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Innate immune responses to infection

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The human host survives many infectious challenges in the absence of preexisting specific (adaptive) immunity because of the existence of a separate set of protective mechanisms that do not depend on specific antigenic recognition. These antigen-independent mechanisms constitute innate immunity.

Antimicrobial peptides are released at epithelial surfaces and disrupt the membranes of many microbial pathogens. Toll-like receptors on epithelial cells and leukocytes recognize a range of microbial molecular patterns and generate intracellular signals for activation of a range of host responses. Cytokines released from leukocytes and other cells exhibit a vast array of regulatory functions in both adaptive and innate immunity. Chemokines released from infected tissues recruit diverse populations of leukocytes that express distinct chemokine receptors. Natural killer cells recognize and bind virus-infected host cells and tumor cells and induce their apoptosis.

Complement, through the alternative and mannose-binding lectin pathways, mediates antibody-independent opsonization, phagocyte recruitment, and microbial lysis. Phagocytes migrate from the microcirculation into infected tissue and ingest and kill invading microbes. These innate immune mechanisms and their interactions in defense against infection provide the host with the time needed to mobilize the more slowly developing mechanisms of adaptive immunity, which might protect against subsequent challenges. (*J Allergy Clin Immunol* 2005;116:241-9.)

Key words: Innate immunity, antimicrobial peptides, Toll-like receptors, chemokines, natural killer cells, complement, phagocytes

It is traditional to organize host responses to infection into separate arms or compartments, such as complement, phagocytes, cytokines, cell-mediated immunity, and humoral immunity. A more current approach has been to consider 2 larger categories: *innate immunity*, incorporat-

Abbreviations used

CXCL:	CXC ligand
HBD:	Human β-defensin
ICAM-1:	Intercellular adhesion molecule 1
LFA-1:	Lymphocyte function-associated antigen 1
MAC:	Membrane attack complex
Mac-1:	Macrophage antigen-1
MBL:	Mannan-binding lectin
NADPH:	Reduced nicotinamide adenine dinucleotide phosphate
NF:	Nuclear factor
NK:	Natural killer
PMN:	Polymorphonuclear leukocyte
TLR:	Toll-like receptor

ing the more rapid and phylogenetically primitive non-specific responses to infection, such as surface defenses, cytokine elaboration, complement activation, and phagocytic responses,¹ and *adaptive immunity*, involving more slowly developing, long-lived, and highly evolved antigen-specific protective responses, such as antibody production and cell-mediated immunity, that exhibit extraordinarily diverse ranges of specificity.^{2,3} However, the components of innate and adaptive immunity engage in a range of interactions that is remarkably diverse and complex. This review attempts to provide an overview of the main innate responses to infection that are available to the human host, including relevant examples of such interactions.

INNATE IMMUNITY

Epithelia, defensins, and other antimicrobial peptides

The epithelium of skin and mucosal tissue functions as a mechanical barrier to the invasion of microbial pathogens. In the last 2 decades, it has become clear that epithelial cells also are a major source of antimicrobial peptides that play important roles in local host defense.^{4,5} Studies of their structure, sources, expression, and actions also have revealed an unexpected range of immunologic activities for these molecules, the functions of which once were considered mainly antimicrobial in nature.⁴

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Epithelial cells of mucous membranes of the airways and intestines, as well as keratinocytes, express the human β -defensins (HBD-1 through HBD-4). These small cationic peptides are similar to the α -defensins stored in the azurophilic granules of neutrophils, and they display antimicrobial activity against a broad range of bacteria, fungi, chlamydiae, and enveloped viruses.^{4,5} Their production by epithelial cells might be constitutive, as for HBD-1, or inducible, as for HBD-2, HBD-3, and HBD-4. For example, recent evidence indicates that epithelial cells of the airway or intestine can produce HBD-2 in response to activation by bacterial products through toll-like receptors (TLRs) 2 or 4 (see below) on the epithelial cells.^{6,7} Stimulation of epithelium by cytokines, including IL-1 or TNF- α , also can induce defensin production.⁴ Defensins have been reported to exert their antimicrobial action either through the creation of membrane pores or through membrane disruption resulting from electrostatic interaction with the polar head groups of membrane lipids, with more evidence now favoring the latter mechanism.^{4,8} Some microorganisms have evolved mechanisms for evading the action of defensins. For example, bacterial polysaccharide capsules might limit access of microbial peptides to the cell membrane,⁹ and an exoprotein of *Staphylococcus aureus*, staphylokinase, neutralizes the microbicidal action of neutrophil α -defensins.¹⁰

