Recognition of microorganisms and activation of the immune response

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The mammalian immune system has innate and adaptive components, which cooperate to protect the host against microbial infections. The innate immune system consists of functionally distinct 'modules' that evolved to provide different forms of protection against pathogens. It senses pathogens through pattern-recognition receptors, which trigger the activation of antimicrobial defences and stimulate the adaptive immune response. The adaptive immune system, in turn, activates innate effector mechanisms in an antigen-specific manner. The connections between the various immune components are not fully understood, but recent progress brings us closer to an integrated view of the immune system and its function in host defence.

Infectious diseases are a leading cause of morbidity and mortality worldwide and are a major challenge for the biomedical sciences. Improved sanitary conditions, clean water supplies and vector control are by far the most effective measures to reduce the incidence of infectious disease. However, the development of vaccines and therapeutics is also important, and this requires an understanding of the host immune system. Recently, much progress has been made towards discovering the mechanisms of microbial pathogenesis and host–microbe symbiosis. And knowledge about the immune system has also been steadily increasing. Yet many challenges remain, perhaps the most daunting being effective vaccine development. Indeed, it is not known how to elicit protective immunity against most pathogens in a safe and practical manner. To achieve this and other goals, such as the safe and efficient blockade of autoimmune and allergic immune responses, further developments in basic research are clearly required.

Here, I provide a general overview of the immune system as it relates to defence against microorganisms, with an emphasis on recent findings.

Host–microbe interactions

All metazoan hosts exist in close association with microbial communities that colonize them. The 'rules of engagement' of host–microbe interactions are incompletely understood, and defining these is clearly important for understanding the evolution and functioning of the immune system.

The host as a set of niches colonized by microorganisms

Mammalian hosts provide a number of niches that can be colonized by microorganisms, including the skin, intestine, upper and lower respiratory tract, urogenital tract and internal organs. Some of these niches (for example, the colon and the skin) are colonized constitutively by an endogenous microbiota. Other niches (for example, the internal organs and the lower respiratory tract) are normally kept sterile (in an immunocompetent host). The effect of microbial colonization on host fitness depends on the microbial adaptation strategy. These effects can be positive, as is the case for the many intestinal bacteria that provide a range of benefits to the host (see pages 804 and 811). In other cases, microbial colonization can be detrimental to the host, and these colonizing bacteria are referred to as pathogens. Such negative effects can depend on the status of the host's immune system: for example, certain pathogens, known as opportunistic pathogens, affect only immunocompromised individuals.

Virulence factors

The adaptation of bacteria to particular host niches depends on the activity of various adaptation factors; for pathogens, these are known as virulence factors. Adaptation factors are often encoded on mobile genetic elements (for example, plasmids and genomic islands) that can be transmitted within and between bacterial species[1] (see page 835), although there are important exceptions (for example, in *Mycobacterium* spp.)[2]. The role of virulence factors is to enable adaptation to the specific environments in the host niches and to promote transmission to another host. In this way, some common themes of virulence-factor activity (and therefore pathogenicity) can be identified[3]. Depending on the niche that they colonize, bacterial pathogens have virulence factors that allow a range of activities: penetration of surface epithelia, attachment to cell surfaces and/or the extracellular matrix, invasion of intracellular compartments, acquisition of iron, evasion of host-defence mechanisms and transmission to another host. Different strategies of pathogenic microbial adaptation are associated with varying degrees of damage to the tissues of the host. Regardless of the degree of virulence, at least some symptoms of infectious disease are side-effects of microbial adaptation to host niches.

Recognition of microorganisms by the immune system

The detrimental effects of microbial infections led to the evolution of a variety of host-defence mechanisms. In jawed vertebrates, there are two types of defence: innate and adaptive (also known as acquired). The main distinction between these is the receptor types used to recognize pathogens. Innate immune recognition is mediated by pattern-recognition receptors (PRRs), which are germline encoded, and each receptor has broad specificities for conserved and invariant features of microorganisms[4]. By contrast, adaptive immune recognition is mediated by antigen receptors: the genes encoding these receptors are assembled from gene segments in the germ line, and somatic recombination of these segments enables the generation of a diverse repertoire of receptors with random but narrow specificities[5]. Antigen receptors are clonally distributed on T and B lymphocytes, which allows clonal selection of pathogen-specific receptors and is the basis for immunological memory. (That is, each lymphocyte expresses antigen receptors of a single specificity, so only specific populations of lymphocytes are selected to expand in response to a pathogen.) Therefore, the innate immune system and the adaptive immune system deal with the molecular diversity of pathogens in fundamentally different ways.

