Review Articles

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INNATE IMMUNITY

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THE immune system has traditionally been divided into innate and adaptive components, each with a different function and role. The adaptive component is organized around two classes of specialized cells, T cells and B cells. Since each lymphocyte displays a single kind of structurally unique receptor, the repertoire of antigen receptors in the entire population of lymphocytes is very large and extremely diverse. The size and diversity of this repertoire increase the probability that an individual lymphocyte will encounter an antigen that binds to its receptor, thereby triggering activation and proliferation of the cell. This process, termed clonal selection, accounts for most of the basic properties of the adaptive immune system.

Clonal expansion of lymphocytes in response to infection is absolutely necessary for the generation of an efficient immune response. However, it takes three to five days for sufficient numbers of clones to be produced and to differentiate into effector cells, which allows more than enough time for most pathogens to damage the host. In contrast, the effector mechanisms of innate immunity, which include antimicrobial peptides, phagocytes, and the alternative complement pathway, are activated immediately after infection and rapidly control the replication of the infecting pathogen. For this reason, containing the infection until the lymphocytes can begin to deal with it has long been considered the main function of innate immunity. However, it has become increasingly clear that the innate immune system has a much more important and fundamental role in host defense. In this article we will outline the ways in which the innate immune system interacts with and controls adaptive immune responses.

The clinical implications of these discoveries are just beginning to emerge, and we expect them to contribute in important ways to our understanding of the body's defense against bacteria, the way in which the adaptive immune system establishes long-lasting antimicrobial protection, and some of the mechanisms used to avoid producing autoimmunity.

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STRATEGIES OF INNATE AND ADAPTIVE IMMUNE RECOGNITION

The main distinction between the innate and the adaptive immune systems lies in the mechanisms and receptors used for the immune recognition. In the adaptive immune system, the T-cell receptor and the B-cell receptor are generated somatically, during the development of T and B cells, in a way that endows each lymphocyte with a structurally unique receptor. Since these receptors are not encoded in the germ line, they are not predestined to recognize any particular antigen. Rather, an extremely diverse repertoire of receptors is generated randomly, and lymphocytes bearing useful receptors (i.e., receptors specific for pathogens) are subsequently selected for clonal expansion by encountering the antigens for which they happen to be specific. These useful receptors, moreover, cannot be passed on to the next generation, even though they might give one's progeny a survival advantage. No matter how beneficial they may be, antigen receptors for common environmental pathogens have to be reinvented by every generation.

Since the binding sites of antigen receptors arise as a result of random genetic mechanisms, the receptor repertoire contains binding sites that can react not only with infectious microorganisms but also with innocuous environmental antigens and self antigens. Activation of the adaptive immune response can be harmful to the host when the antigens are self or environmental antigens, since immune responses to such antigens can lead to autoimmune diseases and allergies. How does the immune system determine the origin of the antigen, and how does it decide whether to induce an immune response? Recent studies demonstrate that the innate immune system has a major role in these decisions.

During evolution, the innate immune system appeared before the adaptive immune system, and some form of innate immunity probably exists in all multicellular organisms. By contrast with adaptive immunity, innate immune recognition is mediated by germ-line–encoded receptors, which means that the specificity of each receptor is genetically predetermined. One advantage of these germ-line–encoded receptors is that they evolved by natural selection to have defined specificities for infectious microorgan-

isms. The problem is, however, that every organism has a limit to the number of genes it can encode in its genome. The human genome, for example, contains only 75,000 to 100,000 genes, most of which have nothing to do with immune recognition. By comparison, there are approximately 10^{14} and 10^{18} different somatically generated immunoglobulin receptors and T-cell receptors, respectively. The total number of receptors involved in innate immune recognition is thought to be in the hundreds. Moreover, microbes are extremely heterogeneous and can mutate at a much higher rate than any of their hosts.

The strategy of the innate immune response may not be to recognize every possible antigen, but rather to focus on a few, highly conserved structures present in large groups of microorganisms.[1] These structures are referred to as pathogen-associated molecular patterns, and the receptors of the innate immune system that evolved to recognize them are called pattern-recognition receptors. The best-known examples of pathogen-associated molecular patterns are bacterial lipopolysaccharide, peptidoglycan, lipoteichoic acids, mannans, bacterial DNA, double-stranded RNA, and glucans. Although these structures are chemically quite distinct, all pathogen-associated molecular patterns have common features.[2,3] First, pathogen-associated molecular patterns are produced only by microbial pathogens, and not by their hosts. For example, lipopolysaccharide is synthesized only by bacteria; pattern-recognition receptors recognize lipopolysaccharide, thus alerting the host to the presence of the infecting organism. Second, the structures recognized by the innate immune system are usually essential for the survival or pathogenicity of microorganisms. Third, pathogen-associated molecular patterns are usually invariant structures shared by entire classes of pathogens. For example, all gram-negative bacteria have lipopolysaccharides, and therefore, the lipopolysaccharide pattern-recognition receptor of the host can detect the presence of virtually any gram-negative bacterial infection.

