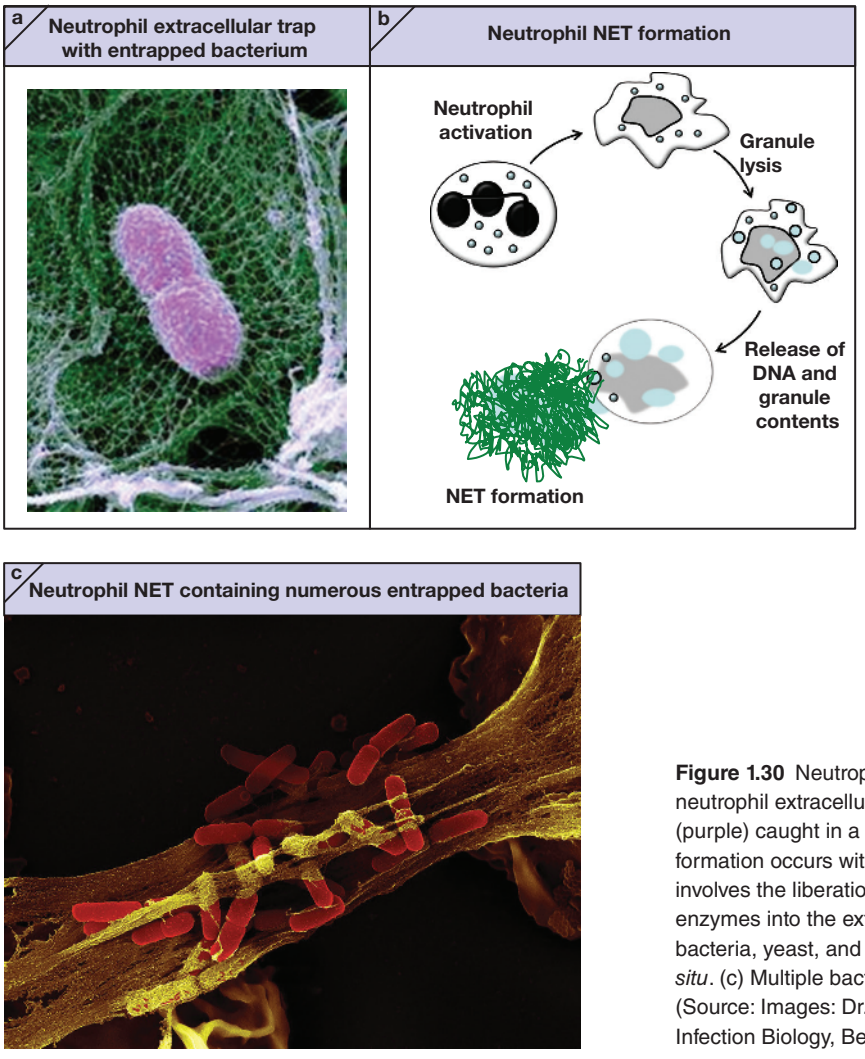


Figure 1.30 Neutrophil activation can lead to the formation of neutrophil extracellular traps (NETs). (a) A *Klebsiella* bacterium (purple) caught in a neutrophil NET (green). (b) Neutrophil NET formation occurs within 1–2 hours after neutrophil activation and involves the liberation of neutrophil DNA, histones, and granule enzymes into the extracellular space where they can ensnare bacteria, yeast, and other extracellular pathogens and kill them *in situ*. (c) Multiple bacteria (red) ensnared on a neutrophil NET. (Source: Images: Dr. Volker Brinkmann, Max Planck Institute for Infection Biology, Berlin, Germany.)

appear to be largely composed of neutrophil DNA with associated histones, along with high concentrations of neutrophil granule proteases such as cathepsin G, elastase, and proteinase-3. The NET is thought to act as a depot for the latter proteases, helping to restrain their off-target activities and also increase their local concentration. Interestingly, histone proteins have also been reported to have potent antimicrobial properties, although how this is achieved is unclear. Macrophages have also been reported to be able to deploy NET-like structures under certain circumstances. Does the immune system have no end to the strategies it will engage in to protect us from harm?

By now, the reader may be excused a little smugness as she or he shelters behind the impressive antimicrobial 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 microorganism; (ii) adhere to it; and (iii) respond by the membrane activation that initiates engulfment. Some bacteria do produce chemical substances, such as the peptide formyl.Met.Leu.Phe, which directionally attract leukocytes, a process known as ***chemotaxis***; many organisms do adhere to the phagocyte surface and many do spontaneously provide the appropriate membrane initiation signal. However, our teeming microbial adversaries are continually mutating to produce new species that may outwit the defenses 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 and bacterial lysis

The complement system comprises a group of some 20 or so plasma proteins that becomes activated in a cascade-like manner upon binding to certain microbial polysaccharides that are not normally present in vertebrates, but are commonly found

on bacterial membranes. Many of the complement factors are proteases that are initially produced as inactive precursors and become activated through the detection of PAMPs, with each protease activating the next in the chain. Complement activation can result in binding of complement to bacterial cell surfaces (called ***opsonization*** in immunological parlance), which can greatly enhance their uptake by phagocytes. Deposition of complement factors onto its surface can also result in ***direct lysis*** of a bacterium that has had the misfortune to trigger this cascade. Just as importantly, certain complement fragments that are produced as byproducts of complement activation can act as ***chemotactic factors*** to guide phagocytic cells (such as neutrophils and macrophages) to the hapless bacterium, resulting in its capture through phagocytosis. The latter complement factors can also ***activate local mast cells*** (as we mentioned earlier) to release molecules that help to recruit neutrophils and other cells of the immune system to the site of infection, through increasing the permeability of local blood vessels. Either way, complement activation spells trouble for our little bacterial foe. The many proteins involved can make the complement system appear daunting initially, but do keep in mind the overall objectives of enhancing phagocytosis, recruitment of other immune cells, and direct lysis of microorganisms, as we proceed through the details.

Complement and its activation

The complement cascade, 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 that 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 kDa and is present in plasma at a concentration of around 1.2 mg/mL.

C3 undergoes slow spontaneous cleavage

Under normal circumstances, an internal thiolester bond in C3 (Figure 1.31) becomes activated spontaneously 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 designated C3i or C3(H_2O). In the presence of Mg^{2+} 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."

C3bBb has an important new enzymic activity: it is a ***C3 convertase*** that can split C3 to give C3a and C3b. We will shortly discuss the important biological consequences of C3 cleavage in relation to microbial defenses, but under normal conditions there must be some mechanism to restrain this process to a "tick-over" level as it can also give rise to more C3bBb, that is, we are dealing with a potentially runaway ***positive-feedback loop*** (Figure 1.32). As with all potentially explosive triggered cascades, there are powerful regulatory mechanisms.

C3b levels are normally tightly controlled

In solution, the C3bBb convertase is unstable and factor B is readily displaced by another component, factor H, to form C3bH, which is susceptible to attack by the C3b inactivator,

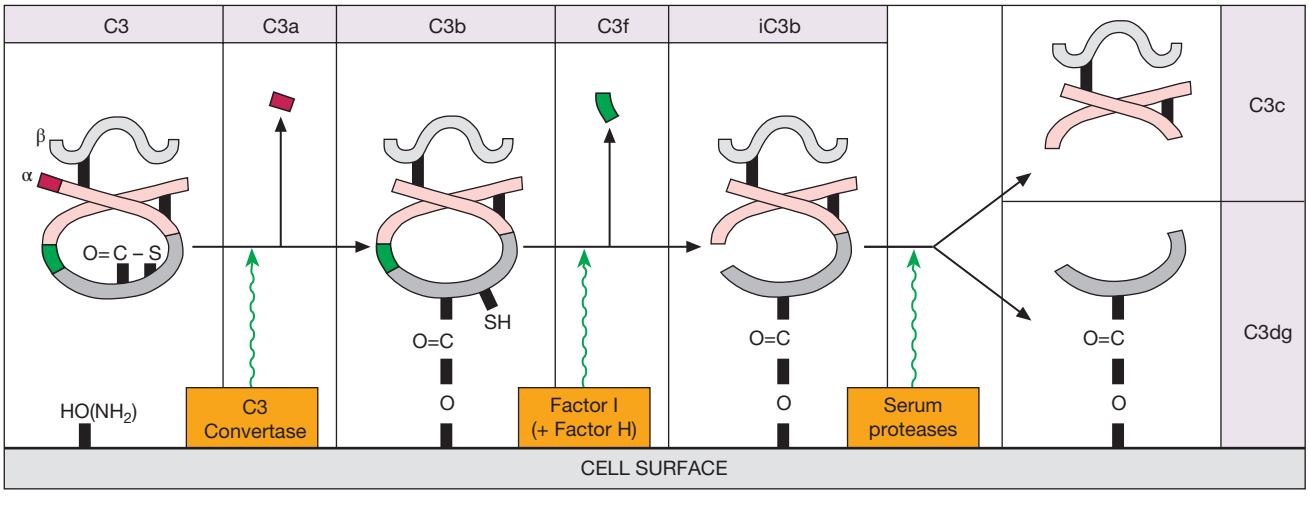


Figure 1.31 Structural basis for the cleavage of C3 by C3 convertase and its covalent binding to ·OH or ·NH_2 groups at the cell surface through exposure of the internal thiolester bonds. Further cleavage leaves the progressively smaller fragments, C3dg and C3d, attached to the membrane. (Adapted from Law S.H.A. and Reid K.B.M. (1988) *Complement*, figure 2.4. IRL Press, Oxford.)