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Innate immune system

Innate immune recognition (also known as pattern recognition) is based on the detection of molecular structures that are unique to microorganisms[4]. Pattern recognition is unusual in that each host receptor (PRR) has a broad specificity and can potentially bind to a large number of molecules that have a common structural motif or pattern. The targets of PRRs are sometimes referred to as pathogen-associated molecular patterns (PAMPs), although they are present on both pathogenic and non-pathogenic microorganisms. PAMPs are well suited to innate immune recognition for three main reasons. First, they are invariant among microorganisms of a given class. Second, they are products of pathways that are unique to microorganisms, allowing discrimination between self and non-self molecules. Third, they have essential roles in microbial physiology, limiting the ability of the microorganisms to evade innate immune recognition through adaptive evolution of these molecules. Bacterial PAMPs are often components of the cell wall, such as lipopolysaccharide, peptidoglycan, lipoteichoic acids and cell-wall lipoproteins. An important fungal PAMP is β-glucan, which is a component of fungal cell walls. The detection of these structures by the innate immune system can signal the presence of microorganisms. The recognition of viruses also partly follows this principle. However, because all viral components are synthesized within host cells, the main targets of innate immune recognition in this case are viral nucleic acids. Discrimination between self (host) and viral nucleic acids occurs on the basis of specific chemical modifications and structural features that are unique to viral RNA and DNA, as well as on the cellular compartments where viral (but not host-derived) nucleic acids are normally found (discussed later). Nevertheless, this discrimination is not perfect and can fail under certain conditions, which can result in the development of autoimmune diseases[6].

An important aspect of pattern recognition is that PRRs themselves do not distinguish between pathogenic microorganisms and symbiotic (non-pathogenic) microorganisms, because the ligands of the receptors are not unique to pathogens. Yet, despite humans being colonized by trillions of symbiotic bacteria, homeostasis is somehow maintained under normal conditions. Furthermore, innate immune recognition of symbiotic microorganisms has an important role in maintaining intestinal homeostasis[7]. And dysregulation of these interactions can lead to the development of inflammatory bowel disease and other disorders.

PRRs and their functions

There are several functionally distinct classes of PRR (Table 1). The best characterized class is Toll-like receptors (TLRs). TLRs are transmembrane receptors that recognize viral nucleic acids and several bacterial products, including lipopolysaccharide and lipoteichoic acids (see ref. 8 for a review). The full range of TLR functions in antimicrobial defence has not yet been determined, but TLRs are known to elicit inflammatory and antimicrobial responses after activation by their microbial ligands.

In terms of the inflammatory response, TLRs activate tissue-resident macrophages to produce pro-inflammatory cytokines, including tumour-necrosis factor (TNF), interleukin-1β (IL-1β) and IL-6, which coordinate local and systemic inflammatory responses. TNF and IL-1β, in turn, activate the local endothelium to induce vasodilation and increase the permeability of the blood vessel, allowing serum proteins and leukocytes to be recruited to the site of infection. In addition, an increase in the amount of tissue factor (also known as coagulation factor III) on the endothelium leads to a local coagulation cascade that helps to prevent microbial dissemination through the blood. Furthermore, IL-1β, together with IL-6, activates hepatocytes to produce acute-phase proteins, including collectins and pentraxins. These proteins, in turn, activate complement and opsonize pathogens for phagocytosis by macrophages and neutrophils. In this way, TLRs indirectly elicit an antimicrobial response.

TLRs also directly trigger such a response, by inducing macrophages to produce antimicrobial proteins and peptides. In mouse macrophages, the activation of TLRs results in transcription of the gene encoding inducible nitric-oxide synthase (iNOS; also known as NOS2), which has an important role in antimicrobial defence[9]. Interestingly, iNOS is not produced in response to the activation of TLRs on human macrophages[10]. Instead, human keratinocytes synthesize vitamin D, which is crucial for antimicrobial activity, partly because of vitamin-D-receptor-dependent induction of the gene encoding the antimicrobial peptide LL37 (also known as CAMP)[11]. Sunlight (a source of UVB radiation) is necessary for vitamin D synthesis, so the difference in vitamin D requirement for antimicrobial defence might reflect the nocturnal and diurnal lifestyles of mice and humans, respectively.

Another well-characterized PRR is dectin 1, a transmembrane receptor that binds to β-glucan[12] and is present on dendritic cells and macrophages. Dectin 1 is a member of a large family of C-type lectins, many of which are present on these same cell types but have unknown functions[13]. Dectin 1 contains an atypical immunoreceptor tyrosine-based activation motif (ITAM) that engages the protein tyrosine kinase SYK, thereby activating a signalling pathway that involves CARD9, Bcl-10 and MALT1 (ref. 14). This PRR has an important role in antifungal defence[15,16], being involved in the phagocytosis of fungal pathogens, the induction of an antimicrobial response (such as activation of NADPH oxidase) and the production of cytokines[17].

In addition to transmembrane receptors on the cell surface and in endosomal compartments, there are intracellular (cytosolic) receptors that function in the pattern recognition of bacterial and viral pathogens. These include NLRs and the intracellular sensors of viral nucleic acids RIG-I (retinoic-acid-inducible gene I; also known as DDX58), MDA5 (melanoma differentiation-associated gene 5; also known as IFIH1) and DAI (DNA-dependent activator of interferon-regulatory factors; also known as ZBP1). NLRs are a large family of about 20 intracellular proteins with a common protein-domain organization but diverse functions[18–21]. All NLRs contain a nucleotide-binding oligomerization domain (NOD) followed by a leucine-rich-repeat domain at the carboxy terminus. At the amino terminus, NLRs have one of three domains and are thereby categorized into three subfamilies: a caspase-recruitment domain (CARD), present in proteins in the NOD subfamily; a pyrin domain, in the NALP subfamily; or a BIR domain (baculoviral inhibitor-of-apoptosis-protein repeat-containing domain), in the NAIP subfamily[18–21]. The N-terminal domains engage distinct signalling pathways, which define the functional properties of the family members.