Several immunoregulatory properties of defensins and related peptides, distinct from their antimicrobial actions, have been documented.⁴ Several such peptides have been shown to facilitate posttranslational processing of IL-1 β .¹¹ Some of the β -defensins have been shown to function as chemoattractants for neutrophils, memory T cells, and immature dendritic cells by binding to the chemokine receptor CCR-6.^{5,12,13} Separately, HBD-2 has been shown to activate immature dendritic cells through a mechanism that requires TLR4.¹⁴ The activation of immature dendritic cells by these mechanisms also promotes their maturation. The β -defensins also act as a chemoattractant for mast cells through an undefined mechanism and can induce mast cell degranulation.¹⁵ HBD-2 and several other antimicrobial peptides can interfere with binding between bacterial LPS and LPS-binding protein.¹⁶

Additional antimicrobial peptides of epithelial cells include lysozyme and cathelicidin. Lysozyme, an antimicrobial peptide also found in neutrophil granules, attacks the peptidoglycan cell walls of bacteria and can be released from cells through mechanisms that involve TLR activation.¹⁷ Cathelicidin, or LL37, like lysozyme, is released from both neutrophils and epithelial cells. It exhibits broad antimicrobial activity and can inhibit lentiviral replication.^{5,18} Cathelicidin also exhibits chemotactic activity for neutrophils, monocytes, and T lymphocytes. This activity is mediated by a formyl peptide receptor-like molecule, FPR1, rather than the chemokine receptor CCR6 bound by β -defensins.¹⁹

The release of defensins in response to activation of TLRs and the many actions of these peptides, including their direct antimicrobial activities, their chemoattractant actions for a wide range of immune cells, and their

activation of dendritic cell maturation, already suggest a highly complex and regulatory role in the development of host defense and immunity. Recent genomic evidence for the possible existence of as many as 25 additional human defensins that have not yet been characterized suggests that current knowledge describes but a small sample of the overall contribution of these peptides to immune responses.²⁰

TLRs

Mononuclear phagocytes, including circulating monocytes and tissue macrophages, other phagocytic cells, and many epithelial cells, express a family of receptors that is highly homologous to the *Drosophila* receptor called Toll.^{6,7,21} These receptors mediate a phylogenetically primitive, nonclonal mechanism of pathogen recognition based on binding not to specific antigens but to structurally conserved pathogen-associated molecular patterns.²¹⁻²³ There are at least 10 human TLRs with a range of microbial ligands, such as gram-negative bacterial LPS, bacterial lipoproteins, lipoteichoic acids of gram-positive bacteria, bacterial cell-wall peptidoglycans, cell-wall components of yeast and mycobacteria, unmethylated CpG dinucleotide motifs in bacterial DNA, and viral RNA.²²⁻²⁴ Gram-positive cell-wall components bind mainly to TLR2, and TLR2 also can bind components of herpes simplex virus.²²⁻²⁵ Gram-negative LPS activates TLR4 indirectly by first binding to LPS-binding protein, which binds in turn to CD14 at the cell surface. The bound CD14 has no transmembrane domain but associates directly with an extracellular domain of TLR4.^{23,24} TLR5 has been identified as the receptor for bacterial flagellin, TLR9 recognizes CpG motifs of bacterial DNA, and TLR3 has been shown to bind synthetic and viral double-stranded RNA.²⁶⁻²⁸

Signalling through TLRs occurs through a well-described pathway in which receptor binding generates a signal through an adaptor molecule, MyD88, that leads to intracellular association with IL-1 receptor-associated kinase. In turn, this leads to activation of TNF receptor-associated factor 6, which results in nuclear translocation of nuclear factor κ B (NF- κ B).^{23,24} NF- κ B is an important transcription factor that activates the promoters of the genes for a broad range of cytokines and other proinflammatory products, such as TNF- α , IL-1, IL-6, and IL-8. This signalling pathway, on the basis of studies with TLR4, is similar but not identical to the signalling pathways activated by other TLRs.²⁴ The activation of cytokine production by TLRs plays an important role in recruiting other components of innate host defense against bacterial pathogens. However, with large-scale cytokine release, the deleterious effects of sepsis or other forms of the systemic inflammatory response syndrome demonstrate that these pathways have both beneficial and potentially harmful effects for the host.²⁴ Genetic polymorphisms in TLRs might play a role in determining the balance of these effects in certain individuals responding to the challenge of systemic infection.^{24,29,30}