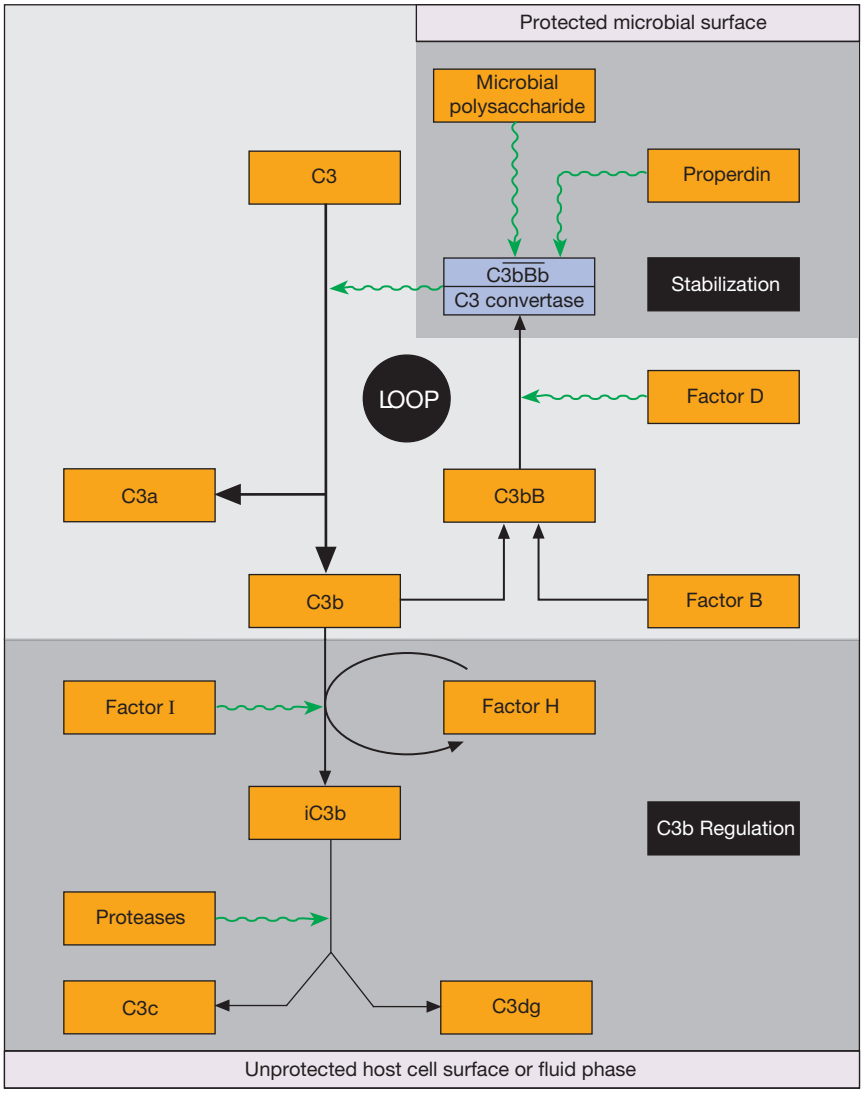


Figure 1.32 Microbial activation of the alternative complement pathway by stabilization of the C3 convertase ($C\overline{3bBb}$) and its control by factors H and I. When bound to the surface of a host cell or in the fluid phase, the C3b in the convertase is said to be "unprotected," in that its affinity for factor H is much greater than for factor B and is therefore susceptible to breakdown by factors H and I. On a microbial surface, C3b binds factor B more strongly than factor H and is therefore "protected" from or "stabilized" against cleavage – even more so when subsequently bound by properdin. Although in phylogenetic terms this is the oldest complement pathway, it was discovered after a separate pathway to be discussed in the next chapter, and so has the confusing designation "alternative." Green wiggly arrow represents an activation process. The horizontal bar above a component designates its activation.

factor I (Figure 1.32). The inactivated iC3b is biologically inactive and undergoes further degradation by proteases in the body fluids. Other regulatory mechanisms are discussed later.

C3 convertase is stabilized on microbial surfaces

A number of microorganisms can activate the $C\overline{3bBb}$ convertase to generate large amounts of C3 cleavage products ***by stabilizing the enzyme on their (carbohydrate) surfaces***, thereby protecting the C3b from factor H. Another protein, properdin, acts subsequently on this bound convertase to stabilize it even further. As C3 is split by the surface membrane-bound enzyme to nascent C3b, it undergoes conformational change and its potentially reactive internal thiolester bond becomes exposed. As the half-life of nascent C3b is less than 100 microseconds, it can only diffuse a short distance before reacting covalently with local hydroxyl or amino groups available at the microbial cell surface (Figure 1.31). Each catalytic site thereby leads to the clustering of large numbers of C3b molecules on the microorganism. This series of reactions leading to C3 breakdown provoked directly by microbes has been called ***the alternative pathway*** of complement activation (Figure 1.32).

The post-C3 pathway generates a membrane attack complex

Recruitment of a further C3b molecule into the $\overline{C3bBb}$ enzymic complex generates a C5 convertase that activates C5 by proteolytic cleavage, releasing a small polypeptide, C5a, and leaving the large C5b fragment loosely bound to C3b. Sequential attachment of C6 and C7 to C5b forms a complex with a transient membrane-binding site and an affinity for the β-peptide chain of C8. The C8α chain sits in the membrane and directs the conformational changes in C9 that transform it into an amphipathic molecule capable of insertion into the lipid bilayer (cf. the colicins) and polymerization to an annular ***membrane attack complex*** (MAC; Figure 1.33). This forms a transmembrane channel fully permeable to electrolytes and water, and because of the high internal colloid osmotic pressure of cells, there is a net influx of Na^+ and water, frequently leading to lysis.

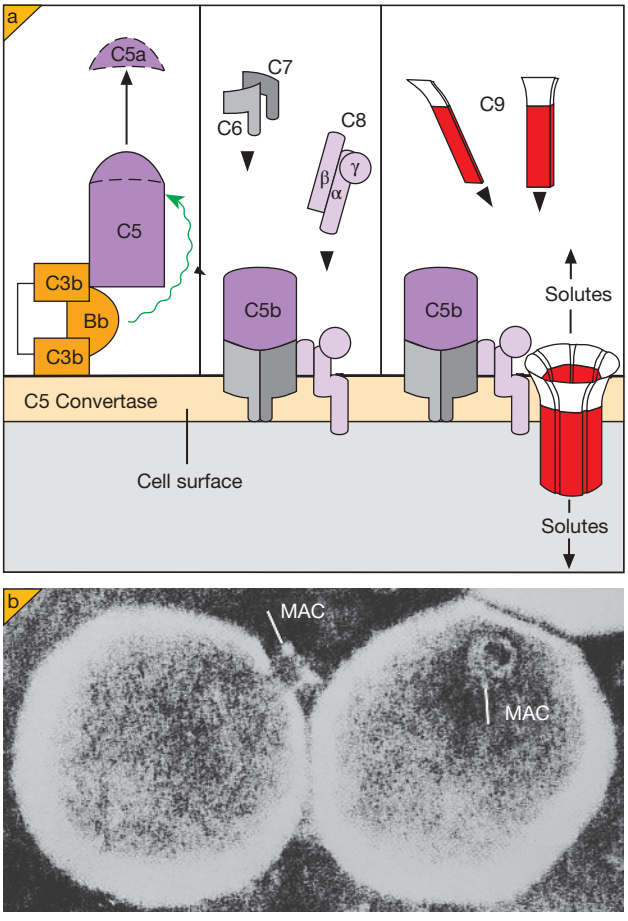


Figure 1.33 Post-C3 pathway generating C5a and the C5b–9 membrane attack complex (MAC). (a) Cartoon of molecular assembly. The conformational change in C9 protein structure that converts it from a hydrophilic to an amphipathic molecule (bearing both hydrophobic and hydrophilic regions) can be interrupted by an antibody raised against linear peptides derived from C9; as the antibody does not react with the soluble or membrane-bound forms of the molecule, it must be detecting an intermediate structure transiently revealed in a deep-seated structural rearrangement. (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. Although in itself a rather splendid structure, formation of the annular C9 cylinder is probably not essential for cytotoxic perturbation of the target cell membrane, as this can be achieved by insertion of amphipathic C9 molecules in numbers too few to form a clearly defined MAC. (Source: Professor J. Tranum-Jensen and Dr. S. Bhakdi. Reproduced with permission.)

Complement has a range of defensive biological functions

These can be grouped conveniently under three headings:

1. ***C3b adheres to complement receptors:*** Phagocytic cells have receptors for C3b (CR1) and iC3b (CR3) that facilitate the adherence of C3b-coated microorganisms to the cell surface (discussed more fully in Chapter 11).
2. ***Biologically active fragments are released:*** C3a and C5a, the small peptides split from the parent molecules during complement activation, have several important actions. Both act directly on phagocytes, especially neutrophils, to stimulate the respiratory burst associated with the production of reactive oxygen intermediates and to enhance the expression of surface receptors for C3b and iC3b. Also, both are ***anaphylatoxins*** in that they are capable of triggering mediator release from mast cells (Figure 1.14 and Figure 1.34) and their circulating counterpart, the basophil (Figure 1.9), a phenomenon of such relevance to our present discussion that we have presented details of the mediators and their actions in Figure 1.14; note in particular the chemotactic properties of these mediators and their effects on blood vessels. In its own right, C3a is a chemoattractant for eosinophils whereas C5a is a potent neutrophil chemotactic agent and also has a striking ability to act directly on the capillary endothelium to produce vasodilatation and increased permeability, an effect that seems to be prolonged by leukotriene B_4 released from activated mast cells, neutrophils and macrophages.
3. ***The terminal complex can induce membrane lesions:*** As described above, the insertion of the MAC into a membrane may bring about cell lysis. Providentially, complement is relatively inefficient at lysing the cell membranes of the autologous host owing to the presence of control proteins.

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

In the first act, $\overline{C3bBb}$ 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.

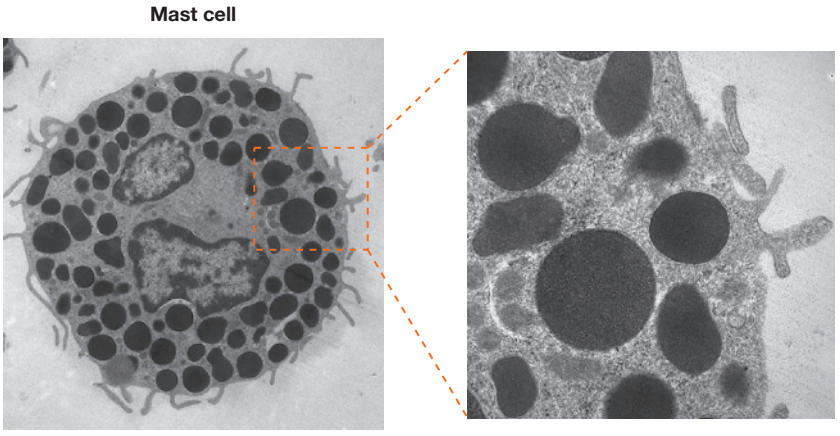


Figure 1.34 The mast cell. Transmission electron micrograph of a resting mouse peritoneal mast cell illustrating the copious membrane-enclosed granules that are filled with inflammatory mediators. Release of the latter mediators may be triggered by direct injury, complement products (C3a, C5a), and through direct stimulation with PAMPs. (Source: Source: Gunnar Pejler, University of Uppsala, Sweden. Reproduced with permission.)

