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REVIEW

Phylogenetic Perspectives in Innate Immunity

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The concept of innate immunity refers to the first-line host defense that serves to limit infection in the early hours after exposure to microorganisms. Recent data have highlighted similarities between pathogen recognition, signaling pathways, and effector mechanisms of innate immunity in *Drosophila* and mammals, pointing to a common ancestry of these defenses. In addition to its role in the early phase of defense, innate immunity in mammals appears to play a key role in stimulating the subsequent, clonal response of adaptive immunity.

It has long been appreciated that the antimicrobial host defense relies both on innate and adaptive components. Overwhelmingly, however, studies on immunity during the last few decades have concentrated on the adaptive response and its hallmarks, that is, the generation of a large repertoire of antigen-recognition receptors and immunological memory. Only quite recently has innate immunity gained renewed interest, particularly as it became apparent that it is an evolutionary, ancient defense mechanism (1, 2).

In this review we will first discuss innate immunity in *Drosophila* where the power of genetics combined with molecular and bio-

chemical approaches has allowed a dissection of pathways required for host defense. With the guidance of paradigms set in *Drosophila*, we will examine the role of innate immunity in mosquitoes and discuss its relevance in reducing transmission of medically important parasites. We will then define the essential characteristics of mammalian innate immunity, namely, its ability to distinguish species self from infectious nonself, and we will illustrate the links between innate and adaptive immunity. A central theme of this review is the marked conservation of innate defenses between insects and mammals, which points to a common ancestry of these systems.

Prototypical Innate Immune Responses in *Drosophila*

Drosophila is particularly resistant to microbial infections. Three mechanisms contribute to this resistance: (i) phagocytosis of invading microorganisms by blood cells, (ii) proteolytic cascades leading to localized blood clotting, melanin formation, and opsoniza-

tion, and (iii) transient synthesis of potent antimicrobial peptides. These reactions all take place within a short period after septic injury. Whereas information on the involvement of blood cells and of proteolytic cascades in *Drosophila* immunity is still fragmentary, much has been learned in recent years about the structure and regulated expression of the inducible antimicrobial peptides, and we will restrict our analysis here to this facet of the host defense (3). The peptides are primarily produced in the fat body (the functional equivalent of the mammalian liver) and are secreted into the blood. In addition to this systemic response, *Drosophila* also produces antimicrobial peptides locally, in barrier epithelia (4).

Since the discovery of inducible antimicrobial peptides in the moth *Hyalophora cecropia* by Boman and associates in 1981 (5), 400 peptides have been reported to participate in innate immunity, not only in insects but in all multicellular organisms that were investigated, including humans and plants. Paramount among these peptides are the defensins, a group of compact (3- to 5-kD) protease-resistant molecules with three or four disulfide bridges. Defensins have wide spectra of activity directed against various bacteria, fungi, and enveloped viruses (6, 7). Four defensin families have been reported in eukaryotes: α -defensins and β -defensins in mammals, insect defensins, and

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plant defensins (Fig. 1). Whereas mammalian defensins consist solely of β sheets linked by disulfide bridges (in a slightly different pattern for α - and β -defensins), insect and plant defensins have an α helix stabilized through disulfide bridging to strongly twisted antiparallel β sheets. Most other antimicrobial peptides are devoid of cysteines. Some, like the insect cecropins and the frog magainins, contain only α helices. Others contain a high content of a given amino acid, for instance His in histatins, Pro in batenecins and drosocin, and Gly in attacins and dipterin (6, 7). Defensins and most other antimicrobial peptides act by permeabilizing the cell membranes of microorganisms, resulting in the efflux of solutes. The molecular mechanisms are not fully understood but may involve the transient appearance of channel-like structures (8). Antimicrobial peptides are cationic and generally not cytotoxic at concentrations where they kill microorganisms (6, 7).