The proteins of the NOD subfamily — NOD1 and NOD2 — are both involved in sensing bacterial peptidoglycans, although they recognize structurally distinct peptidoglycan fragments[18]. The sensing of peptidoglycan by NOD1 or NOD2 triggers the production of pro-inflammatory cytokines and chemokines and the recruitment of neutrophils to the site of infection[19]. In addition, these NOD proteins contribute to the initiation of the adaptive immune response[22,23], and mutations in NOD2 have been implicated in the pathogenesis of Crohn's disease[24]. NOD2 is also crucial for the production of antimicrobial peptides known as defensins by Paneth cells (which are present in the small intestine), and NOD proteins can presumably activate antimicrobial responses in other cell types[23].

The NALP subfamily of NLRs has 14 members, and at least some of these are involved in the induction of the inflammatory response mediated by the IL-1 family of cytokines, which includes IL-1β, IL-18 and IL-33 (ref. 20). These cytokines are synthesized as inactive precursors that need to be cleaved by the pro-inflammatory caspases: that is, caspase 1, caspase 4 and caspase 5 in humans, and caspase 1, caspase 11 and caspase 12 in mice. These caspases are activated in a multisubunit complex called the inflammasome[25]. There are several types of inflammasome, categorized according to their composition and the involvement of a particular NALP or NAIP. The individual inflammasomes are activated in response to a variety of bacterial infections, by mechanisms that have been poorly defined[20]. Why IL-1-family members are activated by such an elaborate mechanism is puzzling. Unlike other pro-inflammatory cytokines, IL-1β production is regulated by two distinct signals: TLR-induced transcription and inflammasome-mediated processing of the precursor protein. It is possible that, in addition to IL-1-family members, the inflammasomes

Table 1 | Modules of the innate immune system

Innate host-defence module	Primary sensors (PRRs)	Prototypical responses
Mucosal epithelia	TLRs and NOD proteins	Production of antimicrobial peptides Production of mucins
Phagocytes	TLRs, dectins and NOD proteins	Production of antimicrobial proteins Production of cytokines: IL-1β, IL-6 and TNF
Acute-phase proteins and complement system	Collectins, pentraxins and ficolins	Lysis or opsonization of pathogens Chemotactic attraction of leukocytes
Inflammasomes	NALPs and NAIPs	Production of IL-1-family members Apoptosis of infected host cells
NK cells	ND	Apoptosis of infected host cells
Type-I-IFN-induced antiviral proteins	RIG-I, MDA5, DAI and TLRs	Induction of an antiviral state Apoptosis of infected host cells
Eosinophils and basophils	ND	Contraction of smooth muscle Production of mucins Peristalsis Production of biogenic amines Production of cytokines: IL-4, IL-5, IL-9, IL-13 and TNF
Mast cells	ND	Contraction of smooth muscle Production of mucins Peristalsis Production of biogenic amines Production of cytokines: IL-4, IL-5, IL-9, IL-13 and TNF

This list of modules, sensors and responses is not comprehensive and has been simplified for clarity. It should be noted that the function of NALPs and NAIPs is not completely understood. In addition, the primary sensors that control expression of NK-cell-receptor ligands, as well as the sensors that activate antihelminthic responses by mast cells, eosinophils and basophils, have not been identified. Certain modules can be co-induced during infection; these modules are functionally linked (for example, phagocytes and the complement system) and co-regulated by the same cytokines. ND, not determined.

process antimicrobial peptides or proteins that have not yet been characterized. NALPs might also contribute to antimicrobial defence by inducing the apoptosis of infected cells[20]. Whether they can also directly induce the expression of antimicrobial genes is unknown.

Intracellular recognition of viral infections is mediated by two types of viral nucleic-acid sensor. Viral RNA in the cytosol is detected by the RNA-helicase-family proteins RIG-I and MDA5 (ref. 26), whereas viral DNA is detected by the recently identified protein DAI[27]. RIG-I and MDA5 recognize different types of viral RNA: single-stranded RNA containing 5′ triphosphate and double-stranded RNA, respectively[28–31]. These structural features are absent from cellular (host) RNAs, which contain either short hairpin structures, in the case of transfer RNAs and ribosomal RNAs, or a 5′-cap structure, in the case of messenger RNA. These structural differences allow discrimination between viral and self RNAs. Activation of RIG-I or MDA5 results in the production of type I interferons (IFNs; IFN-α and IFN-β) and thereby the induction of antiviral immunity[30]. Interestingly, a crucial adaptor involved in RIG-I and MDA5 signalling is associated with the mitochondrial membrane[32], but the reason for this is unclear at present. The details of how viral DNA is recognized in the cytosol, and the signalling pathways induced by the engagement of DAI, are not yet known. It is, however, clear that the RNA-sensing pathway and the DNA-sensing pathway converge on the protein kinase TBK1 (TANK-binding kinase 1) and the transcription factor IFN-regulatory factor 3 (refs 33–35). Type I IFNs are therefore elicited by the engagement of either type of sensor. This results in antiviral immune responses in both cases, through inducing the expression of numerous IFN-inducible genes, the products of which have a broad range of antiviral activities[36].