In addition to their first-responder roles in generating an inflammatory response to invading pathogens, TLRs can network with other components of innate and adaptive immunity. TLR4 function is suppressed by activation of cells through the chemokine receptor CXCR4.³¹ Activation of some TLRs also can induce expression of the costimulatory molecule B7 on antigen-presenting cells, which is required for activation of naive T cells.²¹

Cytokines

A heterogeneous group of soluble small polypeptide or glycoprotein mediators, often collectively called cytokines, form part of a complex network that helps regulate the immune and inflammatory responses. Included in this group of mediators, the molecular weights of which range from about 8 to about 45 kd, are the ILs, IFNs, growth factors, and chemokines (see separately below). Most cells of the immune system, as well as many other host cell types, release cytokines, respond to cytokines through specific cytokine receptors, or both. The range of sources and effects of cytokines and their actions and interrelationships are of such complexity that they cannot be addressed here in detail. A number of them will be addressed individually in sections below, and several excellent reviews are available.³²⁻³⁴ However, 2 cytokines, IL-1 and TNF- α , are of such fundamental importance in acute host responses to infection that they warrant specific attention.

IL-1 and TNF- α are small polypeptides, each with a molecular weight of approximately 17 kd, that exhibit a broad range of effects on immunologic responses, inflammation, metabolism, and hematopoiesis.^{34,35} IL-1 originally was described as "endogenous pyrogen," referring to its ability to produce fever in experimental animals, and TNF- α , which produces some of the same effects produced by IL-1, was originally named "cachectin" after the wasting syndrome it produced when injected chronically in mice.^{34,35} Many of the physiologic changes associated with gram-negative sepsis can be reproduced by injecting experimental animals with these cytokines in the absence of microorganisms. Depending on the doses injected, these effects might include fever, hypotension, and either neutrophilia or leukopenia.^{34,35} In the production of endotoxic shock caused by gram-negative sepsis, IL-1 and TNF- α are produced by mononuclear phagocytes in response to activation of TLRs by bacterial LPS. They in turn activate the production of other cytokines and chemokines, lipid mediators (eg, platelet-activating factor and prostaglandins), and reactive oxygen species. They also induce expression of adhesion molecules of both endothelial cells and leukocytes, stimulating recruitment of leukocytes by inducing release of the chemokine IL-8 and activating neutrophils for phagocytosis, degranulation, and oxidative burst activity.^{24,35} These all are important and usually beneficial host responses to infection. However, at high levels of activation, there sometimes are pathophysiological effects of this proinflammatory cascade, including vascular instability, decreased myocardial contractility, capillary leak, tissue hypoperfusion, coagulopathy, and

multiple organ failure.²⁴ For some systemic actions, notably the production of hemodynamic shock, IL-1 and TNF- α are synergistic. Both IL-1 and TNF- α also induce production of IL-6, a somewhat less potent cytokine that exhibits some of the actions of IL-1 and TNF- α .³⁴ The human host produces several soluble antagonists of IL-1 and TNF- α that can modulate their effects, including IL-1 receptor antagonist, soluble TNF- α receptor, and anti-inflammatory cytokines, especially IL-10.²⁴

The importance of effects mediated by IL-1 and TNF- α in the pathophysiology of septic shock has prompted much active research aimed at blocking their effects to reduce morbidity and mortality. Monoclonal antibodies against TNF- α and other inhibitors of TNF- α or IL-1 have showed early promise *in vitro* and in animal models of septic shock.^{24,34,36,37} However, they have been far more effective at preventing the effects of cytokines than reversing them. More recent attempts to address the issue of the timing of intervention have been directed at the intracellular signaling mechanisms activated through the TNF- α receptor or at mediators that appear later than TNF- α . Lipophilic inhibitors of protein tyrosine kinases, enzymes that propagate the cellular signals through TNF- α receptors, have been found to enhance survival in experimental animals, even when administered 2 hours after systemic injection with endotoxin.³⁸ Additionally, mAbs against a cytokine-like nonhistone nucleoprotein product of macrophages, high-mobility group B1 (which appears much later than TNF- α or IL-1 after LPS stimulation), were found to rescue mice from endotoxin shock when given 2 hours after an otherwise lethal dose of LPS.³⁹ More recently, clinical trials with activated protein C, a regulatory protein in the coagulation cascade, has demonstrated beneficial effects in selected patients with septic shock through mechanisms that might involve inhibition of NF- κ B activation.⁴⁰ To date, despite progress, clinical strategies to interfere with the cytokine-induced cascade that leads to endotoxin-induced shock have continued, overall, to meet with limited success.