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).

Humoral mechanisms provide an additional defensive strategy

Microbicidal factors in secretions

Turning now to those defense systems that are mediated entirely by ***soluble pattern recognition molecules*** (Figure 1.2), we recollect that many microbes activate the complement system and may be lysed by the insertion of the membrane attack complex. The spread of infection may be limited by enzymes released through tissue injury that 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 that splits the exposed peptidoglycan wall of susceptible bacteria (see Figure 11.5).

Like the α-defensins of the neutrophil granules, the human β-defensins are peptides derived by proteolytic cleavage from larger precursors; they have β-sheet structures, 29–40 amino acids, and three intramolecular disulfide bonds, although they differ from the α-defensins in the placement of their six cysteines. The main human β-defensin, hDB-1, is produced abundantly in the kidney, the female reproductive tract, the oral gingiva, and especially the lung airways. As the word has it that we are all infected every day by tens of thousands of airborne bacteria, this must be an important defense mechanism. This being so, inhibition of hDB-1 and of a second pulmonary defensin, hDB-2, by high ionic strength could account for the susceptibility of cystic fibrosis patients to infection as they have an ion channel mutation that results in an elevated chloride concentration in airway surface fluids. Another airway antimicrobial active against Gram-negative and Gram-positive bacteria is LL-37, a 37-residue α-helical peptide released by proteolysis of a cathelicidin (cathepsin L-inhibitor) precursor.

This theme surfaces again in the stomach where a peptide split from lactoferrin by pepsin could provide the gastric and intestinal secretions with some antimicrobial policing. A rather longer two-domain peptide with 107 residues, termed secretory leukocyte protease inhibitor (SLPI), is found in many human secretions. The C-terminal domain is anti-protease but the N-terminal domain is distinctly unpleasant to metabolically active fungal cells and to various skin-associated microorganisms, which makes its production by human keratinocytes particularly appropriate. In passing, it is worth pointing out that many D-amino acid analogs of peptide antibiotics form left-handed helices that retain the ability to induce membrane ion channels and hence their antimicrobial powers and, given their resistance to catabolism within the body, should be attractive candidates for a new breed of synthetic antibiotics.

Lastly, we may mention the two lung surfactant proteins SP-A and SP-D that, in conjunction with various lipids, lower the surface tension of the epithelial lining cells of the lung to keep the airways patent. They belong to a totally different structural group of molecules termed collectins (Figure 1.35) that contribute to innate immunity through binding of their lectin-like domains to carbohydrates on microbes, and their collagenous stem to cognate receptors on phagocytic cells – thereby facilitating the ingestion and killing of the infectious agents.

Acute phase proteins increase in response to infection

A number of plasma proteins collectively termed acute phase proteins show a dramatic increase in concentration in response to early "alarm" mediators such as macrophage-derived

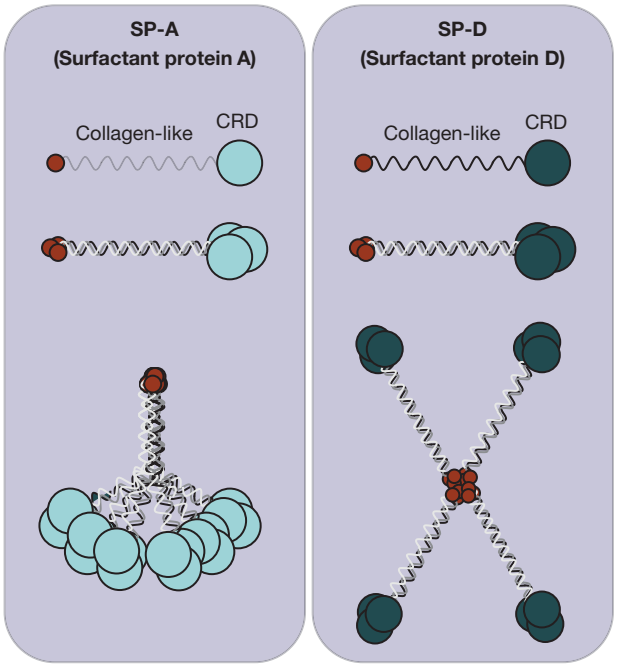


Figure 1.35 Structural features of surfacant proteins A and D. Surfactant proteins are composed of collagen-like and carbohydrate recognition domains (CRD) that are arranged into trimers (middle) and further arranged into higher order multimers of trimers (bottom). Surfactants belong to the collectin family and can recognize nonself carbohydrate moieties on microbes, leading to opsonization followed by phagocytosis.

Table 1.2 Acute phase proteins.

Acute phase reactant	Role
Dramatic increases in concentration	
C-reactive protein	Fixes complement, opsonizes
Mannose binding lectin	Fixes complement, opsonizes
α_1-Acid glycoprotein	Transport protein
Serum amyloid P component	Amyloid component precursor
Moderate increases in concentration	
α_1-Protease inhibitors	Inhibit bacterial proteases
α_1-Antichymotrypsin	Inhibit bacterial proteases
C3, C9, factor B	Increase complement function
Ceruloplasmin	$\cdot O_2^-$ scavenger
Fibrinogen	Coagulation
Angiotensin	Blood pressure
Haptoglobin	Bind hemoglobin
Fibronectin	Cell attachment

interleukin-1 (IL-1) released as a result of infection or tissue injury. These include C-reactive protein (CRP), mannose-binding lectin (MBL), and serum amyloid P component (Table 1.2). Expression levels of the latter proteins can increase by as much as 1000-fold in response to proinflammatory cytokines such as IL-1 and IL-6. Other acute phase proteins showing a more modest rise in concentration include α_1-antichymotrypsin, fibrinogen, ceruloplasmin, C9, and factor B.

The acute phase proteins are a relatively diverse group of proteins belonging to several different families (including, but not limited to, the ***pentraxin***, ***collectin***, and ***ficolin*** families) that have a number of functional effects in common. All of these proteins act as soluble pattern recognition molecules and are capable of binding directly to infectious agents to function as opsonins (i.e., "made ready for the table"), thereby enhancing uptake of microorganisms by macrophages and neutrophils. Many of these proteins also have the ability to activate complement and the assembly of a membrane attack complex. The ability to agglutinate microorganisms, thereby impeding their spread within the infected tissue, is another common theme. Some of these molecules can also form heterocomplexes, extending the range of PAMPs that can be detected.

These soluble pattern recognition molecules are frequently synthesized by activated macrophages upon stimulation of their pattern recognition receptors, or are stored within neutrophil granules available for immediate release via degranulation in response to infection. The liver is another major source of many acute phase proteins that are released into the circulation as a result of the systemic effects of the major proinflammatory cytokines IL-1 and IL-6. Let us look at some examples further.

Pentraxins

Pentraxins, so-called because these agents are made up of five identical subunits, constitute a superfamily of conserved proteins typified by a cyclic multimeric structure and a C-terminal 200-amino-acid-long pentraxin domain. CRP, serum amyloid P component (SAP), and pentraxin 3 are members of this family (Figure 1.36). Human CRP is composed of five identical polypeptide units noncovalently arranged as a cyclic pentamer around a calcium (Ca)-binding cavity, was the first pentraxin to be described, and is the prototypic acute phase response protein. Pentraxins have been around in the animal kingdom for some time, as a closely related homolog, limulin, is present in the hemolymph 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, as a pattern recognition molecule, to a number of microorganisms that contain phosphorylcholine in their membranes, the complex having the useful property of activating complement (by the classical and not the alternative pathway with which we are at present familiar). This results in the deposition of C3b on the surface of the microbe that thus becomes opsonized for adherence to phagocytes.

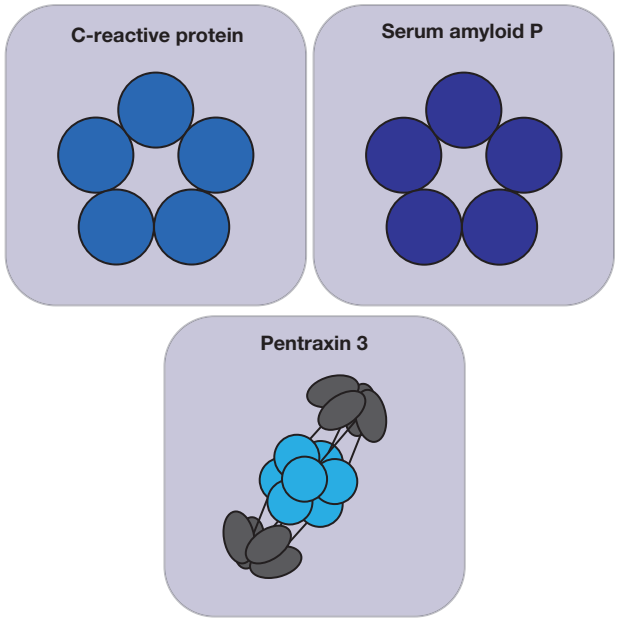


Figure 1.36 Higher order features of pentraxins. Pentraxins, such as C-reactive protein (CRP), serum amyloid P (SAP) and pentraxin 3, as depicted, are all composed of five identical subunits with a cyclic structure. Pentraxins act as soluble PRRs and can opsinize bacteria as well as promote complement activation.