Adaptive immune system

Adaptive immune recognition is mediated by two types of antigen receptor: T-cell receptors and B-cell receptors. The genes encoding antigen receptors are assembled from variable and constant fragments through recombination-activating gene (RAG)-protein-mediated somatic recombination[5], a process that yields a diverse repertoire of receptors. This diversity is further increased by additional mechanisms, such as non-templated nucleotide addition, gene conversion and (in the case of B cells) somatic hypermutation, generating a highly diverse repertoire of receptors with the potential to recognize almost any antigenic determinant in a specific manner[5].

There are two types of lymphocyte that express antigen receptors: conventional lymphocytes and innate-like lymphocytes. In the case of conventional lymphocytes — that is, conventional T cells (most αβ T cells) and B cells (also known as B2 cells) — antigen receptors are assembled essentially at random. By contrast, for innate-like lymphocytes — that is, B1 cells, marginal-zone B cells, natural-killer T cells and subsets of γδ T cells — the diversity of antigen receptors is restricted and not entirely random. Their specificities are skewed towards a predefined set of ligands[37].

The specificities of the receptors of conventional lymphocytes are not predetermined and neither, therefore, is the site where these cells might encounter their cognate antigen (that is, the antigen specifically recognized by the receptor) or the effector response they need to elicit on activation. So these lymphocytes circulate through the lymph nodes, which drain most of the body's tissues and organs, and the spleen, which filters the blood, until they encounter an antigen that they are specific for. Microbial antigens are taken up by antigen-presenting cells in the peripheral tissues and are delivered to the lymph nodes or spleen through the lymph or blood, respectively, where they are recognized by conventional lymphocytes. Because the specificity of each antigen receptor is not directly linked to the origin of the antigen, conventional lymphocytes need to be able to differentiate into several types of effector cell, depending on the class of pathogen they recognize (discussed later). The differentiation of conventional lymphocytes into a particular effector-cell type and their localization to the site of infection are regulated by the instructions provided by the innate immune system, generally in the form of cytokines and chemokines, respectively.

There are two types of conventional αβ T cell: T-helper (T_H) cells, which are marked by the co-receptor CD4 on the cell surface; and cytotoxic T cells, which express CD8. These cells recognize antigenic peptides bound to major histocompatibility complex (MHC) class II and class I molecules, respectively. Conventional B cells can recognize almost any antigen by binding to a specific three-dimensional molecular determinant (or epitope).

Innate-like lymphocytes differ from conventional lymphocytes in several important ways. Although the antigen receptors of innate-like lymphocytes are assembled in a similar manner to those of conventional lymphocytes, their assembly process is not entirely random. Receptor diversity is biased towards a characteristic set of specificities for each subset of innate-like lymphocytes[37]. Accordingly, the effector functions of these lymphocytes and the sites where they reside are often predetermined. The effector responses of innate-like lymphocytes therefore do not generally require the same types of instruction that are provided by the innate immune system to conventional lymphocytes.

The innate-like B cells known as B1 cells reside in the peritoneal and pleural cavities and produce mainly antibodies of the IgM class with specificities skewed towards some common bacterial polysaccharides and some self antigens[38]. Innate-like T cells recognize non-classical MHC molecules (also known as MHC class Ib molecules), which can present bacteria-specific ligands: for example, bacterial lipids or formylated peptides in the case of the CD1 and H–2M3 families, respectively. In a way, these MHC-like molecules function as PRRs, presenting microbial ligands to specialized T cells[39]. Some non-classical MHC molecules might themselves be ligands for T-cell receptors, without presenting any other molecules. In this case, the expression of these molecules is thought to be inducible by the engagement of PRRs on specific cell types, such as mucosal epithelial cells[40].

Modules of the innate immune system

Unlike the adaptive immune system, the innate immune system is not a single entity. It is a collection of distinct subsystems, or modules, that appeared at different stages of evolution and carry out different functions in host defence. Some of the main modules found in mammals and how these function in innate host defence are described in this section (Table 1).

Mucosal epithelia

All metazoans have mucosal epithelia, one of the most ancient and universal modules of innate immunity. Together with the skin, the mucosal epithelia are the main interface between the host and the microbial world (including both pathogenic and symbiotic microorganisms). Mucosal epithelia have many important functions in protecting the host from pathogen invasion, as well as in establishing a symbiotic relationship with the human microbiota. Accordingly, mucosal epithelial cells and skin keratinocytes have specialized antimicrobial functions: for example, producing antimicrobial peptides, which limit the viability and multiplication of pathogens and symbiotic microorganisms that colonize these sites. The production of these antimicrobial molecules is induced by engagement of TLRs and NOD proteins and, presumably, other PRRs. Epithelial cells at the mucosal surface also produce mucins, which help to prevent the attachment and entry of pathogens.

Phagocytes

The phagocytic uptake of pathogens is crucial for host defence and is carried out by macrophages and neutrophils. These phagocytes are equipped with multiple antimicrobial mechanisms that are activated on initial contact with pathogens. They have a crucial role in defence against both intracellular bacteria and extracellular bacteria, as well as fungal pathogens. Phagocytosis is facilitated by opsonins, which are host products of the acute-phase response and the complement systems (discussed in the next section), through their ability to bind to both the cell walls of microorganisms and the opsonin receptors present on phagocytes.