The strong and rapid induction of antimicrobial peptide genes in the *Drosophila* fat body cells after a septic injury has served as a model system for the analysis of innate immunity in this species. *Drosophila* produces at least seven distinct antimicrobial peptides. Drosomycin is potentially antifungal, whereas the others (cecropins, dipterin, drosocin, attacin, defensin, and metchnikowin) act primarily on bacteria. The upstream sequences of all these genes contain binding sites for transcription factors of the Rel and nuclear factor kappa B (NF- κ B) family of inducible transactivators. When κ B-related binding sites were first reported in *Drosophila* antimicrobial peptide genes (3), the only known Rel protein was Dorsal, which plays a key developmental role in dorsoventral patterning of the early embryo (9). Genetic and biochemical studies had already established that a signaling cascade involving 11 maternally expressed genes controls whether the Dorsal protein is retained in the cytoplasm by binding to the inhibitor Cactus, an inhibitor of kappa B (I- κ B)-like protein, or is translocated into the nucleus to act as a transcription factor. The extracellular portion of this cascade comprises four serine pro-

teases that act in sequence and ultimately cleave the protein Spaetzle, a member of the cystine-knot family of growth factor- and cytokine-like proteins. It is assumed that cleaved Spaetzle is a ligand that binds to the transmembrane receptor Toll, which has an extracytoplasmic leucine-rich domain (LRR) and an intracytoplasmic domain homologous to that of the mammalian interleukin-1 (IL-1) receptor, or a so-called TIR (Toll/IL-2 receptor) homology domain. Activation of Toll leads to a cascade of cytoplasmic events that implicate the Tube protein and the serine-threonine kinase Pelle, and culminate in the phosphorylation and subsequent degradation of Cactus, releasing Dorsal for translocation into the nucleus (9). The similarities with the cytokine-induced, NF- κ B-dependent activation of acute-phase response genes in mammals (Fig. 2 and see below) prompted molecular and genetic studies to probe whether the dorsoventral signaling cascade is reused in larvae and adults of *Drosophila* to control antimicrobial peptide production. It was indeed found that the genes of the Spaetzle-Toll-Cactus cassette are also expressed in fat body cells, and that their expression is up-regulated by immune challenge (10). Furthermore, analysis of dorsoventral mutants demonstrated that this cassette controls the expression of the antifungal peptide drosomycin after septic injury (10). Induction of antibacterial peptides requires an input from one or several additional pathways depending on the *imd* (for immune deficiency) and *ird* (for immune response deficient) genes, which are not yet fully characterized (11). Mutations at the two extremes of the dorsoventral signaling cascade, that is, in the upstream genes encoding the protease zymogens and in the Rel protein Dorsal itself, do not affect drosomycin induction (10). Evidently, either Dorsal is not the transactivator for the drosomycin gene, or other Rel proteins can substitute for its function.

Two additional proteins with a Rel homology domain have been identified recently in *Drosophila*: Dif, which is closely related to Dorsal and binds to Cactus, and Relish, which contains in addition to the Rel homol-

ogy domain an ankyrin repeat domain (12). It is surmised that the presence of the latter domain retains Relish in the cytoplasm and that nuclear translocation requires proteolytic cleavage as in the mammalian p105 Rel protein. Experimental evidence now indicates that both of these Rel proteins participate in the control of antimicrobial peptide gene expression. Similarly, the observation that the embryonic protease zymogens are dispensable for drosomycin induction suggests that other proteolytic enzymes can cleave the Spaetzle protein after an immune challenge. The current view is that several proteolytic cascades are activated in the hemolymph by septic injury and lead to the cleavage of Spaetzle (and probably other Spaetzle-like proteins), generating ligands that interact with Toll and other transmembrane receptors to activate intracellular signaling pathways. It is of interest in this context that *Drosophila* has several genes encoding Toll-like receptors, notably 18-Wheeler, which has been implicated in the control of the antibacterial peptide attacin (13).

Drosophila is capable of discriminating between classes of invading microorganisms, for instance bacteria versus fungi, and of responding by preferentially producing peptides that target destruction of the recognized pathogen. Flies, for example, that are naturally infected by entomopathogenic fungi exhibit an adapted response by selectively activating the Toll pathway to produce peptides with antifungal activities (14). By analogy with the situation in mammals, we propose that distinct proteins recognize characteristic molecular patterns associated with particular classes of pathogens and preferentially activate the production of peptides that kill the relevant pathogen. Proteins that recognize and bind bacterial or fungal cell wall components and activate protease zymogens have been characterized in other invertebrates, that is, in the coagulation cascade of the horseshoe crab and the prophenoloxidase cascade of crustaceans [(15, 16); see below].