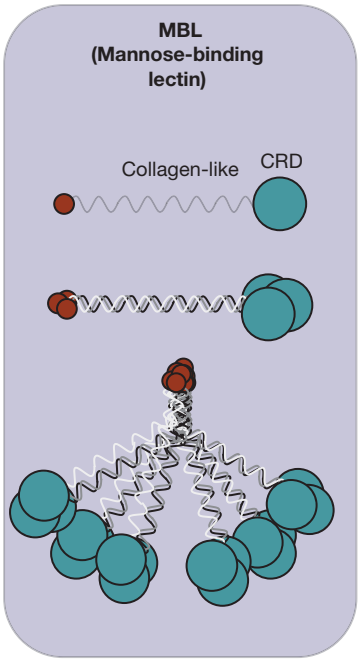


Figure 1.37 Structural features of mannose-binding lectin. Mannose-binding lectin (MBL) is a multiple of trimeric complexes, each unit of which contains a collagen-like and lectin-binding domain (or carbohydrate recognition domain, CRD). MBL can react with a wide variety of bacterial carbohydrates, such as mannose, leading to opsonization of bacteria for uptake through phagocytosis, or can activate the lectin pathway to complement activation (which will be discussed in detail in Chapter 2) through the actions of two associated serine proteases (MASP-1 and MASP-2).

SAP can complex with chondroitin sulfate, a cell matrix glycosaminoglycan, and subsequently bind lysosomal enzymes such as cathepsin B released within a focus of inflammation. The degraded SAP becomes a component of the amyloid fibrillar deposits that accompany chronic infections – it might even be a key initiator of amyloid deposition. SAP also binds several bacterial species via LPS and, similar to CRP, can also activate the classical complement pathway. CRP and SAP represent the main acute phase reactants in human and mouse, respectively.

Collectins

Nine members of the collectin family have been described in vertebrates to date, the most intensively studied of which is ***mannose-binding lectin (MBL)***. MBL can react not only with mannose but several other sugars, so enabling it to bind with an exceptionally wide variety of Gram-negative and Gram-positive bacteria, yeasts, viruses, and parasites; its subsequent ability to trigger the classical C3 convertase through two novel associated serine proteases (MASP-1 and MASP-2) is the basis of what is known as the ***lectin pathway*** of complement activation. (Please relax, we unravel the secrets of the classical and lectin pathways in the next chapter.)

MBL is a multiple of trimeric complexes, each unit of which contains a collagen-like region joined to a globular lectin-binding domain (Figure 1.37). This structure places it in the family of collectins (***col***lagen + ***lectin***) that have the ability to recognize "foreign" carbohydrate patterns differing from "self" surface polysaccharides, normally terminal galactose and sialic acid groups, whereas the collagen region can bind to and activate phagocytic cells through complementary receptors on their surface. The collectins, especially MBL and the alveolar surfactant molecules SP-A and SP-D mentioned earlier (Figure 1.35), have many attributes that qualify them for a first-line role in innate immunity as soluble PRRs. These include the ability to differentiate self from nonself, to bind to a variety of microbes, to generate secondary effector mechanisms, and to be widely distributed throughout the body including mucosal secretions. They are of course the soluble counterparts to the cell surface C-type lectin PRRs described earlier.

Interest in the collectin conglutinin has intensified with the demonstration, first, that it is found in humans and not just in cows, and second, that it can bind to *N*-acetylglucosamine; being polyvalent, this implies an ability to coat bacteria with C3b by cross-linking the available sugar residue in the complement fragment with the bacterial proteoglycan. Although it is not clear whether conglutinin is a member of the acute phase protein family, we mention it here because it embellishes the

general idea that the evolution of lectin-like molecules that bind to microbial rather than self polysaccharides, and which can then hitch themselves to the complement system or to phagocytic cells, has proved to be such a useful form of protection for the host.

Ficolins

These proteins are structurally and functionally related to collectins (Figure 1.38), and can also recognize carbohydrate-based PAMPs on microorganisms to activate the lectin pathway of complement activation. Ficolins typically recognize *N*-acetylglucosamine residues in complex-type carbohydrates in addition to other ligands. Three ficolins have been identified in humans, ficolin-1, -2, and -3 (also known as M-, L-, and H-ficolin, respectively), and a role as opsonins for the enhancement of phagocytosis has also been demonstrated for these proteins. Ficolins can also interact with CRP to widen the range of bacteria recognized by the latter and also to enhance complement-mediated killing. The range of bacterial structures recognized by ficolins and MBL are complementary and recognize different but overlapping bacterial species.

Interferons inhibit viral replication

Recall from our earlier discussion of pattern recognition receptors (PRRs) that engagement of many of these receptors by PAMPs results in the production of cytokines and chemokines that act to amplify immune responses by binding to cells in the vicinity. An important class of cytokines induced by viral as well as bacterial infection is the type I ***interferons*** (IFNα and IFNβ). These are a family of broad-spectrum antiviral agents present in birds, reptiles, and fish 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, the genes for all of which have been isolated. There are at least 14 different α-interferons (IFNα) produced by leukocytes, while fibroblasts, and probably all cell types, synthesize IFNβ. We will keep a third type (IFNγ), which is not directly induced by viruses, up our sleeves for the moment.

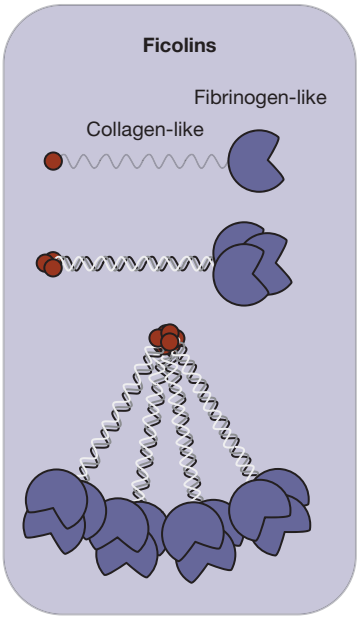


Figure 1.38 Structural features of ficolins. Ficolins are composed of collagen-like and fibrinogen-like domains (top), that are further arranged into trimers (middle), and then multimerize into higher order structures (bottom). Ficolins can bind to carbohydrate-based PAMPs to activate the lectin pathway to complement activation, or can opsonize bacteria for uptake through phagocytosis.

Cells synthesize interferon when infected by a virus and secrete it into the extracellular fluid, where it binds to specific receptors on uninfected neighboring cells. As we saw earlier, engagement of several members of the TLR family, as well as the RIG-like helicase receptors and the cytoplasmic DNA sensors, with their cognate PAMPs results in the induction of members of the interferon-regulated factor (IRF) family of transcription factors (Figure 1.22 and Figure 1.23). In combination with NFkB, another transcription factor activated by engagement of several of the PRRs, IRFs induce expression of type I interferons that are secreted and bind to cells in the vicinity. Long double-stranded RNA molecules, which are produced during the life cycle of most viruses, are particularly good inducers of interferons. The bound interferon now exerts its antiviral effect in the following way. At least two genes are thought to be derepressed in the interferon-binding cell, allowing the synthesis of two new enzymes. The first, a protein kinase called ***protein kinase R*** (PKR), catalyzes the phosphorylation of a ribosomal protein and an initiation factor (eIF-2) necessary for protein synthesis. The net effect of this is to dramatically reduce protein translation as a means of reducing the efficiency of virus production. Another gene product induced by interferons, ***oligoadenylate synthetase***, catalyzes the formation of a short polymer of adenylic acid which activates a latent endoribonuclease; this in turn degrades both viral and host mRNA. This is another clever adaptation that is designed to reduce the production of viral products. Another consequence of the downturn in protein synthesis is a reduction in the expression of major histocompatibility complex (MHC) proteins, making cells susceptible to the effects of ***natural killer*** cells.

The net result is to establish a cordon of uninfectable 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.

Natural killer cells kill virally infected cells

Thus far, we have dealt with situations that deal primarily with infectious agents that reside in the extracellular space. But what if an infectious agent manages to enter cells of the host, where they are protected from the attentions of the soluble PRRs (e.g., complement) and are also shielded from phagocytosis by macrophages and neutrophils? To deal with this situation, another type of immune cell has evolved – the natural killer (NK) cell, which is endowed with the ability to inspect host cells for signs of abnormal patterns of protein expression that may indicate that such cells might be harboring a virus. NK cells are also capable of killing cells that have suffered mutations and are on the way to malignant transformation into tumors. Note that although NK cells constitute a component of the innate response, under certain circumstances they exhibit immunological memory, a feature usually confined to adaptive responses.

Natural killer (NK) cells kill host cells that appear abnormal

NK cells are large granular leukocytes with a characteristic morphology. They choose their victims on the basis of two major criteria. The first, termed "***missing self***," relates to the fact that practically all nucleated cells in the body express molecules on their surface called ***major histocompatibility complex (MHC)*** proteins. The latter molecules have a very important role in activating cells of the adaptive immune system, which we will deal with later in this chapter, but for now, it is sufficient to know that a cell lacking MHC molecules is not a good proposition from the perspective of the immune system. NK cells exist as a countermeasure to such an eventuality and cells lacking the normal pattern of expression of MHC molecules are swiftly recognized and killed by NK cells. As we saw in the previous section dealing with interferons, one way in which the expression of MHC molecules can be reduced is as a consequence of interferon-responsive gene products that can interfere with protein translation within cells infected by viruses, or in the vicinity of such cells.

In addition to reduced or absent MHC expression, NK cells are also capable of inspecting cells for the expression of MHC-related molecules (called nonclassical MHC molecules) and other proteins that are not normally expressed on cells, but become so in response to certain stresses such as DNA damage. This scenario represents "***altered self***" and also results in such cells being singled out for the attentions of NK cells, culminating in swift execution. NK receptors have also been found to be capable of detecting certain viral proteins directly, such as hemagglutinin from the influenza virus, that qualifies such receptors as another class of PRRs. There are additional receptors on the surfaces of NK cells that enable these cells to recognize infected or transformed cells that we will discuss in Chapter 4. Clearly an NK is not a cell to get on the wrong side of.

NK cells kill target cells via two different pathways

Upon recognition of a target cell, through either of the mechanisms mentioned in the preceding section, the NK cell has two main weapons at its disposal, either of which is sufficient to kill a target cell within a matter of 30–60 minutes (see Videoclip 3). In both cases the target cell dies through switching on its own cell death machinery as a result of encounter with the NK cell; thus, NK killing represents a type of assisted cellular suicide. During NK-mediated killing, killer and target are brought into close apposition (Figure 1.39) as a result of detection of either missing self or altered self on the target cell. This can engage either the ***death receptor pathway*** or the ***granule-dependent pathway*** to apoptosis (Figure 1.40). We shall consider these in turn, although the outcomes are very similar.