PATTERN-RECOGNITION RECEPTORS

The receptors of the innate immune system that are encoded in the germ line differ from antigen receptors in several important ways. They are expressed on many effector cells of the innate immune system, most importantly on macrophages, dendritic cells, and B cells — the professional antigen-presenting cells. The expression of pattern-recognition receptors is not clonal, in that all such receptors displayed by cells of a given type (e.g., macrophages) have identical specificities. Moreover, once the pattern-recognition receptors identify a pathogen-associated molecular pattern, the effector cells are triggered to perform their effector functions immediately rather than after they have proliferated. This fact accounts for the rapid kinetics of innate immune responses.

Structurally, pattern-recognition receptors belong to several families of proteins. Leucine-rich repeat domains, calcium-dependent lectin domains, and scavenger-receptor protein domains, for example, are often involved in pattern recognition.[3,4] Functionally, pattern-recognition receptors can be divided into three classes: secreted, endocytic, and signaling. Secreted pattern-recognition molecules function as opsonins by binding to microbial cell walls and flagging them for recognition by the complement system and phagocytes.

The best-characterized receptor of this class is the mannan-binding lectin,[5,6] a member of the calcium-dependent lectin family that binds to microbial carbohydrates to initiate the lectin pathway of complement activation. Mannan-binding lectin and surfactant proteins form a structurally related family of collectins, so named because they consist of a collagenous domain linked to the calcium-dependent lectin domain. Mannan-binding lectin is synthesized in the liver and is secreted into the serum as a component of the acute-phase response. It can bind to carbohydrates on gram-positive and gram-negative bacteria and yeast, as well as some viruses and parasites.[5] Mannan-binding lectin is associated with two serine proteases, mannan-binding lectin–associated proteases 1 and 2. These proteases are related to C1r and C1s, the serine proteases of the classic complement pathway. Similar to C1r and C1s, mannan-binding lectin–associated protease, once activated, ultimately leads to the cleavage of the third component of complement (C3) and to the activation of C3 convertase, which results in an amplified cascade of complement activation.[5] However, unlike C1 proteases, which require antigen–antibody complexes for their activation, mannan-binding lectin–associated proteases are activated by the binding of microbial ligands to mannan-binding lectin (Fig. 1).

Endocytic pattern-recognition receptors occur on the surface of phagocytes. On recognizing pathogen-associated molecular patterns on a microbial cell, these receptors mediate the uptake and delivery of the pathogen into lysosomes, where it is destroyed. Pathogen-derived proteins can then be processed, and the resulting peptide can be presented by major-histocompatibility-complex (MHC) molecules on the surface of the macrophage.

The macrophage mannose receptor, which is also a member of the calcium-dependent lectin family, is an endocytic pattern-recognition receptor. It specifically recognizes the carbohydrates with large numbers of mannoses that are characteristic of microorganisms and mediates their phagocytosis by macrophages.[6] Another endocytic pattern-recognition receptor, the macrophage scavenger receptor, binds to bacterial cell walls and is an essential part of the clearance of bacteria from the circulation.[7,8]