Acute-phase proteins and complement

A variety of secreted proteins that function in the circulation and tissue fluids — acute-phase proteins and the complement system — constitute another module. Acute-phase proteins are secreted by hepatocytes in response to the pro-inflammatory cytokines IL-1β and IL-6, and the serum concentration of acute-phase proteins increases markedly at the early stages of infection. A key component of this response is the secreted PRRs: collectins, ficolins and pentraxins[41–43]. Their main functions are opsonizing microbial cells for phagocytosis and activating the complement system. Whereas collectins and ficolins initiate the lectin pathway of complement activation, pentraxins activate the classical pathway, which is also induced by antibodies[41–43]. Complement activation itself has several consequences, including the following: opsonization of pathogens, through the covalent attachment of C3 fragments; recruitment of phagocytes to the site of infection, through the release of proteolytic fragments of C4 and C5 that have chemotactic activity; and direct killing of pathogens, through the formation of the membrane-attack complex, which is the terminal component of the complement cascade[44].

Inflammasomes

Inflammasomes are protein complexes that activate pro-inflammatory caspases[25]. The activation of caspase 1, in particular, is required for processing the IL-1 family of cytokines, including IL-1β, IL-18 and IL-33. These complexes might also process proteins other than pro-inflammatory cytokines. Inflammasomes are activated by the NALP and NAIP subfamilies of NLRs (discussed earlier) in response to bacterial infections and some forms of cellular stress. IL-1-family cytokines have diverse functions in inflammation and host defence.

Natural killer cells

Natural killer (NK) cells are specialized in defence against intracellular pathogens, mainly viruses. These cells have two main functions: inducing the apoptosis of infected cells and producing cytokines, particularly IFN-γ. They express two types of receptor, activating and inhibitory, and these receptors recognize their cognate, host-encoded ligands on infected (target) cells[45]. The balance of expression of activating and inhibitory ligands by a target cell is thought to determine whether it is killed or spared by a particular NK cell. The mechanisms that control the production of these ligands are poorly understood but might involve cell-autonomous viral recognition by intracellular sensors of infection or cell-autonomous detection of excessive cellular stress. Recognition of viral infection by the infected cells themselves, through RIG-I or MDA5, and by plasmacytoid dendritic cells, through TLRs, also controls NK-cell activity, by eliciting the production of type I IFNs either directly or indirectly through the expression of IL-15. IL-15 also regulates NK-cell maintenance[46].

Type I IFNs and IFN-induced proteins

Type I IFNs and IFN-induced proteins have a crucial role in defence against viruses. Type I IFNs are produced in response to viral infections, and these proteins trigger the expression of more than 100 genes, the products of which have diverse antiviral activities[47]. Type-I-IFN production can be elicited in two ways: first, by intracellular sensors of infection (as described in the previous section); and, second, by TLR3, TLR7 and TLR9 (which are located intracellularly, on endosomes). The first mode of production is ubiquitous and occurs in virally infected cells. It results in autocrine or paracrine IFN-mediated signalling, which confers an antiviral state on the infected cell and neighbouring cells. By contrast, the second mode of production involves the engagement of TLR7 or TLR9, which results in specialized type-I-IFN-producing cells, known as plasmacytoid dendritic cells, producing systemic levels of IFN-α[48]. In almost all cases, type I IFNs are produced in response to viral or bacterial nucleic acids. The only exception to this seems to be IFN-β production in response to TLR4 ligands, which are not nucleic acids.

Eosinophils, basophils and mast cells

Eosinophils, basophils and their products form a host-defence module involved in protection against multicellular parasites, such as helminths. Mast cells are also a component of this module, although their function is not restricted to protection against parasites[49]. Mast cells reside in mucosal and connective tissues, whereas eosinophils and basophils are recruited to the sites of infection from the circulation. During bacterial infection, mast cells can be activated directly by TLRs[49]. The way in which parasites activate mast cells, basophils and eosinophils is largely unknown. Recently, however, one of the main parasite-associated cell-wall components, chitin, was found to induce eosinophil recruitment[50]. Interestingly, the main defensive strategy that components of this module use against parasites does not seem to target these pathogens directly (although direct effects do occur). Instead, it is the host tissues, particularly the mucosal epithelia, smooth muscles and vasculature, that are the main targets of the immune response. These tissues are affected by the mediators released by mast cells and basophils in a way that limits

the spread of parasites and promotes their expulsion from the host. The function of this module is regulated by several cytokines, including IL-4, IL-5, IL-9 and IL-13 (ref. 51).

Evolution and functional organization of the innate immune system

The various modules of the innate immune system evolved at different stages of phylogeny in response to specific challenges imposed by different classes of pathogen. The appearance of distinct innate host-defence modules during evolution also reflects changes in anatomy and physiology as animals evolved. In addition, a given module is not the same in different animals and might have been expanded or contracted during evolution in response to specific needs. The composition of the innate host-defence modules in any given animal species is therefore one of many possible configurations, presumably the optimal one for affording maximum protection in the specific physiological context. Thus, the innate immune system, although ancient in origin, is not equivalent in different animal phyla or classes or even between different species of the same class. For example, the number, expression and regulation of the antimicrobial molecules known as defensins varies between mammalian species.