Chemokines

A specialized group of small cytokine-like polypeptides, chemokines, which all share the feature of being ligands for G protein-coupled, 7-transmembrane segment receptors, plays an increasingly complex role in the immune response as cellular activators that induce directed cell migration, mainly of immune and inflammatory cells.⁴¹⁻⁴⁴ The chemokines and their receptors have been classified into 4 families on the basis of the motif displayed by the first 2 cysteine residues of the respective chemokine peptide sequence. Each of at least 16 CXC chemokines binds to 1 or more of the CXCRs (CXCR1 through CXCR6). Examples of CXC chemokines include IL-8 and Gro- α . Similarly, at least 28 CC chemokines, such as macrophage inflammatory protein 1 α , RANTES, and eotaxin-1, 2, and 3, bind to one or more of the CCRs (CCR1 through CCR10). The sole CX3C chemokine, fractalkine, or neurotaxin, binds to CX3CR1, currently the only receptor in its family. The 2 XC chemokines,

including lymphotaxin, bind to the sole receptor in this family, XCR1. A new nomenclature has been proposed to designate each of the chemokines as a numbered ligand for its respective receptor family. In this system Gro- α is CXC ligand (CXCL) 1 (or CXCL-1), and IL-8 now becomes CXCL-8. Similarly, RANTES becomes CCL-5, fractalkine is CX3CL-1, and lymphotactin is XCL-1.^{41,43} An update of this nomenclature system recently has been published, tabulating each of the families with their respective ligands and receptors, as well as the traditional names in both human and murine systems.⁴³

Virtually every cell type of the immune system expresses receptors for one or more of the chemokines. The cells of most inflamed tissues can release a variety of chemokines, and tissues infected with different bacteria or viruses release chemokines that recruit characteristic sets of immune cells.^{44,45} For example, whereas rhinoviruses induce the release of chemokines that result mainly in recruitment of neutrophils (early in the course of infection), EBV induces a set of chemokines that result in recruitment of B cells, natural killer (NK) cells, and both CD4 $^{+}$ and CD8 $^{+}$ T cells.⁴⁵ It is of interest that almost mutually exclusive sets of chemokines are induced by cytokines associated with T_H1 (IL-12 and IFN- γ) versus T_H2 (IL-4 and IL-13) immune responses, indicating a tight interplay between cytokines and chemokines in determining the type of immune response to specific infectious challenges generated under different conditions.⁴⁶ The specificity of such cellular responses is strongly influenced by the chemokines released from specific tissues, the vascular adhesion molecules expressed in those tissues, the chemokine receptors expressed by different populations of leukocytes, and the specific adhesion molecules expressed by leukocytes.⁴⁴⁻⁴⁶

Modulation of chemokine function can occur through several mechanisms. Chemokines themselves can be potentiated or inactivated by tissue proteases, including tissue peptidases and matrix metalloproteases.⁴⁷ Heparin sulfate-related proteoglycans on endothelial cell surfaces tether chemokines locally, where they can most efficiently activate circulating leukocytes for adhesion (see below). However, similar proteoglycans free in the extracellular environment can bind and sequester chemokines, keeping them from interacting with their cellular receptors.^{48,49} Finally, in addition to the well-described use of chemokine receptors as coreceptors for viral entry by HIV-1, other viruses, especially members of the herpesvirus family, encode soluble decoy receptors that compete with native host receptors for chemokine binding, thereby disrupting normal host responses.^{49,50}

NK cells

NK cells are an important cellular feature of innate immunity. They are lymphoid cells that do not express clonally distributed receptors, such as T-cell receptors or surface immunoglobulin, for specific antigens.⁵¹ They respond in an antigen-independent manner to help contain viral infections before the development of adaptive immune responses, and they aid in the control of malignant

tumors. NK cells are found in the peripheral circulation and in the spleen and bone marrow. Like many other leukocytes, they can be recruited to sites of inflammation by chemokines and other chemoattractants. They appear to be important for the control of tumors *in vivo* and serve a critical function in host defense against viral infections, especially those caused by members of the herpesvirus family.^{51,52} Activated NK cells also are an important source of IFN- γ , which limits tumor angiogenesis and promotes the development of specific protective immune responses.^{51,52}