Signaling receptors recognize pathogen-associated

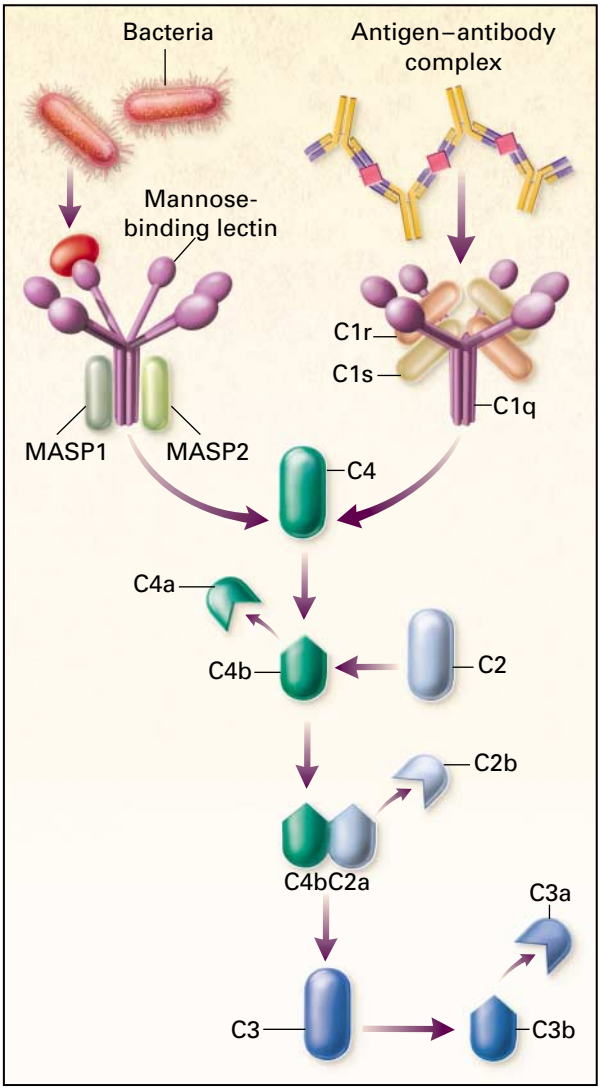

Figure 1. The Lectin Pathway of Complement Activation.

Activation of the lectin pathway of complement is mediated by mannose-binding lectin, which is a pattern-recognition receptor specific for microbial carbohydrates. Mannose-binding lectin is associated with the serine proteases mannan-binding lectin–associated proteases 1 and 2 (MASP1 and MASP2). Binding of mannose-binding lectin to its microbial ligands activates these proteases, which leads to the cleavage of the complement components C2 and C4. The cleavage products C2a and C4b then form a C3 convertase, which initiates the complement cascade by cleaving the C3 protein. The complex of mannose-binding lectin and its proteases functions similarly to the C1 complex of the classic complement cascade. It is important to note, however, that the C1r and C1s serine proteases are activated by the binding of C1q to the antibody–antigen complex and are thus dependent on antibody responses, whereas activation of the complement pathway is triggered directly by microbial recognition and is therefore independent of adaptive immune responses.

molecular patterns and activate signal-transduction pathways that induce the expression of a variety of immune-response genes, including inflammatory cytokines. The recently identified receptors of the toll family appear to have a major role in the induction of immune and inflammatory responses.

TOLL RECEPTORS

The first receptor of the toll family was identified in drosophila as a component of a signaling pathway that controls dorsoventral polarity in fly embryos.[9] Analysis of the sequence of the *toll* gene revealed that it encodes a transmembrane protein with a large extracellular domain containing leucine-rich repeats. Remarkably, the sequence of the cytoplasmic domain of the toll protein turned out to be similar to the cytoplasmic domain of the mammalian interleukin-1 receptor.[10] Moreover, both the interleukin-1 receptor in humans and toll in drosophila induce signal-transduction pathways that lead to the activation of transcription factors of the nuclear factor-κB (NF-κB) family.[11] Members of this family have a key role in the induction of immune and inflammatory responses in mammals.[12] In drosophila, microbial infection triggers the rapid up-regulation of a variety of peptides with antimicrobial activity.[13] Interestingly, the promoter regions of the genes encoding these peptides, like many mammalian genes involved in inflammation and immune responses, contain NF-κB–binding sites.

These findings suggested that drosophila toll, in addition to its role in embryonic development, is involved in the immune responses of adult flies. This was demonstrated in elegant studies by Hoffmann's group.[14] Drosophila with a loss-of-function mutation in the *toll* gene were shown to be highly susceptible to fungal infection. Interestingly, however, inactivation of the *toll* gene did not impair responsiveness to bacterial infections. Since there are eight toll-like proteins in drosophila,[15] other members of the toll family may be programmed to recognize bacterial pathogens and induce antibacterial responses.

Homologues of drosophila toll have been identified in mammals and are referred to as toll-like receptors (TLRs).[16,17] The first human toll to be characterized (now referred to as toll-like receptor 4, or TLR4) was shown to induce, like its drosophila homologue (Fig. 2), the activation of the NF-κB signaling pathway. Through this pathway, the activation of TLR4 induces the expression of a variety of cytokines and costimulatory molecules that are crucial to adaptive immune responses.[16] These findings suggested that TLRs may function as receptors of the innate immune system.[18] This is now known to be the case for at least two members of the toll family, TLR4 and TLR2.