The availability of mutations of the regulatory pathways controlling the expression of the antimicrobial peptides served to assess not only their induction but also their relevance in the host defense of insects. In particular, mutations affecting the Toll pathway, notably expression of the antifungal peptide drosomycin, lower the resistance to fungi but not to bacteria. Conversely, mutations affecting predominantly the induction of antibacterial peptides result in reduced survival to bacterial challenge, with a less marked effect in the case of fungal infection (10). Although these data underline the role that the selective induction of antifungal and antibacterial peptide synthesis plays in the resistance to infection in *Drosophila*, results obtained with mutants defective in hematopoiesis and mela-

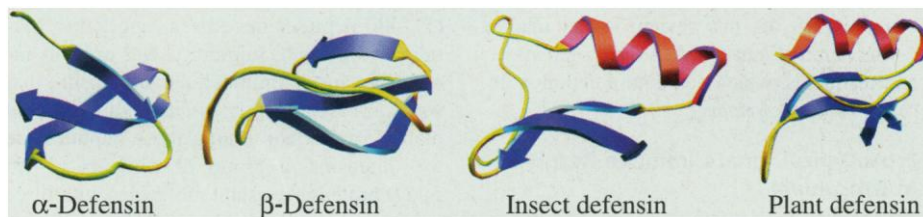


Fig. 1. Three-dimensional structures of eukaryotic defensins. Mammalian defensins are all β sheets, and the α and β forms differ by the array of disulfide bridges. Insect and plant defensins have an α -helix (red) linked to the β sheet (blue). Mammalian and insect defensins have three disulfide bridges and plant defensins have four (not shown). Structures were drawn from coordinates in Protein Data Bank where the codes are as follows: α -defensin, 1DFN; β -defensin, 1BNB; insect defensin, 1ICA; plant defensin, 1AYJ.

nization confirm that blood cells and the phenoloxidase cascade significantly contribute to this resistance (17).

The Innate Immune System of Mosquitoes

The order Diptera, to which *Drosophila* belongs, includes numerous hematophagous species that are vectors of major human diseases such as malaria, trypanosomiasis, and dengue fever. The African mosquito *Anopheles gambiae*, for example, is the major carrier of human malaria, a disease that afflicts hundreds of millions of people and kills about 2 million children each year. Historically, successful antimalarial efforts have required mosquito control measures. To be transmitted to the vertebrate host, the *Plasmodium* malaria parasite must complete development over 2 to 3 weeks as it traverses the midgut epithelium, the hemolymph, and the salivary gland of the mosquito vector. Huge losses of parasite numbers occur during this process, partially compensated by proliferation during the midgut-associated oocyst stage (18). At the extreme, the mosquito does not permit survival and transmission of the parasite: in genetically selected refractory mosquito strains, the parasites may be lysed as they traverse the midgut, or may be encapsulated and melanized at the early oocyst stage (19).

With the *Drosophila* model as a guide, the innate immune system of mosquitoes and other disease vectors has recently been submitted to intensive study (20). Components such as transcription factors, antimicrobial defensins and cecropins, binding proteins, and other putative members of innate immune cascades have been isolated by homology cloning, or by the empirical criterion of up-regulation upon immune challenge. With the use of these components as markers, it has become clear that the mosquito vector mounts a succession of multisite immune reactions—both systemically and locally in the traversed epithelia—during parasite development. The effect of these reactions on parasite survival remains to be fully evaluated, although clear indications exist that some reactions are functionally important, for example the up-regulation of nitric oxide synthase. The melanotic encapsulation form of refractoriness is a classical case of insect innate immune response, entailing both coagulation and phenoloxidase activation cascades that are as yet poorly defined. Immune-responsive and phenoloxidase-secreting hemocyte-like cell lines have recently been obtained and are a promising tool for unraveling the mechanisms of immune cascade regulation. Undoubtedly, the intellectual input from comparative studies on innate immunity will be invaluable in advancing the field, to the point that intervention through the vector immune system can be considered as part of an inte-

grated approach to the control of malaria and other parasitic diseases.

Innate Immunity in Mammals: Limiting Infectious Challenge

As in insects, a key feature of innate immunity in mammals is the ability to limit the infectious challenge rapidly. This is based on the capacity to discriminate species self from infectious nonself. Mammals have provided important paradigms for understanding the molecular basis of this recognition.