Regulation of NK cell activity involves a balance between activating and inhibitory signals. Several cytokines can activate NK cell proliferation, cytotoxicity, or IFN- γ production, including IL-12, IL-15, IL-18, IL-21, and IFN- $\alpha\beta$.⁵¹ Activating signals through other receptors on NK cells, such as NKG2D, can lead to cytotoxicity, cytokine production, or both, depending on the receptor's association with distinct intracellular adaptor proteins that signal through different kinases.^{51,53} Other molecules on NK cells can act as either costimulatory or adhesion receptors, including CD27, CD28, CD154 (CD40 ligand), and lymphocyte function-associated antigen 1 (LFA-1) (CD11a/CD18).⁵¹⁻⁵³ Additionally, Fc γ RIII (CD16) can contribute to NK cell cytotoxic activity through mechanisms that include antibody-dependent cell cytotoxicity.⁵¹ NK cells are able to distinguish normal cells of self origin through receptors that recognize specific MHC class I molecules. Activation of such receptors provides an inhibitory signal that protects healthy host cells from NK cell-mediated lysis. Virus-infected cells and malignant cells often express MHC class I molecules at reduced levels and thus are less able to generate inhibitory NK cell signals, rendering them more susceptible to attack by NK cells.^{51,52} NK cell inhibitory receptors, which are not well characterized, appear to contain intracytoplasmic tyrosine-based inhibition motifs and antagonize NK cell activation pathways through protein tyrosine phosphatases.^{51,54} Thus the regulation of the phosphorylation state of specific tyrosine residues by activating kinases and inhibitory phosphatases appears to be a pivotal determinant of NK cell activation.

NK cells kill infected or malignant cells through the release of perforin and granzymes from granular storage compartments and through binding of the death receptors Fas and TRAIL-R on target cells through their respective NK cell ligands.^{51,55} The mechanisms by which perforin and granzymes mediate target cell death are not fully understood. The best available evidence suggests that perforin and one or more of 5 human granzymes released along with perforin from cytotoxic granules of NK cells associate with the cell membranes of target cells, either by binding through the mannose 6-phosphate receptor or through another mechanism that remains to be defined. One or more of the granzymes appears to activate intracellular pathways leading to target apoptosis through pathways that involve the mitochondria, caspases, or both.⁵⁶ Separately, binding of the death receptors also activates caspases, causing target cell apoptosis.⁵¹ It is

notable that although some tumor cells do not express Fas, NK cells can induce Fas expression on these targets by releasing IFN- γ and then proceed to kill them by binding to the newly expressed Fas.⁵¹

NK cells engage in several kinds of interactions with other cells of the immune system, including dendritic cells and other antigen-presenting cells. Dendritic cells can influence the proliferation and activation of NK cells both through release of cytokines, including IL-12, and through cell-surface interactions, including CD40/CD40 ligand, LFA-1/intercellular adhesion molecule 1 (ICAM-1), and CD27/CD70.⁵⁷ In return, NK cells can provide signals that result in either dendritic cell maturation or apoptosis.^{51,52}

The complement system

The complement system is made up of at least 30 proteins in serum or at cell surfaces. Most of these proteins are made in the liver or, to a lesser extent, by mononuclear phagocytes. Activation of the complement cascade by one or more of 3 distinct pathways leads to the evolution of its main effector functions: microbial opsonization, phagocyte recruitment, and bacteriolysis. All 3 activation pathways act at a microbial surface to assemble a convertase that cleaves C3 to form C3b, which in turn binds to the target surface, either as an opsonin or to help activate C5 and the remainder of the cascade.⁵⁸⁻⁶¹

Complement activation pathways. The classical pathway ordinarily is activated by IgG or IgM bound to microbial surface antigens. Antigen binding makes a site on the Fc portion of the antibody available to bind C1q, which in turn binds C1r and C1s. C1qrs catalyzes the cleavage and binding of C4 and C2, in the form of C4b2a, the classical pathway C3 convertase. This convertase then cleaves C3 to form C3a, which is released, and C3b, which remains bound on the target surface, and the cascade beyond C3 continues.⁵⁹

The recently characterized mannan-binding lectin (MBL) pathway is similar to the classical pathway but does not involve antibodies.⁶⁰ MBL is a serum protein of the collectin family with structural and functional similarities to C1q and binds to mannose-containing carbohydrates on microbial surfaces. Subsequently, MBL and the MBL-associated serine proteases, which have structural and functional similarities to C1r and C1s, form a complex that activates C4, with sequential binding of C4b and C2a and formation of C4b2a, the classical pathway C3 convertase.⁶⁰ C3 is activated, and the cascade proceeds as described.