Death receptor-dependent cell killing

Death receptors are a subset of the TNF receptor superfamily, which includes the receptors for Fas, TNF, and TRAIL, and these molecules derive their name from the observation that ligation of such receptors with the appropriate ligand can result in death of the cell bearing the receptor (Figure 1.40). When this observation was first made, it was a fairly astonishing proposition as it suggested that a cell could be killed through the simple expedient of tickling a membrane receptor in the correct way. Clearly, this is a very different type of killing compared with that seen upon exposure of a cell to a toxic chemical or physical stress that can kill through disruption of normal cellular processes. Here we have a physiological receptor/ligand system that exists for the purpose of killing cells on demand – something it has to be said that the immune system does a lot of. Naturally, this sparked a lot of investigation directed towards understanding how ligation of Fas, TNF, and related receptors culminates in cell death and this is now understood in fine detail as a consequence. Engagement of Fas or TNF receptors with their trimeric ligands results in the recruitment of a protease, called ***caspase-8***, to the receptor complex that becomes activated as a result of receptor-induced aggregation of this protease that now undergoes autoactivation (Figure 1.41). Activation of caspase-8 at the receptor then results in propagation of the signaling cascade in two possible ways, either via proteolysis of Bid, which routes the signal through mitochondria, or by direct processing of other ***effector caspases*** (caspases-3 and -7) downstream. In each case, activation of the effector caspases culminates in death of the cell via apoptosis, which, as we mentioned earlier in this chapter, represents a programmed mode of cell death. NK cells can kill target cells in a Fas ligand-dependent manner, but can also kill through the related TNF ligand to some extent.

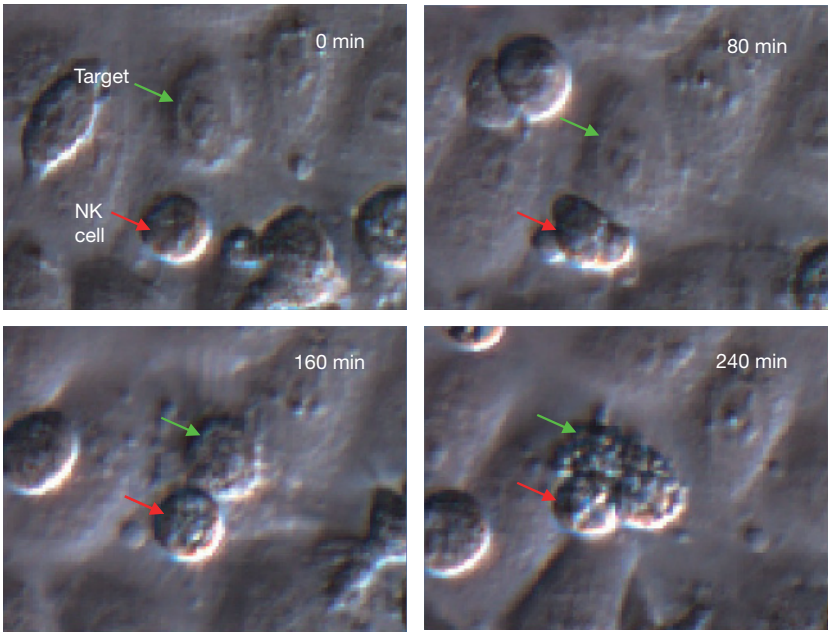


Figure 1.39 Cytotoxic lymphocyte killing. In this time-lapse series, an NK cell (red arrows) is observed to come into close contact with a target cell (green arrows), which is rapidly followed by rounding up and vigorous membrane blebbing within the target cell as it undergoes apoptosis. The interval between each frame is 80 minutes. (Source: Dr. Sean Cullen, Martin Laboratory, Trinity College Dublin, Ireland. Reproduced with permission.)

Granule-dependent cell killing

NK cells also possess cytotoxic granules that contain a battery of serine proteases, called ***granzymes***, as well as a pore-forming protein called ***perforin***. Activation of the NK cell leads to polarization of granules between nucleus and target within minutes, and extracellular release of their contents into the space between the two cells followed by target cell death. Polarization of the granules towards the target cell takes place as a result of the formation of a synapse between the killer and target that is composed of an adhesion molecule called LFA-1 and its cognate receptor ICAM-1.

Perforin bears some structural homology to C9; it is like that protein, but without any help other than from Ca^{2+} it can insert itself into the membrane of the target, apparently by binding to phosphorylcholine through its central amphipathic domain. It then polymerizes to form a transmembrane pore with an annular structure, comparable to the complement membrane attack complex (Figure 1.41). This pore then facilitates entry of the additional cytotoxic granule constituents, the granzymes, which do the actual killing. Perforin-deficient animals are severely compromised in terms of their ability to kill target cells, as the granule-dependent pathway no longer functions in the absence of a mechanism to deliver the granzymes into the target.

Granzymes kill through proteolysis of a variety of proteins within the target cell. Most of the killing potential resides in granzymes A and B, with the function of several additional granzymes (H, K, and M in humans) still unclear. The mode of action of ***granzyme B*** is particularly well understood and it has been found that this protease in essence mimicks the action of caspase-8 in the death receptor pathway to apoptosis, as described above. Thus, upon entry into the target cell, granzyme B can initiate apoptosis by cleaving Bid or through directly processing and activating the downstream effector caspases (Figure 1.41). Both routes result in the activation of the effector caspases that coordinate the dismantling of the cell through restricted proteolysis of hundreds of key cellular proteins.

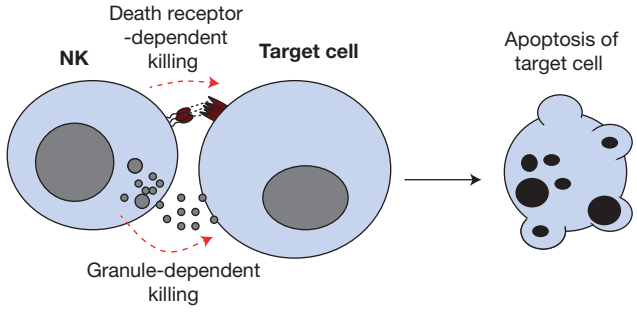


Figure 1.40 Natural killer (NK) cells can kill target cells by two major mechanisms: the death receptor and granule-dependent pathways. In both cases, the target cell dies as a result of the activation of a battery of cytotoxic proteases within the target cell, called caspases. See Figure 1.41 for further details of the molecular mechanisms of killing in either case.

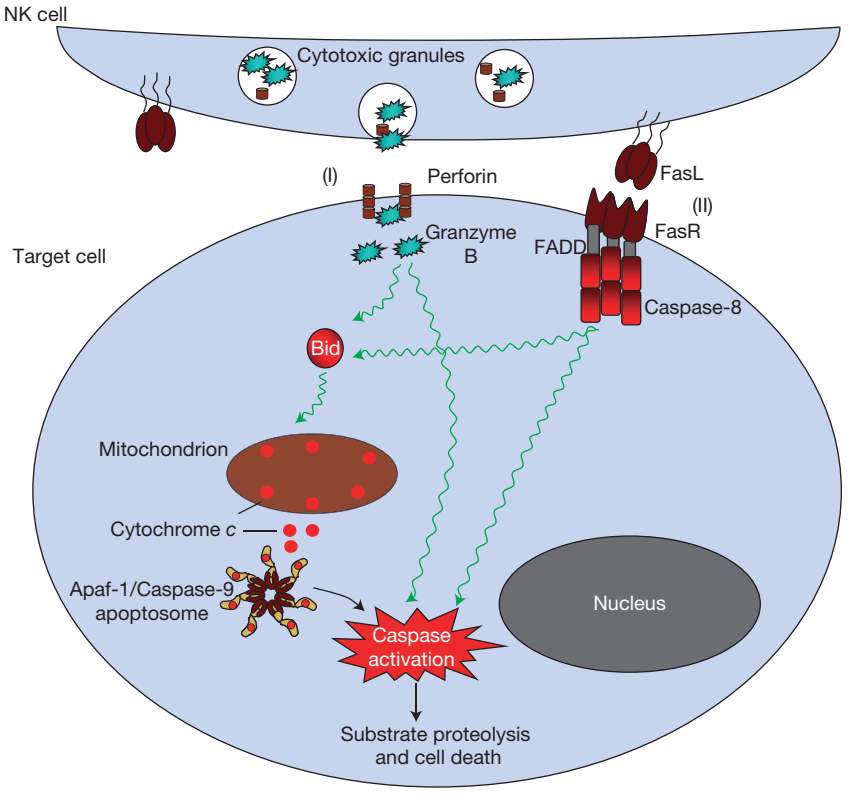


Figure 1.41 Signal transduction events involved in natural killer (NK) cell-mediated apoptosis. NK cells can kill target cells by two major pathways (I) or (II) as shown. In the cytotoxic granule-dependent pathway (I), binding of the NK receptors to the surface of the virally infected cell triggers the extracellular release of perforin (a pore-forming protein) and granzymes (which are a diverse collection of proteases) from the NK cell cytotoxic granules; perforin polymerizes within the target cell membrane to form transmembrane channels that permit entry of granzymes into the target cell. Granzymes induce apoptotic cell death through activation of the caspase protease cascade, either by directly processing and activating caspases, or through release of cytochrome *c* from mitochondria that activates the "apoptosome" pathway to caspase activation. In the second pathway (II) to cell death (called the death receptor pathway), membrane-bound Fas ligand (FasL) on the NK cell engages and trimerizes surface Fas receptors on the target cell. Engagement of Fas receptors recruits the adaptor protein FADD, followed by caspase-8, which then becomes activated at the receptor. Caspase-8 can then promote further caspase activation through directly processing other caspases, or via the mitochondrial apoptosome pathway similar to granzymes. In both pathways, the final common pathway to apoptosis occurs as a result of the activation of several "executioner caspases" that coordinate cell death through restricted proteolysis of hundreds of cellular proteins.