The first evidence linking TLR4 to the innate immune system was the demonstration that it is the receptor for lipopolysaccharide in the mouse. Mice

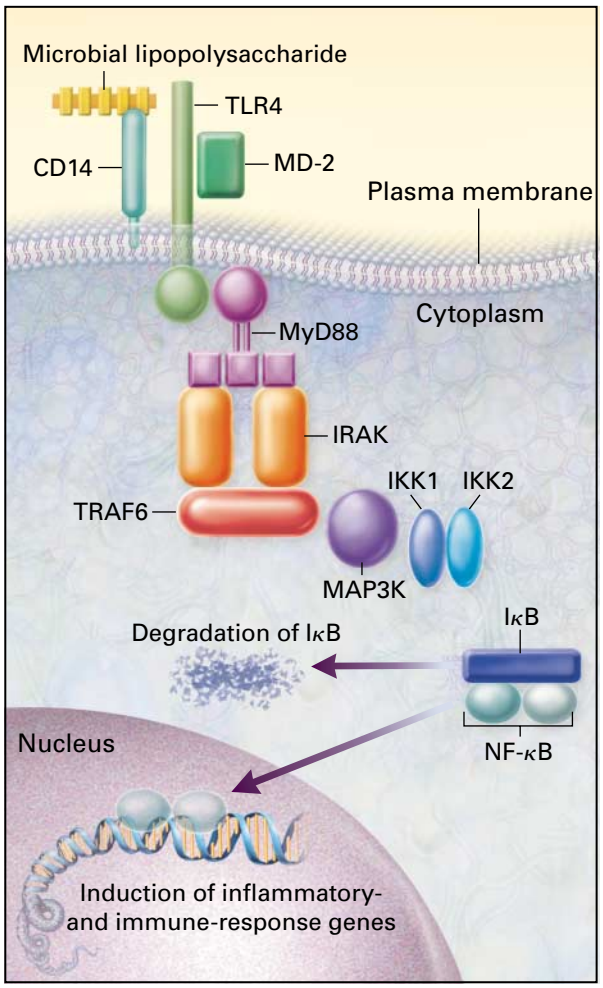

Figure 2. The Signaling Pathway of Toll-like Receptors.

Some of the toll-like receptors (TLRs) function as pattern-recognition receptors of the innate immune system. Their recognition of microbial products leads to the activation of the nuclear factor-κB (NF-κB) signaling pathway. In this example, the recognition of lipopolysaccharide is mediated by three different gene products: CD14, toll-like receptor 4 (TLR4), and MD-2. The binding of lipopolysaccharide to CD14 presumably leads to the association of CD14 with the TLR4–MD-2 complex and is thought to induce the dimerization of TLR4. Once TLR4 is activated, it recruits the adapter protein MyD88, which is associated with the serine–threonine protein kinase interleukin-1 receptor–associated kinase (IRAK). IRAK is then phosphorylated and associated with the tumor necrosis factor–associated factor 6 (TRAF-6) adapter protein. Oligomerization of TRAF-6 is thought to activate a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family, which directly or indirectly leads to the activation of IκB kinase 1 (IKK1) and IκB kinase 2 (IKK2). These kinases phosphorylate IκB on serine residues, thus targeting IκB for degradation and releasing NF-κB, which moves into the nucleus and induces the transcriptional activation of a wide variety of inflammatory- and immune-response genes.

with either a spontaneous mutation of the *tlr4* gene or a targeted disruption of the gene have no response to lipopolysaccharide and are thus resistant to endotoxic shock.[19-21] By contrast, mice with a targeted deletion of the *tlr2* gene have a normal response to lipopolysaccharide.[22] It is therefore clear that TLR4, but not TLR2, is essential for the recognition of lipopolysaccharide.

TLR4 is not the only protein involved in the recognition of lipopolysaccharide, however. Lipopolysaccharide first interacts with a serum protein called lipopolysaccharide-binding protein, which transfers lipopolysaccharide to CD14, a receptor on macrophages and B cells that is anchored to the cell surface by a glycosylphosphoinositol tail.[23,24] Another protein, MD-2, is required for TLR4-mediated recognition of lipopolysaccharide,[25] making it likely that the lipopolysaccharide-recognition complex has at least three components — CD14, TLR4, and MD-2. TLR4 and MD-2 are constitutively associated with each other, whereas CD14 is presumably recruited to the complex after binding lipopolysaccharide (Fig. 2).