The alternative pathway is vital to the host as a separate means by which C3 can be activated before development of specific antibodies.^{59,61} A low constitutive level of hydrolysis of the thioester of C3 in the fluid phase produces an activated form of C3, C3(H₂O). This activated form of C3 can bind factor B, and the latter is cleaved by factor D to form the fluid-phase C3 convertase C3(H₂O)Bb. The constitutive presence of small amounts of this convertase in the fluid phase ensures that some C3b always is available to initiate the alternative pathway at microbial surfaces.^{59,61} Factor B binds to surface

C3b and undergoes proteolytic cleavage by factor D to form C3bBb, the alternative pathway C3 convertase. Properdin stabilizes the convertase, which produces more C3b, establishing the C3 amplification loop of the alternative pathway and activating the remainder of the cascade.⁵⁹ Microbes that bear large amounts of surface sialic acid are usually poor activators of the alternative pathway because factor H outcompetes factor B for C3b binding at such surfaces.^{59,61} No convertase or amplification loop is created because factor H allows C3b cleavage by factor I to form iC3b, the only function of which is opsonic.⁵⁹ Nonactivators of the alternative pathway are some of the most successful pathogens in nonimmune hosts. They include *K1 Escherichia coli*, groups A and B streptococci, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type b, and some salmonellae.⁶²

Complement effector functions. Opsonization (from the Greek “to cater or prepare”) facilitates the removal of microorganisms from the circulation by macrophages in the liver and spleen and from tissue sites by neutrophils and tissue macrophages.^{58,59} Recognition and attachment of surface-bound C3b and iC3b on microbes by the type 1 and type 3 phagocytic complement receptors, CR1 (CD35) and CR3 (CD11b/CD18), respectively, activates ingestion and intracellular killing of the organisms.⁵⁹ Antibodies, themselves important opsonins, direct the localization of C3b binding on microorganisms through the classical pathway. This is important for encapsulated bacteria because the capsule’s presence as a barrier means that only C3b bound at the capsular surface by specific anticapsular antibody will be accessible to phagocyte receptors.⁶³

The free cleavage fragments of C3 and C5 can promote host inflammatory responses. C3a stimulates marrow release of granulocytes, and C5a serves as a potent chemoattractant for monocytes, neutrophils, and eosinophils. C5a also stimulates expression of CR1 and CR3, aggregation, and microbicidal activity of phagocytes. C5a-induced neutrophil aggregation and stasis in the pulmonary circulation can contribute to the respiratory distress syndrome associated with sepsis.⁶⁴ C4a, C3a, and especially C5a are anaphylotoxins that can induce release of histamine from mast cells and basophils, causing increased vascular dilatation and permeability.⁶⁴ In large amounts, they can contribute to the pathophysiology of septic shock.⁶⁴

When C5a is released by the classical or alternative pathway C5 convertase, C5b is bound at the target surface. The terminal complement proteins C5b, C6, C7, C8, and C9 assemble sequentially to form the membrane attack complex (MAC), which can kill and lyse target cells, especially gram-negative bacteria, by penetrating their outer membranes.^{59,61} The C5b-C8 complex serves as a polymerization site for several molecules of C9.^{61,65} Although C9 is not essential to membrane penetration, its presence as poly-C9 allows it to proceed more efficiently.^{59,65} The MAC also can lyse certain virus-infected host cells and some enveloped viruses directly.⁶⁶

Complement fragments can modulate other parts of the immune response, both directly by binding to CR1, CR2, and CR3 on the surfaces of T cells, B cells, and other cells involved in antigen recognition and indirectly by stimulating the synthesis and release of cytokines.⁶⁷ For example, the C3b cleavage product, C3dg, when covalently bound to antigen, brings the antigen close to B cells by binding to B-cell CR2 (CD21); C3 influences antigen localization within germinal centers, anamnestic responses, and isotype switching; and C1, C2, C4, and C3 are important for normal antibody responses.^{68,69}

Phagocytes

The first recognized cellular mechanism of host defense was the accumulation of phagocytic host cells around a foreign body in starfish observed by Metchnikoff.⁷⁰ Polymorphonuclear leukocytes (PMNs), the most abundant circulating phagocytes in the human host, will serve as a model for discussing phagocyte functions. These cells constitute a major line of defense against invading bacterial and fungi. The proliferation of myeloid marrow progenitors and their differentiation into mature progeny are regulated by specific growth factors and cytokines.^{71,72} The normal half-life of circulating PMNs is approximately 8 to 12 hours.⁷³ In the absence of active infection, most PMNs leave the circulation through the gingival crevices and the lower gastrointestinal tract, where the resident flora stimulate ongoing local extravasation of PMNs, a process that helps maintain the integrity of these tissues.⁷⁴ In response to invasive bacterial infection, circulating PMNs engage in 3 major functions: (1) migration to the site of infection, (2) recognition and ingestion of invading microorganisms, and (3) killing and digestion of these organisms.