More marked changes in the structure of the innate immune system can be seen at different stages of phylogeny. NK cells, type I IFNs, eosinophils and basophils are all unique to vertebrates. Moreover, the elimination of virally infected cells by NK cells is a viable defensive strategy only in complex metazoans, which have renewable tissues, and not in invertebrates, which consist of post-mitotic cells, most of which do not self-renew. (The extreme tissue and organ autonomy and regenerative capacity in plants might similarly explain why immune-response-associated cell death is one of the main host-defence strategies in plant immunity.) In addition, mast-cell-mediated and basophil-mediated defence against multicellular parasites is based, in part, on the effects of these cells on the vasculature, which is absent in invertebrates with an open circulatory system. Also, most arthropods are not suitable hosts for helminths because they are not large enough to accommodate them. Accordingly, the components of innate immunity against parasites are absent from most or all invertebrate phyla.

Another important aspect of the organization of the innate immune system is that modules that are co-induced by an infection tend to develop functional links and are usually co-regulated by the same inducible signals, most commonly cytokines. For example, phagocytes, the complement system and acute-phase proteins are functionally linked through opsonization-dependent phagocytosis, and these two modules are co-induced during many bacterial infections. A more specific example is that TLR-activated macrophages produce IL-6, which induces hepatocytes to secrete opsonins during the acute-phase response. Similarly, NK cells and type I IFNs are co-induced by viral infections and are functionally coupled. Not all modules of the innate immune system are co-induced by a given infection, however. The modules that are not functionally coupled (for example, NK cells and basophils) are triggered by distinct pathways and are not co-regulated by the same cytokines.

The modules of innate host defence are activated by primary sensors of infection, in most cases by PRRs (as discussed earlier). TLRs can activate multiple modules (mucosal epithelium, phagocytes, acute-phase proteins and type I IFNs), whereas other PRRs seem to be more specialized.

Innate control of adaptive immune responses

In addition to direct activation of innate host-defence mechanisms, some PRRs are coupled to the induction of adaptive immune responses. As discussed earlier, conventional lymphocytes (most αβ T cells and B2 cells) express antigen receptors with random specificities and therefore recognize antigens that lack any intrinsic characteristics indicative of their origin. Therefore, conventional lymphocytes require instructions indicating the origin of the antigen they recognize. These instructions come from the innate immune system in the form of specialized signals inducible by PRRs[4], which can sense infection because of their specificity for products of microbial origin. Therefore, the basic principle of innate control of adaptive immunity is based on establishing an association between the antigens recognized by lymphocytes and the microbial products (that is, PAMPs) recognized by PRRs.

For T cells, this association is interpreted by dendritic cells. Dendritic cells reside in most peripheral tissues, where they monitor the tissue

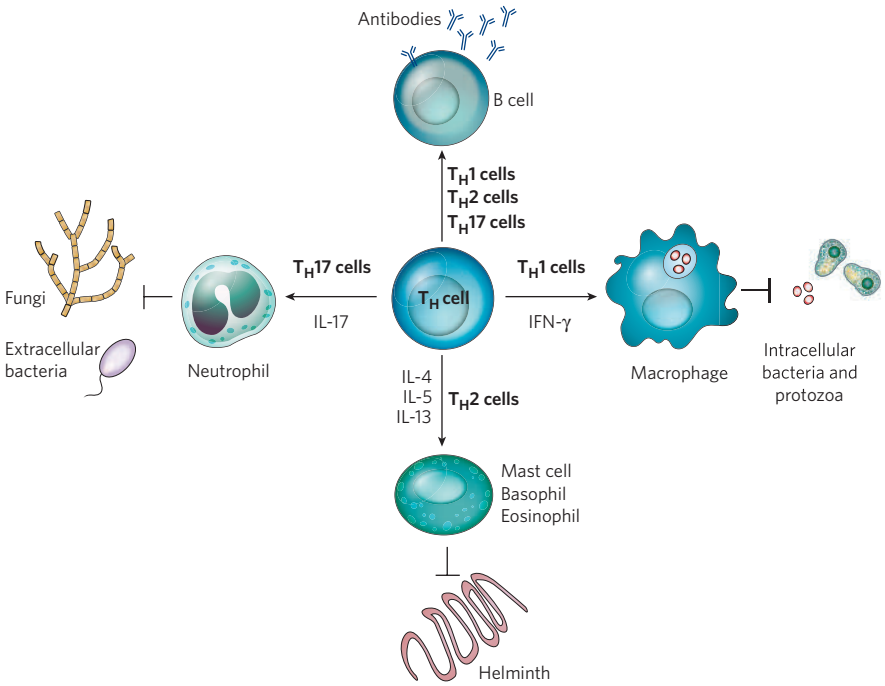

Figure 1 | Effector T_H-cell lineage and pathogen class. When circulating 'naive' T_H cells first recognize their cognate antigen, they differentiate into one of several effector-cell lineages (listed in bold), depending on the infecting pathogen. T_H1, T_H2 and T_H17 cells are the known types of effector T_H cell; however, other types of effector T_H cell probably exist. Each T_H-cell lineage is characterized by the cytokines that are produced and by the innate immune effector mechanism that is activated (denoted by arrows). It is possible (but has not been proved) that every module of the innate immune system is controlled by a dedicated effector T_H-cell lineage.