Mice in which the *tlr2* gene is deleted have no response to two major bacterial pathogen-associated molecular patterns: peptidoglycan and lipoproteins.[22,26] Since at least 10 mammalian TLRs have been identified, some if not all of them are probably involved in the recognition of the major microbial patterns that trigger innate immune responses. It is therefore likely that alterations of the *TLR* genes will profoundly affect the immune system. The strain of mice (C3H/HeJ) with a loss-of-function mutation in the *tlr4* gene, for example, is highly susceptible to gram-negative bacterial infections. Conceivably, polymorphisms in the human *TLR4* gene are correlated with an increased susceptibility to gram-negative infection.

Although information about allelic variants of human *toll* genes is still very limited, mutations in both the ectodomains and the cytoplasmic domains of TLR4 have been identified.[27] It remains to be seen whether these mutations affect TLR4-mediated recognition of lipopolysaccharides and susceptibility to infection.

INNATE IMMUNE RECOGNITION AND CONTROL OF ADAPTIVE IMMUNE RESPONSES

As discussed earlier, the adaptive immune system has a tremendous capacity to recognize almost any antigenic structure, but because antigen receptors are generated at random, they bind to antigens regardless of their origin — bacterial, environmental, or self. The receptors of the innate immune system, by contrast, are specific for structures found exclusively in microbial pathogens (pathogen-associated molecular patterns), which is why they function to signal the presence of infection. The signals induced on recogni-

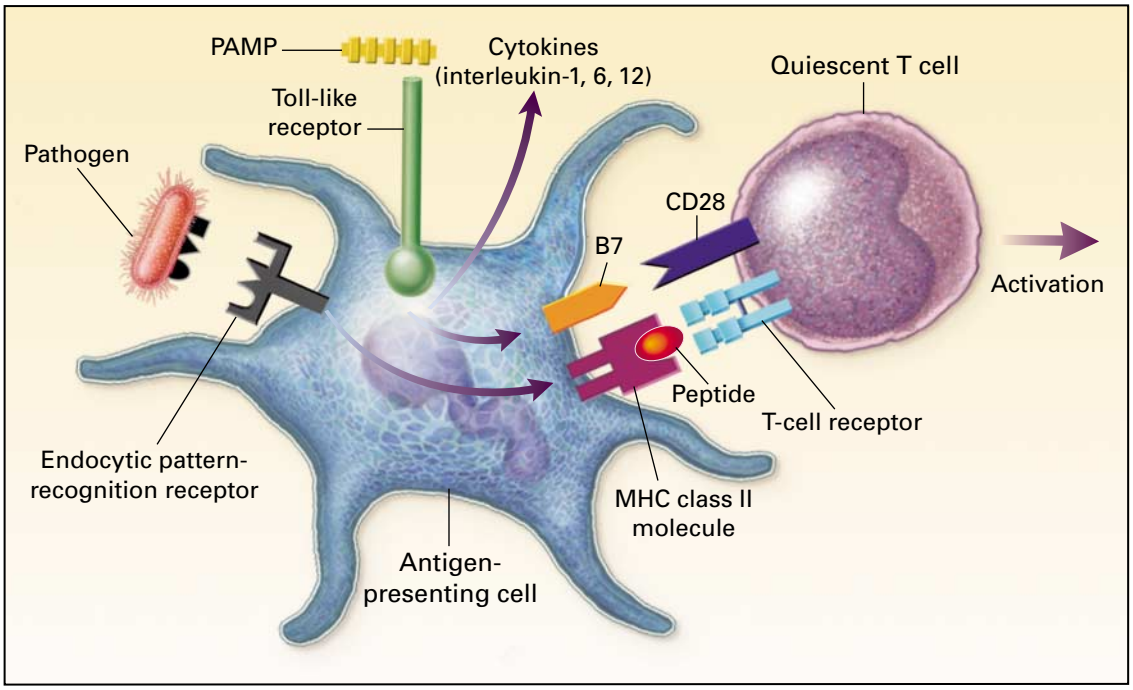

Figure 3. The Receptors Involved in the Interplay of the Innate and Adaptive Immune Systems.