NK cell activity can be enhanced by PAMPs as well as type I interferons

NK cells also express a subset of the TLRs that are focused towards detecting PAMPs, such as double-stranded RNA, that are typically associated with viruses. TLR3, TLR7, and TLR8 all appear to be functional in NK cells and upon engagement of these receptors, NK cells become activated and their killing potential is enhanced. ***Interferon-α and interferon-β*** are also important activators of NK cells, the effects of which can increase the killing activity of such cells by up to 100-fold (Figure 1.42). Recall from our earlier discussion of PRRs, especially those that detect intracellular infections such as the cytoplasmic DNA sensor, STING, and the viral RNA sensors within RIG-I-like receptor family (Figure 1.22 and Figure 1.23), that activation of these PRRs induces the expression of Type I interferons, such as IFN-α and IFN-β. This is an excellent example of cooperation between cells of the innate immune system, where cytokines produced by macrophages or other cells upon detection of a pathogen results in the activation of other cells, NK cells in the present context, that may be better adapted to dealing with the infectious threat.

Activated NK cells can amplify immune responses through production of IFNγ

Another consequence of the activation of NK cells is the production of another type of interferon, IFNγ, a very important cytokine that has a set of activities distinct from that of IFNα and IFNβ. Macrophages respond to IFNγ by greatly enhancing their microbicidal activities and also by producing other cytokines (such as IL-12) that shape the nature of the ensuing

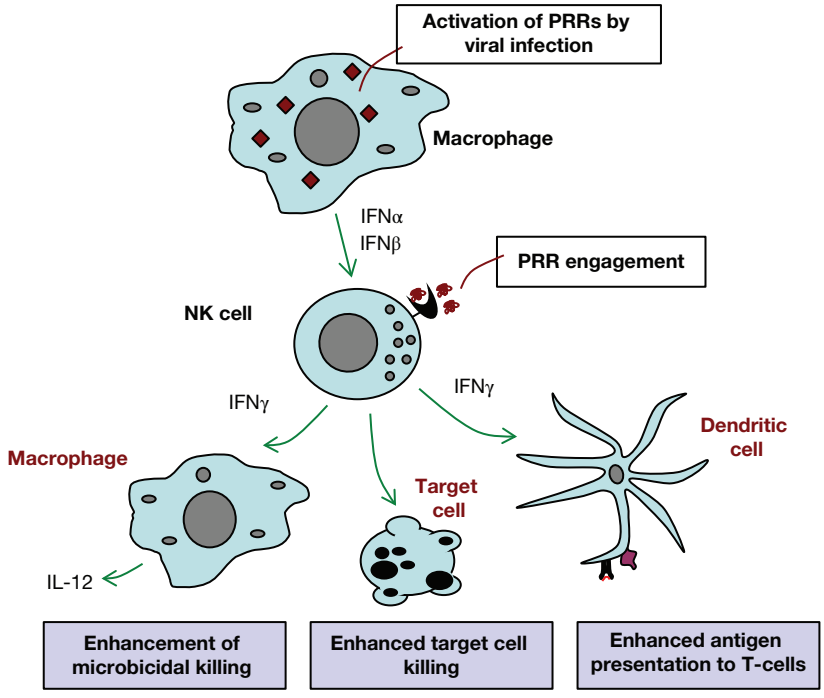


Figure 1.42 Type I interferons, or direct PAMP-mediated stimulation, activates NK cells leading to IFNγ secretion. Activated macrophages can produce type I interferons, as shown, leading to 100-fold enhancement of NK killing activity. NK cells can also be activated through direct stimulation with PAMPs. In turn, activated NK cells are an important source of IFNγ, which greatly enhances killing of intracellular microbes by macrophages and also leads to production of IL-12 by the latter. As we shall see in Chapter 8, IL-12 is an important T-cell polarizing cytokine. Production of IFNγ by NK cells also enhances antigen presentation by dendritic cells.

immune response by T-cells within the adaptive immune system (Figure 1.42). Another effect of IFNγ is to enhance the ***antigen presentation*** function of dendritic cells, which is also important for activation of the adaptive immune system. This cytokine can also influence the type of adaptive immune response that is mounted by helping to polarize T-cells towards a particular response pattern; we shall discuss this issue at length in Chapter 8.

Dealing with large parasites

Because most infectious agents are physically much smaller than the average macrophage or neutrophil, phagocytosis of such agents is a sensible strategy for their removal. But what happens in situations where the invading organism hopelessly dwarfs the phagocytic cells of the immune system? A close cousin of the neutrophil, the eosinophil (Figure 1.9), is important in such cases.

Eosinophils

Large parasites such as helminths cannot physically be phagocytosed and extracellular killing by eosinophils would seem to have evolved to help cope with this situation. These polymorphonuclear "cousins" of the neutrophil have distinctive granules that stain avidly with acid dyes (Figure 1.9) and have a characteristic appearance in the electron microscope (see Figure 11.25). A major basic protein 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 arylsulfatase 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. Not satisfied with that, Nature has also armed the cell with granule proteins capable of producing a transmembrane plug in the target membrane like C9 and the NK perforin. Quite a nasty cell.

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. If this contact should lead to activation, the eosinophil will launch its extracellular attack, which includes the release of the major basic protein and especially the cationic protein which damages the parasite membrane.

The innate immune system instigates adaptive immunity

As we have seen throughout this chapter, any infectious agent that manages to enter the body faces a formidable array of defensive weapons, ranging from macrophage-and neutrophil-mediated phagocytosis, to complement-mediated attack, membrane perforation by defensins, and digestion by extracellular enzymes. As if all of this were not enough, the innate

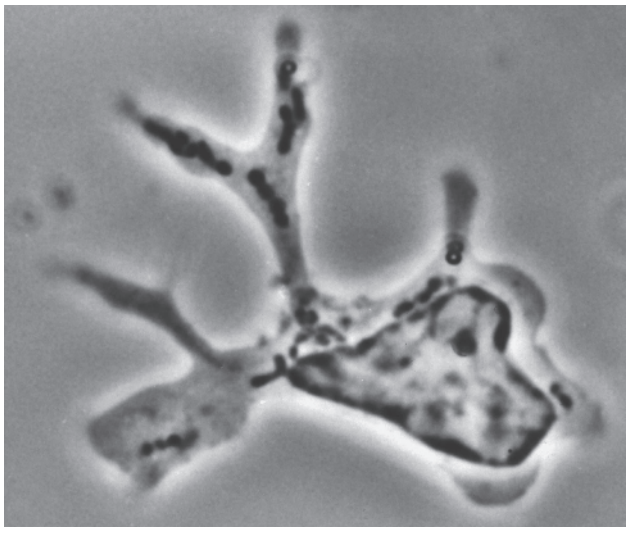
immune system also plays a critical role in initiating an immune response that is uniquely tailored to the ongoing infection. This is achieved by calling upon cells of the adaptive immune system and instructing these cells in the nature of the particular antigens that are giving cause for concern. This function, called ***antigen presentation***, is carried out largely, but not exclusively, by a cell that has relatively recently come to the fore as being of critical importance as a conduit between the innate and adaptive immune systems: the ***dendritic cell*** (DC).

Dendritic cells, which were discovered by Steinman and Cohn in 1973, are produced primarily in the bone marrow and derive their name from the multiple long membrane projections or dendrites that these cells possess (Figure 1.43). These cells share a common progenitor with macrophages, with the result that both macrophages and DCs have somewhat overlapping functions. DCs effectively grant permission for ***T-cells*** of the adaptive immune system to become involved in fighting an infection. They achieve this by providing such cells with ***two signals*** that are essential for a ***naive T-cell*** (i.e., one that has not previously been engaged in an immune response) to become activated and to undergo clonal expansion and differentiation to a fully fledged ***effector T-cell*** (i.e., capable of mounting immune responses). We will look at the role of the T-cell in the immune response in much greater detail in Chapter 8; for now it is sufficient to know that activated T-cells carry out a range of functions that reinforce the efforts of the innate immune system, by providing cytokines to help activate macrophages and attract neutrophils. Some T-cells also have functions very similar to NK cells and can detect and kill virally infected cells, while other T-cells assist in the production of antibodies, the functions of which we will deal with in the next chapter.

Dendritic cells provide a conduit between the innate and adaptive immune systems

Similar to macrophages, DCs migrate to the tissues where they reside in a quiescent state, continuously sampling their environment by phagocytosis and pinocytosis. These cells have been given various names depending on the tissue they are found in; for example the DCs in the skin are called Langerhans cells. DCs are equipped with a battery of TLRs and other PRRs and, similar to macrophages, perform a function as sentinels, waiting and watching for signs of infection or tissue damage (i.e., engagement of any of their PRRs). However, unlike the macrophage, DCs do not stand and fight upon PRR engagement but rather take flight to the nearest lymph node (which acts as a kind of army barracks for lymphocytes) to carry out a special function, called ***antigen presentation***, which awakens cells of the adaptive immune system (Figure 1.44 and Figure 1.45). We will discuss this in much more detail in Chapter 5, but will quickly summarize events now as it is important that the reader is aware of the central role of DCs in adaptive immunity from the outset.

(a)



(b)

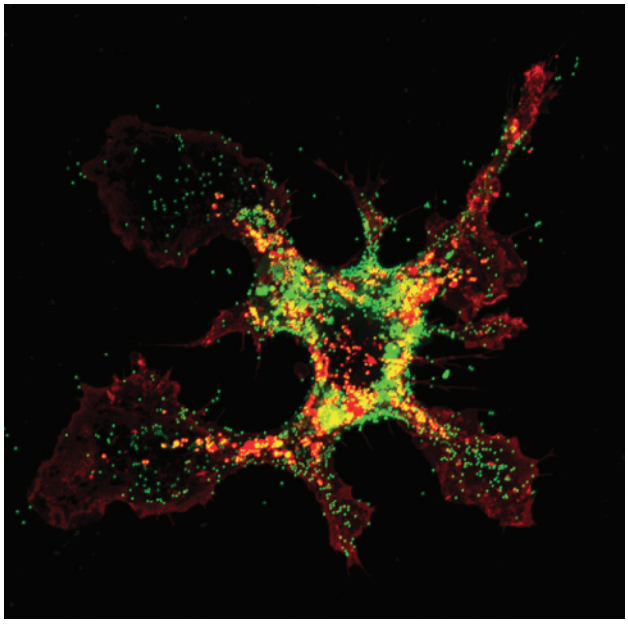


Figure 1.43 Dendritic cell morphology. (a) Phase-contrast image of an unstained dendritic cell with characteristic "dendron tree." (Source: Dr. Ralph Steinman, The Rockefeller University, New York, USA and first published in *Mononuclear Phagocytes in Immunity, Infection, and Pathology* (ed. R. van Furth), Blackwell Scientific (1975), p. 96. Reproduced with permission of Wiley.) (b) Confocal fluorescence microscopy image of a dendritic cell that has phagocytosed green fluorescent microparticles, followed by staining the plasma membrane with Alexa-594-conjugated wheat germ agglutinin (red) to decorate surface carbohydrate. (Source: Dr. Jim Harris and Dr. Ed Lavelle, Trinity College Dublin, Ireland.)