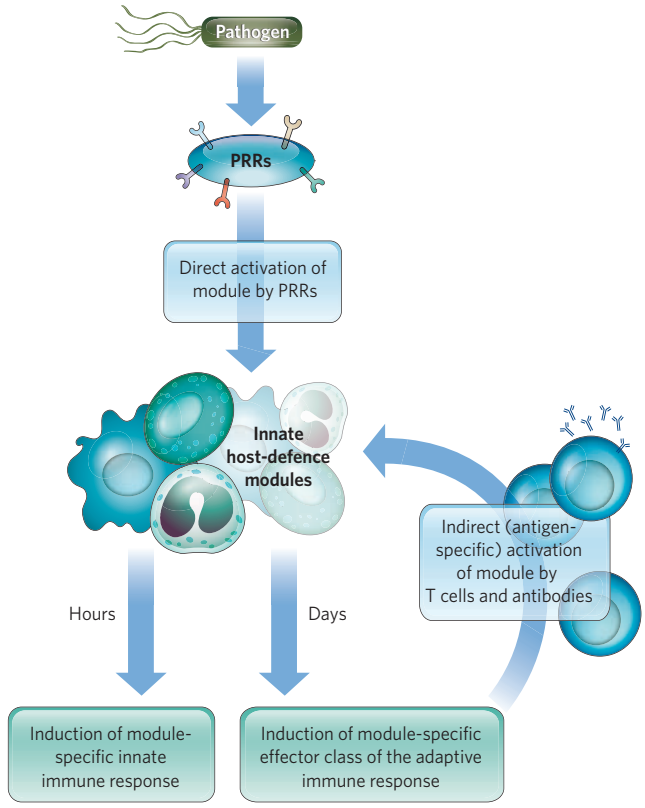

Figure 2 | Activation of host-defence mechanisms. Host-defence mechanisms can be induced directly, by engagement of PRRs, or indirectly, by T cells and/or antibodies. Each module is characterized by distinct antimicrobial defence mechanisms and can instruct the adaptive immune system to mount a response involving a module-specific effector class. After an adaptive immune response has been initiated, it results in antigen-specific activation of the same innate immune module that instructed the adaptive immune response. For example, macrophages can be activated either directly by TLRs or indirectly by T_H1 cells, through IFN-γ, CD40 ligand and other signals. Eosinophils can be activated either directly by an unidentified PRR or indirectly by T_H2 cells. And the classical pathway of complement activation can be induced either directly by pentraxins or indirectly by antibodies. Antigen-specific activation of the innate host-defence modules is more efficient than direct activation and is often required for pathogen clearance.

environment for the presence of pathogens by using various PRRs. When a pathogen is encountered by a dendritic cell, it is taken up by phagocytosis, and its protein constituents are processed into antigenic peptides, which are presented at the cell surface by MHC class I and/or class II molecules. For MHC class II molecules, the antigenic peptides selected for presentation derive from the phagosome in which the pathogen was internalized in response to the triggering of TLRs or other PRRs[52]. A similar mechanism might also operate for MHC class I molecules. Therefore, the association between an antigen and a PAMP is established as a result of their presence in the same phagocytosed 'cargo' (for example, a bacterial cell). PRRs also activate dendritic cells, inducing them to produce cytokines and express cell-surface signals and to migrate to the lymph nodes through the lymphatic vessels that drain the site of infection. When these dendritic cells reach the lymph nodes, they present the pathogen-derived antigens, together with PRR-induced signals (cytokines and cell-surface-associated molecules), to T cells. This results in T-cell activation and, in the case of T_H ($CD4^+$) cells, differentiation into one of several types of effector T_H cell[53].

For B cells, the association between an antigen and a PAMP can be established directly, when the two are physically linked in a single molecule or particle. This presumably occurs through co-engagement of a B-cell receptor and a PRR. In the extreme case, a TLR ligand (for example, lipopolysaccharide or flagellin) is itself recognized by the B-cell receptor and by a corresponding TLR expressed by a B cell. Antigens of this class, which combine ligands for both innate and adaptive immune recognition, are called T-independent antigens, because they can elicit B-cell responses without 'help' from T_H cells. When an antigen and a PAMP are not physically linked, their association is established through effector T_H cells that have previously been activated by dendritic cells. Antigens of this class (usually proteins) are called T-dependent antigens.

The antigen receptors of innate-like lymphocytes are skewed towards the recognition of microbial products, so the activation of these cells does not require the same elaborate mechanisms as for conventional lymphocytes. Indeed, B1 cells can be activated directly by PRRs and are programmed to produce antibodies with a broad specificity for common bacterial antigens[38]. Innate-like T cells recognize microbial antigens (such as lipids, glycolipids and formylpeptides) presented by non-classical MHC molecules. In certain cases, these cells recognize MHC-like molecules that do not seem to present any antigens but whose expression is inducible by PRRs. In such cases, the production of T-cell-receptor ligands in response to microbial products might be sufficient to signal the presence of infection.