Recognition of the pathogen-associated molecular pattern (PAMP) by pattern-recognition receptors, such as the toll-like receptors, generates signals that activate the adaptive immune system. Endocytic pattern-recognition receptors, such as the macrophage mannose receptor, bind to components of microbial cell walls and mediate the uptake and phagocytosis of pathogens by antigen-presenting cells (macrophages and dendritic cells). Proteins derived from the microorganisms are processed in the lysosomes to generate antigenic peptides, which form a complex with major-histocompatibility-complex (MHC) class II molecules on the surface of the macrophage. These peptides are recognized by T-cell receptors. In the case of the signaling class of pattern-recognition receptors, the recognition of pathogen-associated molecular patterns by toll-like receptors leads to the activation of signaling pathways that induce the expression of cytokines, chemokines, and costimulatory molecules. Therefore, pattern-recognition receptors have a role in the generation of both the peptide–MHC-molecule complex and the costimulation required for the activation of T cells.

tion by the innate immune system, in turn, control the activation of adaptive immune responses; the adaptive immune system responds to a pathogen only after it has been recognized by the innate immune system.

For example, T cells use their antigen receptors to recognize a ligand in the form of a peptide bound to an MHC class II molecule on the surface of an antigen-presenting cell. However, these peptides can be either self peptides or peptides derived from a microbial pathogen. And because the antigen receptor was randomly generated, the T cell cannot distinguish self from nonself on the basis of peptide recognition alone. Indeed, the recognition of the peptide–MHC ligand by the antigen receptor is not sufficient to activate T cells. T cells require at least two signals to become activated: one is the complex of a peptide and an MHC molecule, and the other is a costimulatory signal mediated by, for example, the CD80 and CD86 molecules on the surface of the antigen-presenting cell. It is only when the antigen-presenting cell expresses both antigen and CD80 or CD86 molecules that the T cell can be activated. Recognition of an antigen in the absence of CD80 or CD86 molecules leads to permanent inactivation or apoptosis of the T cell.

The expression of CD80 and CD86 molecules on the surface of the antigen-presenting cell is controlled by the innate immune system. Receptors such as TLRs induce these molecules to appear on the antigen-presenting cell when they recognize their cognate pathogen-associated molecular patterns.[16] Since pathogen-associated molecular patterns occur only on pathogens, TLRs induce CD80 and CD86 molecules only in the presence of infection. A T cell, in turn, receives both of the signals required for activation only if its receptor binds to the peptide that was derived from the pathogen that induced the expression of CD80 or CD86 molecules through its pathogen-associated molecular patterns (such as lipopolysaccharide) (Fig. 3).

Self antigens, on the other hand, are not recognized by receptors of the innate immune system and, therefore, do not induce the expression of CD80 or CD86 molecules. This mechanism ensures that, normally, only pathogen-specific T cells are activated. After activation, helper T cells control other components of adaptive immunity, such as the activation of cytotoxic T cells, B cells, and macrophages. Innate immune recognition therefore appears to control all the major aspects of the adaptive immune responses through the recognition of infectious microbes and the induction of signals required for the triggering of adaptive immunity.

INNATE IMMUNITY AND DISEASE

Given the essential role of the innate immune system in regulating all aspects of immunity, it is conceivable that dysfunction of the components of innate immunity can contribute to diseases. Two general types of genetic alterations could lead to immunologic abnormalities: mutations that inactivate the receptors or signaling molecules involved in innate immune recognition and mutations that render them constitutively active.

The first type of mutation would be expected to result in various types of immunodeficiencies. The second type of mutation would trigger inflammatory reactions and could thus contribute to a wide variety of conditions with an inflammatory component, including asthma, allergy, arthritis, and autoimmunity. Indeed, mutations in macrophage mannose receptors and mannan-binding lectin of both humans and mice have been associated with increased susceptibility to infection by a variety of pathogens.[5,6]

So far, little is known about mutations in *TLR* genes, and the search for *TLR* gene polymorphisms is likely to provide new insights into the cause of immune and inflammatory disorders. A dramatic example of the effect of mutational inactivation of an unknown component in the toll and interleukin-1 receptor signaling pathways was described recently in a patient with increased susceptibility to bacterial infection.[28]

CONCLUSIONS

Innate immunity, an ancient form of host defense, must have appeared early in the evolution of multicellular organisms, because many genes involved in innate host defense occur not only in vertebrate and invertebrate animals but also in plants. Higher-order vertebrates also have an adaptive immune system whose principles of operation are quite different from those of innate immunity. The random generation of a highly diverse repertoire of antigen receptors allows the adaptive immune system to recognize virtually any antigen. But the price of this diversity is the inability to distinguish foreign antigens from self antigens. The innate immune system, by contrast, deploys a limited number of receptors with specificity for conserved microbial structures. Recognition of these structures by the innate immune system induces costimulators, cytokines, and chemokines, which recruit and activate antigen-specific lymphocytes and initiate adaptive immune responses.

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