Microbes display molecular arrays or patterns that are recognized by pattern recognition molecules or receptors (PRM or PRR, respectively) (1, 21). These patterns seem to be shared among groups of pathogens; the lipopolysaccharides (LPS) of Gram-negative bacteria, the glycolipids of mycobacteria, the lipoteichoic acids of Gram-positive bacteria, the mannans of yeast, and double-stranded RNAs of viruses are representative examples. To limit infection, the mammalian host uses a wide armamentarium of pattern recognition molecules. These include complement, collectins, and a battery of antimicrobial peptides that act together with effector cells to combat the infectious challenge.

Recognition of endotoxin or LPS is an important function of innate immunity and may have profound consequences for the host. Failure to contain the infection can result in Gram-negative sepsis and septic shock as a result of the release of LPS (22). Whereas there are many descriptions of mammalian LPS-binding proteins (23), two homologous LPS-binding proteins, bactericidal/perme-

ability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBP) are of particular importance because LPS binding results in markedly different functional outcomes (23, 24). The role for BPI is directly antimicrobial. BPI is a 55-kD neutrophil granular pattern recognition molecule that has selective toxicity against Gram-negative bacteria. BPI consists of two functionally distinct domains, one that binds endotoxin and is antimicrobial and the other that is opsonic. BPI appears to be most effective when it acts at sites of inflammation in the context of the phagocytosing neutrophil in synergy with defensins (see below) and the membrane attack complex of complement. In contrast, LBP greatly enhances sensitivity to LPS, allowing effector cells to be triggered by subpicomolar concentrations of LPS. LBP recognizes lipid-A, the biologically reactive moiety of endotoxin (25). LBP plays an important role in the clearance of bacteria from the circulation that is mediated by CD14, as illustrated by data from LBP-deficient and CD14-deficient mice (25). Recent experiments (described below) indicate that mammalian Toll-like receptors are critical in LPS-mediated signaling in association with LBP and CD14.

A second family of first-line host defense molecules, the collectins, have collagen and lectin domains and a spectrum of activity broader than that of LBPs that includes microbes and viruses (26). Members of this family include the lung surfactant protein SP-A. Increased susceptibility of SP-A-deficient animals to a variety of pathogens indi-

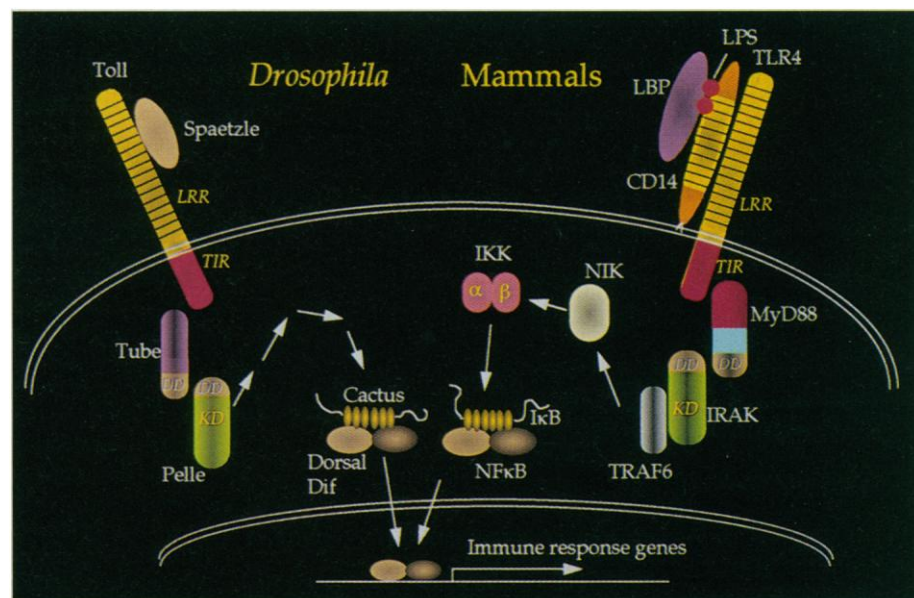


Fig. 2. Conserved pathways in innate immunity in *Drosophila* and mammals. Examples chosen are, left, the induction of the antifungal gene drosomycin by binding of processed Spätzle protein to the transmembrane receptor Toll and, right, activation of costimulatory protein genes by binding of a LPS-LBP-CD14 complex to a human Toll homolog, TLR4. DD, death domain; KD, kinase domain; LRR, leucine-rich domain; TIR, Toll/IL-1 receptor homology domain.