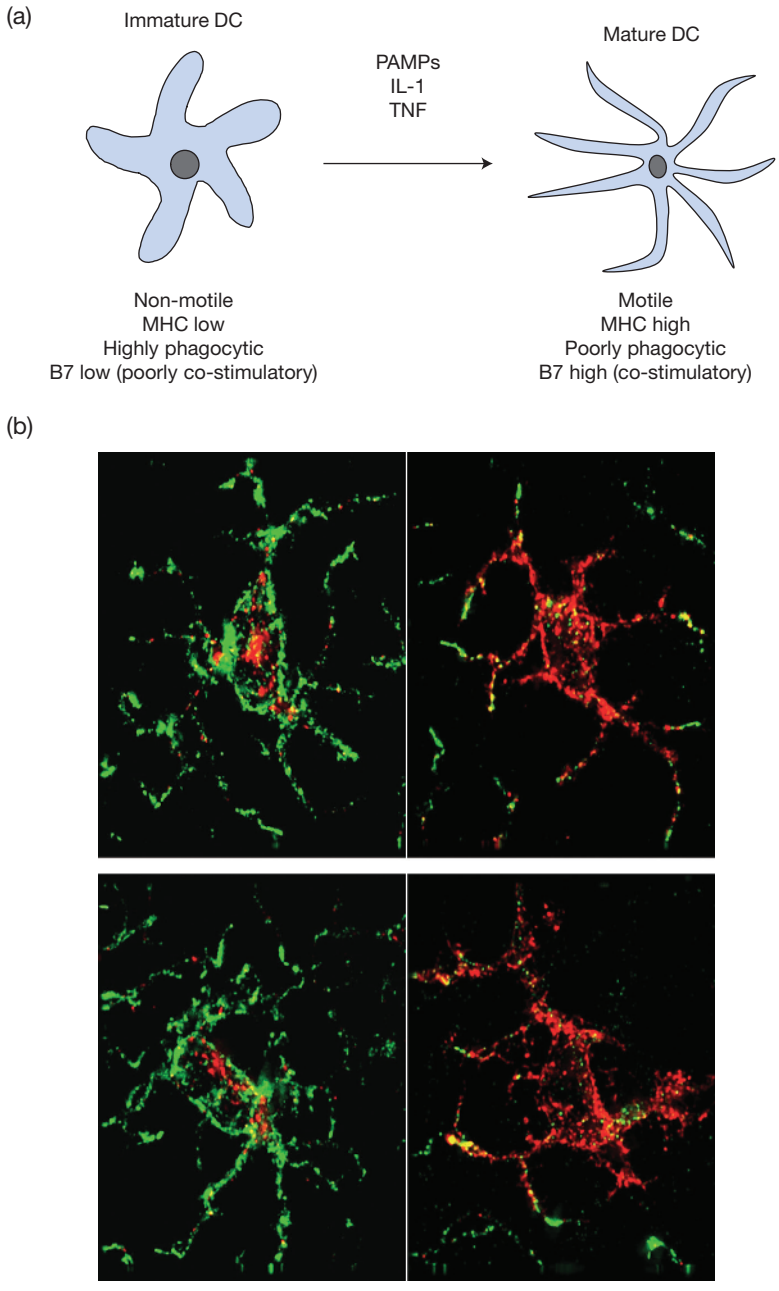


Figure 1.44 Dendritic cell maturation is induced by PAMPs and other signs of infection. (a) Immature dendritic cells (DCs) undergo maturation and become equipped to present antigen and provide co-stimulatory signals upon activation by a pathogen-associated molecular patterns (PAMPs) (or danger-associated molecular pattern (DAMP)), as this leads to a dramatic increase in the expression of surface MHC and B7 molecules on the DC. The expression of B7 family proteins is controlled by NFkB, which is activated downstream of many PRRs. Whereas immature DCs are relatively nonmotile, mature DCs are highly motile and migrate to secondary lymphoid tissues to present antigen to T-cells. (b) Mouse epidermal Langerhans cells (i.e., DCs of the skin) were stained for langerin (green) and MHC class II (red) either before (left) or after maturation (right). Note that before DC maturation MHC class II (red) is present intracellularly, whereas after maturation it is readily detected on the cell surface. (Source: (b) Dr. Ralph Steinman and Dr. Juliana Idoyaga, The Rockefeller University, New York, USA.)

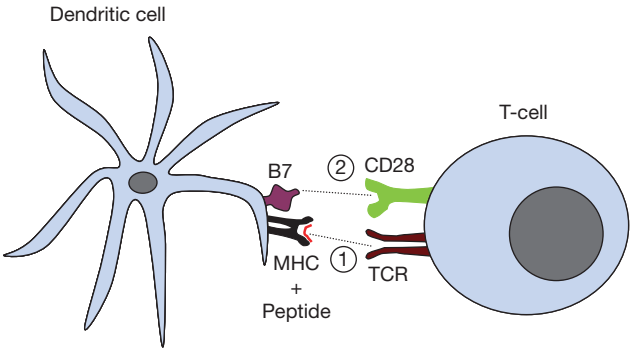


Figure 1.45 Dendritic cells (DCs) present antigen to T-cells of the adaptive immune system. MHC molecules on DCs function as serving platforms for dismembered proteins (i.e., peptides). T-cells can only "see" antigen when presented within the cleft of an MHC molecule; this represents signal 1. In addition to presenting antigen to T-cells in the correct format, DCs also give permission for T-cells to undergo clonal expansion (i.e., proliferation to increase their numbers) by providing co-stimulatory signals in the form of the membrane ligands, B7–1 and B7–2 (also called CD80/CD86), that engage with CD28 on the surface of the T-cell; this represents signal 2.

DCs present antigen to T-cells and provide co-stimulatory signals

Whereas cells of the innate immune system can directly sense nonself molecules using their panoply of PRRs, the T-lymphocytes of the adaptive immune system need to have antigen "presented" to them in a special format. Typically this involves protein antigens becoming internalized and broken down into small peptide fragments by an ***antigen-presenting cell*** (APC), such as a DC. Antigen presentation by the DC is achieved via a membrane complex called the ***major histocompatibility complex*** (MHC), which was originally discovered for its role in graft rejection (hence the unwieldy name). In essence, MHC molecules function as serving platforms for dismembered proteins and T-cells can only "see" antigen when presented within the cleft of an MHC molecule; this represents ***signal 1*** (Figure 1.45). T-cells inspect antigen presented on DCs using their membrane-borne ***T-cell receptors*** (TCRs), which are specialized for the recognition of peptide–MHC complexes. Successful triggering of a TCR results in activation and the acquisition of various immune-related functions by the T-cell (see Chapters 7 and 8). Although DCs are the most efficient APCs for presenting antigen to T-cells, macrophages and B-cells can also perform this important function.

In addition to presenting antigen to T-cells in the correct format, DCs also give permission for T-cells to undergo clonal expansion by providing ***co-stimulatory signals*** in the form of the membrane ligands, B7–1 and B7–2 (also called CD80/CD86), that engage with CD28 on the surface of the T-cell; this represents ***signal 2*** (Figure 1.45).

Co-stimulation (i.e., signal 2) is not some afterthought on the part of the DC, for if it is absent the T-cell refuses to respond in the correct manner and will often kill itself through programmed cell death (apoptosis). Just to be sure that we are perfectly clear here, because this is critical for activation of the adaptive immune system, ***naive T-cells require both signal 1 and 2 from an APC to become successfully activated***.

Engagement of PRRs equips DCs to provide co-stimulation

Because of the requirement for signals 1 and 2 for proper T-cell activation, knowing when to provide co-stimulation is a critical feature of the role of an APC. The astute reader will now be wondering how a DC knows when to provide co-stimulation, as this essentially dictates whether the adaptive immune system will be engaged or not.

Once again, PRRs provide the key to knowing when the immune system should respond or not. DCs only become equipped to provide co-stimulatory signals upon activation by a PAMP (or DAMP), as this leads to a dramatic increase in the expression of surface B7 molecules on the DC; the expression of B7 family proteins are also controlled by NFkB, which is activated downstream of many PRRs. DCs that present antigen acquired in the absence of PAMP-mediated stimulation are overwhelmingly likely to be presenting molecules derived from self and will therefore fail to provide the proper co-stimulatory signals required to activate naive T-cells (Figure 1.45).

The upshot of all of this is that the adaptive immune system is heavily reliant on cells of the innate immune system for the purposes of knowing when to initiate a response and what to respond to.

SUMMARY

The ability to recognize and respond to "nonself" as well as "hidden self" is central to immunity

- Immune responses are initiated through detection of pathogen-associated molecular patterns (PAMPs) representing nonself or danger-associated molecular patterns (DAMPs) that represent hidden self.
- Immune responses need to be proportional to the threat.
- Pattern recognition receptor molecules (PRRs), which can be either soluble (humoral) or cell-associated, are used by the immune system to detect the presence of PAMPs or DAMPs.
- PRR engagement leads to a diversity of responses that are aimed at directly killing or engulfing microorganisms via phagocytosis, and also results in amplification of immune responses through release of a range of messenger molecules such as cytokines and chemokines.
- Interleukins represent an important class of cytokines used by leukocytes to initiate and amplify immune responses.

Immune responses are tailored towards particular types of infection

- There are different classes of pathogen (intracellular versus extracellular bacteria, viruses, yeasts, parasitic worms, unicellular parasites, fungi, etc.) and this dictates that different types of immune responses are necessary.
- PRRs decipher the molecular fingerprint of particular pathogens, thereby shaping the appropriate immune response downstream.
- Cytokines that are produced downstream of PRR engagement help to activate and trigger maturation of the appropriate classes of immune effector cells to deal with a particular type of infection.