Phagocyte recruitment to infected sites. Activation of endothelial cells that line the microvessels of acutely infected tissue occurs through locally produced cytokines, eicosanoid compounds, and microbial products.⁷⁵ As a result, the endothelial cells rapidly upregulate their surface expression of P-selectin and then E-selectin.^{75,76} These selectins engage in lectin-like interactions with the fucosylated tetrasaccharide moiety sialyl Lewis X, which is presented on constitutively expressed glycoproteins on PMNs, including L-selectin and P-selectin glycoprotein ligand 1.⁷⁶ These early interactions slow the PMNs in this first adhesive phase of leukocyte recruitment, sometimes described as "slow rolling."^{75,77} Within several hours, newly synthesized ICAM-1 is expressed at the endothelial surface.^{75,77,78} The slowly rolling PMNs are activated by transient selectin-mediated interactions and locally produced mediators, especially endothelium-derived chemokines, such as IL-8.⁷⁷ These chemokines are most effective in activating the PMNs when they are bound by complex proteoglycans at the endothelial cell surface.⁴⁸ The activated PMNs then increase the surface expression, binding avidity, or both of the β_2 -integrins LFA-1 and macrophage antigen-1 (Mac-1) that interact with endothelial cell ICAM-1 in this second, firm adhesion phase mediated by integrin-ICAM interactions, which is also

necessary for transendothelial migration of the PMNs.^{75,77,79,80} Other chemoattractants, such as C5a, N-formyl bacterial oligopeptides, and leukotrienes (eg, leukotriene B₄) that diffuse from the site of infection further activate PMNs and provide a chemotactic gradient for PMN migration into tissue.^{41,80,81} The receptors for these chemoattractants, like the chemokine receptors, are G protein-coupled and share a 7-transmembrane domain structure.^{41,81} They constitute important sensory mechanisms of the PMNs for activating adhesion, directional orientation, and the contractile protein-dependent lateral movement of adhesion sites in the PMN membrane necessary for migration.^{41,81-83} Although the specific stimuli and adhesion molecules might vary, this general scheme applies to the local recruitment of virtually all circulating cells of the immune system.⁴⁴⁻⁴⁶

Phagocytosis. After PMNs reach the site of infection, they must recognize and ingest, or phagocytose, the invading bacteria. Opsonization, especially with IgG and fragments of C3, greatly enhances phagocytosis.^{58,63} Although nonopsonic phagocytosis can occur, only opsonin-mediated phagocytosis is considered here. CR1 and CR3 are the main phagocytic receptors for opsonic C3b and iC3b, respectively.⁷⁹ When PMNs are activated by chemoattractants or other stimuli, CR1 and CR3 are rapidly translocated to the cell surface from intracellular storage compartments, increasing surface expression up to 10-fold.⁷⁹ Note that CR3 is identical to the adhesion-mediating integrin Mac-1.⁷⁹ CR1 and CR3 act synergistically with receptors for the Fc portion of antibodies, especially IgG.^{58,59} Phagocytic cells can express up to 3 different types of IgG Fc receptors, or Fc γ Rs, all of which can mediate phagocytosis.⁸⁴ Fc γ RI (CD64) is a high-affinity receptor that is expressed mainly on mononuclear phagocytes.⁸⁴ The 2 Fc γ Rs ordinarily expressed on circulating PMNs are Fc γ RII (CD32) and Fc γ RIII (CD16).⁸⁴ Fc γ RII is conventionally anchored in the cell membrane, exhibits polymorphisms that determine preferences for binding of certain IgG subclasses, and can directly activate PMN oxidative burst activity.^{84,85} Fc γ RIII is expressed on PMNs as a glycolipid-anchored protein, although it is anchored conventionally on NK cells and macrophages.⁸⁴ Most phagocytes also express IgA receptors. The best characterized, CD89, binds monomeric IgA and promotes phagocytosis and killing of IgA-opsonized bacteria.⁸⁶

The engagement of phagocyte receptors with opsonins bound on microbes locally activates cytoskeletal contractile elements, leading to invagination of the membrane at the site of initial engagement and extension of pseudopods around the microbe. The ligation of additional opsonin-receptor pairs leads to engulfment of the microbe within a sealed phagosome.⁸⁷ This is followed by fusion of the phagosome with lysosomal compartments containing the phagocyte's array of microbicidal products.