T_H cells can differentiate into several types of effector cell: T_H1, T_H2 and T_H17 cells[54] (Fig. 1). These cells are characterized by the production of distinct sets of cytokines[55,56]. T_H1 cells produce IFN-γ and activate macrophages and other cell types to trigger defence against intracellular pathogens. T_H1-cell-derived IFN-γ also instructs B cells to produce antibodies of the IgG2 subclass. T_H2 cells are involved in protection against multicellular parasites and produce IL-4, IL-5 and IL-13 (ref. 51). These cytokines control the function of eosinophils, basophils and the mucosal epithelia. IL-4 also instructs B cells to produce antibodies of the IgE class, which are important in defence against parasites through their effects on mast-cell and basophil activation[57]. Finally, T_H17 cells produce IL-17, which induces non-haematopoietic cell types, including epithelial cells, to produce chemokines that recruit neutrophils to the site of infection[58]. T_H17-cell responses are involved in protection against extracellular bacteria and fungi[14]. The differentiation of naive T_H cells (which have not previously encountered their cognate antigen) into the three effector-cell lineages, T_H1, T_H2 and T_H17 cells, is controlled by transcriptional master regulators, in this case T-bet, GATA-binding protein 3 (GATA3) and retinoic-acid-receptor-related orphan receptor-γt (RORγt), respectively[59]. The expression of these master regulators is controlled by cytokines produced by antigen-presenting cells (such as dendritic cells) in response to PRR activation.

The effector response in each case is thus dictated by the innate immune system. In terms of T_H cells, TLR engagement induces IL-12 production, which directs T_H cells to differentiate into T_H1 cells. By contrast, TLR-induced IL-6, together with transforming growth factor-β (from an unknown cellular source), induces differentiation into T_H17 cells[58,59]. And dectin-1 engagement results in the production of IL-23, which is required for T_H17-cell function and/or maintenance[17,60]. The mechanisms of T_H2-cell generation are unknown but presumably follow a similar principle, with the dedicated cytokines likely to be IL-4 and thymic stromal lymphopoietin (TSLP), produced in response to engagement of an unidentified sensor after helminth infection[61]. For other cell types, type I IFNs (which are produced in response to TLR engagement or RIG-I, MDA5 or DAI engagement during viral infections) regulate the function of cytotoxic T cells and NK cells, either directly or indirectly by inducing IL-15 production[62].

Importantly, the adaptive immune response ultimately results in an antigen-specific activation of the effector mechanisms of the innate immune system. Thus, the effector T_H cells produce the appropriate effector cytokines that activate a specific module of the innate immune system (Fig. 2), including activation of macrophages by T_H1 cells, activation of neutrophils by T_H17 cells and activation of eosinophils, mast cells and basophils by T_H2 cells[54,63]. Similarly to NK cells, cytotoxic T cells induce apoptosis of infected cells, except that the T-cell response is antigen specific. Likewise, antibodies activate the modules of the innate immune system in a class-dependent (and antigen-dependent)

manner. IgG activates complement and opsonizes pathogens to aid their phagocytosis by macrophages and neutrophils, whereas IgE activates mast cells and basophils. Each of the innate effector responses can therefore be activated either directly, by the appropriate PRRs at the early stages of infection, or indirectly, by T cells and antibodies (in an antigen-specific manner) at the later, effector, stages of the immune response (Fig. 2). Furthermore, each effector mechanism of the adaptive immune system might have evolved to activate the appropriate host-defence module of the innate immune system.

The relative contributions of the innate immune system and the adaptive immune system during bacterial infections have been investigated extensively. One important principle that has emerged from these studies is that, although innate host defence is crucial for controlling an infection, it is often insufficient for pathogen clearance[64]. For example, to clear a *Listeria monocytogenes* infection requires functional T-cell responses[64]. It therefore seems that the innate immune system in vertebrates evolved to depend, to some extent, on antigen-specific (adaptive) immunity. This might explain why the mammalian innate immune system, unlike that of arthropods, is not self-sufficient at affording protection against many infections. It should be noted, however, that our understanding of host defence might be biased because almost all studies are based on symptomatic infections. Asymptomatic infections are presumably common, and many of these infections might be cleared efficiently by innate host-defence mechanisms.

Conclusions and perspectives

The adaptation of microorganisms to host niches can benefit the host, as is the case with many symbiotic microorganisms. In some cases, however, adaptation negatively affects tissue physiology and might directly damage tissues, resulting in symptomatic infectious disease. The symptoms of an infectious disease can also be caused by excessive immune and inflammatory responses, and these can often be more damaging to the host than the virulence activity of the pathogen that elicited them. Thus, it is just as crucial for the host to limit the immunopathology as it is to protect against the infecting pathogen. The trade-off between immunopathology and protection against infection has presumably resulted in an optimal balance of the sensitivity and intensity of the immune response. This balance is probably not hard-wired, because the immune response needs to vary in intensity and duration depending on the infecting pathogen. One possible solution to the conundrum of how the balance is achieved would be that the tissue damage caused directly by the pathogen can be distinguished from the damage inflicted by the immune response. If this is the case and the two types of tissue damage are differentially detected by the host, then the extent of the damage might negatively control the intensity and duration of the immune response. Understanding the balance between the two conflicting causes of infectious-disease symptoms is crucial for the development of appropriate therapeutic strategies[65].

Another area of great importance is understanding the principles of protective immunity. What matters to the host organism is not the induction of an immune response but whether the immune response protects against a given infection. Not all immune responses are protective. Only immune responses of the correct effector class directed at particular antigens can provide protection against infection. It is generally assumed that the effector response depends on the pathogen type. However, in most cases, the crucial pathogen features that determine the effector immune response are unclear. Similarly, it is not known how the relevant features of pathogens are translated into the appropriate set of signals that determine the effector response. Understanding these principles is vital for the development of vaccines that can elicit protective immunity. ■

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