Three levels of immune defense operate in vertebrates

- The skin and mucosal surfaces represent physical barriers to infection.
- The innate immune system is composed of a conglomeration of soluble factors and cells that detect and respond to infectious agents through binding to relatively invariant structures (PAMPs) common to many pathogens.
- The adaptive immune system is composed of T- and B-lymphocytes that recognize highly specific structures (antigens) on microorganisms via highly diverse membrane receptors that are generated randomly and are uniquely tailored to individual pathogens.
- Innate immune responses to infection are rapid (minutes) whereas adaptive immune responses are delayed (days). Innate immune responses are broadly similar between individuals within a population and do not improve upon repeated exposure to infectious agents. Adaptive immune responses differ between individuals and improve upon a second or subsequent encounter with the same antigen.
- Innate and adaptive immune responses are interdependent and cooperate to kill infectious agents.

Cells of the immune system

- The innate immune system is composed predominantly of myeloid cells, including macrophages (and their monocyte precursors), mast cells, dendritic cells, and granulocytes (neutrophils, eosinophils, and basophils). Natural killer cells, although technically lymphocytes, are also part of the innate immune system.
- Cells of the innate immune system use conserved ("hard-wired") PAMP receptors (PRRs) that very reliably recognize highly conserved features of common pathogens, as these have been selected over millions of years of evolution.
- Innate immune cells act as sentinels of infection (macrophages, mast cells, dendritic cells) and deal with infection through phagocytosis (macrophages, neutrophils) or can escalate immune responses through the release of soluble mediators (cytokines, chemokines, vasoactive amines) that recruit additional cells to the site of infection.
- The adaptive immune system comprises T- and B-lymphocytes that can generate new receptors for antigen in response to each new pathogen that enters the body. The antigen receptors of T- and B-lymphocytes are highly variable and are therefore prone to recognizing self and must be authenticated before use.

Barriers against infection

- Microorganisms 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 pattern recognition molecules such as lysozyme and complement, as well as by phagocytosis followed by intracellular digestion.

Initiation of an immune response

- Macrophages play an important role in the initiation of immune responses through release of cytokines and chemokines upon detection of PAMPs. One of the effects of these cytokines and chemokines is to activate the local endothelium to permit the ingress of neutrophils and plasma proteins to the site of infection.
- There are several classes of PRR, including: Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors, RIG-I-like receptors (RLRs), and cytoplasmic DNA sensors (CDSs).
- PRR engagement leads to activation of phagocyte functions and to secretion of a range of cytokines and chemokines, many of which are expressed in an NFkB- and IRF-dependent manner.
- Mast cells play an important role in facilitating the vasodilation and vascular permeability that permits the recruitment of immune cells and soluble mediators to the site of infection.
- The classic inflammatory reaction has several cardinal signs (redness, swelling, pain, and heat) that are the consequence of the release of cytokines and vasoactive amines (e.g., histamine) by activated macrophages and mast cells, leading to increased plasma fluid and neutrophils/monocytes at the inflamed site, which contribute to the swelling seen.
- The combination of PRRs that are engaged at the beginning of an immune response help to decode the nature of infection. Engagement of several PRR classes simultaneously may be required for the initiation of robust immune responses.

Phagocytic cells recognize and kill microorganisms

- The main phagocytic cells are polymorphonuclear neutrophils and macrophages.
- The phagocytic cells use their membrane-localized pattern recognition receptors (PRRs) to recognize and adhere to pathogen-associated molecular patterns (PAMPs) on the microbe surface.
- Organisms adhering to the phagocyte surface activate the engulfment process and are taken inside the cell where they fuse with cytoplasmic granules.
- A formidable array of microbicidal mechanisms then comes into play: the conversion of O_2 to reactive oxygen intermediates, the synthesis of nitric oxide, and the release of multiple oxygen-independent factors from the granules.
- Neutrophils (and macrophages) can also deploy neutrophil extracellular traps (NETs), a meshwork of chromatin and granule-derived proteases that can immobilize and kill microbes.

Complement facilitates phagocytosis and lysis of microorganisms

- The complement system, a multicomponent triggered enzyme cascade, is used to attract phagocytic cells to the microbes and engulf them. Complement activation also leads to a membrane attack complex (MAC) that perforates microorganisms.
- In what is known as the alternative complement pathway, 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 microorganism and acts as an opsonin.
- 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 neutrophils and greatly increases capillary permeability.
- C3a and C5a act on mast cells causing the release of further mediators, such as histamine, leukotriene B_4, and tumor necrosis factor (TNF), with effects on capillary permeability and adhesiveness, and neutrophil chemotaxis; they also activate neutrophils.

Humoral mechanisms provide an additional defensive strategy

- A multitude of soluble pattern recognition molecules belonging to several protein families (e.g., pentraxins, collectins, ficolins) serve to detect conserved PAMPs on microorganisms. Mechanisms of action common to these soluble PRRs upon binding their targets include: opsonization, complement activation, enhanced phagocytic uptake, and agglutination.
- In addition to lysozyme, peptide defensins, and the complement system, other humoral defenses involve the acute phase proteins, such as C-reactive and mannose-binding proteins, whose synthesis is greatly augmented by infection. Mannose-binding lectin generates a complement pathway that is distinct from the alternative pathway in its early reactions, as will be discussed in Chapter 2. It is a member of the collectin family that includes conglutinin and surfactants SP-A and SP-D, notable for their ability to distinguish microbial from "self" surface carbohydrate groups by their pattern recognition molecules.
- Recovery from viral infections can be effected by the interferons that block viral replication.

Natural killer cells instruct abnormal or virally infected cells to commit suicide

- NK cells can identify host cells that are expressing abnormal or altered patterns of proteins.
- Upon selection of an appropriate target cell, NK cells can kill by engaging either the death receptor or cytotoxic granule pathway to apoptosis.
- Both the death receptor and granule-dependent pathways to apoptosis involve activation of a group of proteases, called caspases, within the target cell that coordinate the internal dismantling of critical cellular structures, thereby killing the cell.

Dealing with large extracellular parasites

- Large infectious agents that are physically too big to be readily phagocytosed by macrophages and neutrophils are treated to a bombardment with noxious enzymes by eosinophils.
- Extracellular killing by C3b-bound eosinophils may be responsible for the failure of many large parasites to establish a foothold in potential hosts.

The innate immune system instigates adaptive immunity

- Dendritic cells (DCs) provide a conduit between the innate and adaptive immune systems by presenting antigen to T-lymphocytes within lymph nodes.
- Mature DCs present peptide fragments of antigens to T-cells via surface MHC molecules (signal 1) and also provide co-stimulatory signals via B7 family ligands (signal 2). Both signals are required for efficient T-cell activation.
- PAMP-mediated stimulation of DCs triggers their maturation (i.e., the ability to efficiently present antigen and provide co-stimulation) and promotes their migration to lymph nodes.

Now visit **www.roitt.com** to test yourself on this chapter.

FURTHER READING

Banchereau J. and Steinman R.M. (1998) Dendritic cells and the control of immunity. *Nature* **392**, 245–252.

Bottazzi B., Doni A., Garlanda C., and Mantovani A. (2010) An integrated view of humoral innate immunity: pentraxins as a paradigm. *Annual Review of Immunology* **28**, 157–183.

Brinkmann V. and Zychlinsky A. (2012) Neutrophil extracellular traps: is immunity the second function of chromatin? *Journal of Cell Biology* **198**, 773–783.

Cullen S.P. and Martin S.J. (2008) Mechanisms of granule-dependent killing. *Cell Death and Differentiation* **15**, 251–262.

Gay N.J., Symmons M.F., Gangloff M., and Bryant C.E. (2014) Assembly and localization of Toll-like receptor signalling complexes. *Nature Reviews Immunology* **14**, 546–558.

Hornung V., Hartmann R., Ablasser A., and Hopfner K.P. (2014) OAS proteins and cGAS: unifying concepts in sensing and responding to cytosolic nucleic acids. *Nature Reviews Immunology* **14**, 521–528.

Hornung V. and Latz E. (2010) Intracellular DNA recognition. *Nature Reviews Immunology* **10**, 123–130.

Iwasaki A. and Medzhitov R. (2010) Regulation of adaptive immunity by the innate immune system. *Science* **327**, 291–295.

Iwasaki A. and Medzhitov R. (2015) Control of adaptive immunity by the innate immune system. *Nature Immunology* **16**, 343–353.

Janeway C.A. Jr and Medzhitov R. (2002) Innate immune recognition. *Annual Review of Immunology* **20**, 197–216.

Lamkanfi M. and Dixit V.M. (2012) Inflammasomes and their roles in health and disease. *Annual Reviews in Cell and Developmental Biology* **28**, 137–161.

Matzinger P. (1994) Tolerance, danger, and the extended family. *Annual Review of Immunology* **12**, 991–1045.

Matzinger P. (2002) The danger model: a renewed sense of self. *Science* **296**, 301–305.

Medzhitov R. (2008) Origin and physiological roles of inflammation. *Nature* **454**, 428–435.

Sayed B.A., Christy A., Quirion M.R., and Brown M.A. (2008) The master switch: the role of mast cells in autoimmunity and tolerance. *Annual Review of Immunology* **26**, 705–739.

Schenten D. and Medzhitov R. (2011). The control of adaptive immune responses by the innate immune system. *Advances in Immunology* **109**, 87–124.

Steinman R.M. and Idoyaga J. (2010) Features of the dendritic cell lineage. *Immunological Reviews* **234**, 5–17.

Tamura T., Yanai H., Savitsky D., and Taniguchi T. (2008) The IRF family transcription factors in immunity and oncogenesis. *Annual Review of Immunology* **26**, 535–584.

Taylor R.C., Cullen S.P., and Martin S.J. (2008) Apoptosis: controlled demolition at the cellular level. *Nature Reviews Molecular Cell Biology* **9**, 231–241.

Van Gorp H., Kuchmiy A., Van Hauwermeiren F., and Lamkanfi M. (2014) NOD-like receptors interfacing the immune and reproductive systems. *FEBS Journal* **281**, 4568–4582.