Phagocyte microbicidal mechanisms. The microbicidal mechanisms of PMNs usually are categorized as either oxygen dependent or oxygen independent. Oxygen-dependent microbicidal mechanisms of phagocytes depend on a complex enzyme, reduced nicotinamide adenine

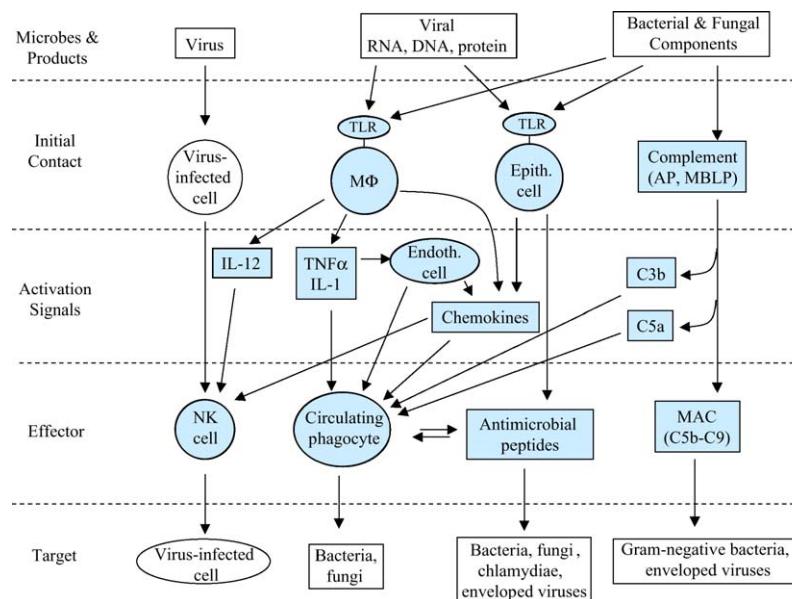


FIG 1. Innate immunity: responses to first contact. Diagrammed are important host responses to infection that are independent of specific cell-mediated immunity or antibodies. Initial contact between the host and microbes or their products results in a range of activating signals that mobilize both cellular and humoral effectors for attack on their respective microbial targets. Components of the host response are highlighted in blue. *MΦ*, Macrophages; *AP*, alternative pathway; *MBLP*, mannose-binding lectin pathway.

dinucleotide phosphate (NADPH) oxidase, which converts molecular oxygen (O_2) into superoxide anion (O_2^-).⁸⁸ This enzyme is assembled at the activated cell membrane from 6 or more components that include a cytochrome (α - and β -subunits, designated gp91 phox and p22 phox , respectively), a flavoprotein, and a quinone, all of which are associated with the cell membrane, and at least 2 cytoplasmic proteins, p47 phox and p67 phox ("phox" refers to phagocyte oxidase), that assemble with the membrane-associated components to form the active enzyme complex.^{88,89} Each of the main oxidant products derived from NADPH activity and subsequent reactions exhibits microbicidal activity, including the earliest products, O_2^- and H_2O_2 , which are less potent than the downstream products hypochlorite (ClO^-) and chloramines (NH_3Cl and RNH_2Cl), with chloramines being the most stable.^{90,91}

Oxygen-independent microbicidal activity of PMNs resides mainly in a group of proteins and peptides stored within primary (azurophilic) granules. Lysozyme is contained in both the primary and the secondary (specific) granules of PMNs.⁹² It cleaves important linkages in the peptidoglycan of bacterial cell walls and can act in concert with the complement MAC.⁵⁸ The primary granules contain several cationic proteins with important microbicidal activity. A 59-kd protein, bactericidal/permeability-increasing protein, is active against only gram-negative bacteria.⁹³ Smaller arginine- and cysteine-rich peptides, the α -defensins, similar to the β -defensins of epithelial cells, are active against a range of bacteria, fungi, chlamydiae, and enveloped viruses.⁶ Other related molecules include cathelicidin and a group of peptides called p15s.^{4,94}

SUMMARY

An overview of most of the main features of innate immunity discussed above, along with some of their important interactions, is diagrammed in Fig 1. Several levels of interaction are depicted, from initial host-pathogen contact, through a variety of activating signals, to the attack by host effector mechanisms on pathogenic targets. Initial contact between the host and microbes or their products might result in viral infection of cells, activation of TLRs on macrophages and epithelial cells, and activation of the alternative pathway or mannose-binding lectin pathway of complement. The resulting activation signals, including cytokines (eg, IL-12, TNF- α , and IL-1), chemokines, and products of the complement cascade mobilize both cellular (NK cells and phagocytes) and humoral (antimicrobial peptides and MAC) effectors that attack their respective microbial targets.

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