cates that this molecule acts locally to limit lung infection (27). Another collectin, the mannose-binding protein (MBP), has provided the most detailed understanding of recognition of molecular micro- and macropatterns. Human MBP is synthesized in the liver as an acute-phase reactant and is deployed to sites of infection where it interacts with the complement system (see below). MBP is considered as an "ante-antibody" with broad binding activity (28). MBP selectively recognizes the carbohydrate patterns that decorate microorganisms such as bacteria, yeast, parasites, mycobacteria, and certain viruses (28). Yet, despite this apparent promiscuity of ligand recognition, MBP does not recognize the sugars that decorate self glycoproteins. The explanation for this paradox has been provided by recent structural studies that define the micropattern recognized by MBP as the equatorial orientation of the C3-OH and C4-OH groups of the sugar moiety (29, 30) (Fig. 3A). This configuration is represented in the hexoses *N*-acetylglucosamine, glucose, and fucose as well as in mannose. The common feature of diverse cell wall structures like LPS, lipoteichoic acid, and mannans appears to be combinations of these sugars in the form of exposed saccharides that decorate the respective microorganisms; this pattern is broadly represented across microbial phyla. It is noteworthy that the configurations of OH groups in galactose and sialic acid, the penultimate and ultimate sugars that usually decorate mammalian glycoproteins, are not accommodated by the carbohydrate recognition domain (CRD) of MBP (29, 30).

On the basis of the three-dimensional

structure of human and rat MBP-CRD, it is clear that ligands have to span a distance of 45 Å between binding sites to achieve high-affinity binding (10^{-10} M) (Fig. 3B). Modeling experiments indicate that, in contrast to microbial cell walls, this macropattern is absent from even complex self glycoproteins. Furthermore, the ability of the multipronged binding sites in MBP (and other multipronged pattern recognition molecules) to recognize microbial structures may depend on the highly repetitive structure of the ligands in microbes. This repetitive structure permits all the prongs to engage. In contrast, the glycoproteins of higher animals are not arranged repetitively in the membrane and may be more mobile.

Mammalian Effector Molecules

As in *Drosophila*, antimicrobial peptides, phagocytosis, and proteolytic cascades concur in mammals to destroy the invading microorganism. Phagocytosis is a critical component, but a detailed description of its molecular mechanisms is beyond the scope of this review [see (31) for an update]. A rich array of antimicrobial peptides counter infection in mammals (6, 7). α -Defensins (Fig. 1) are major constituents of the microbicidal granules of blood granulocytes and are also abundantly expressed in intestinal epithelial cells specialized for host defense functions (Paneth cells). A constitutively expressed human epithelial β -defensin is abundant in the kidney and the urogenital tract, and an infection- or cytokine-inducible β -defensin is abundant in the skin. In addition to defensins,

mammals produce cathelicidins, a group of myeloid antimicrobial peptides that vary significantly by sequence, structure, and length and include α -helical, Cys-rich, Pro- and Arg-rich, and Trp-rich peptides (7).

Proteolytic cascades triggered by nonself recognition also have major roles in mammalian innate immunity. Paradigmatic is the complement cascade, which is activated either directly or indirectly by microorganisms and results in their opsonization for phagocytosis or the assembly on their surface of a pore-forming membrane attack complex (2, 32). There are three pathways of complement activation that differ in the initiation of the cascade leading to cleavage of the third complement component, C3. The classical pathway requires antibody and the first complement components, the alternative pathway is activated directly by the microorganism, and the lectin pathway requires MBP. The engagement of ligands by MBP results in the activation of the MBP-associated proteases, MASP1 and MASP2, which in turn activate the C3 convertase (33). MASPs have been identified in lamprey and tunicates and C3 in tunicates and sea urchins (34). This leads to the prediction that MBP, MASP and C3 may be the minimum ancestral components of complement. In this connection, studies on the invertebrate horseshoe crab *Limulus* (15) provide us with an even earlier link between recognition of microbial molecular patterns, proteolytic cascades, and activation of host defense. In this species, the serial activation of several serine protease zymogens by LPS or β (1-3) glucan results in the formation of an insoluble coagulin gel that limits the infection. The upstream LPS-activatable zymogen in this cascade has consensus repeats that are found in mammalian complement proteins, suggesting an early common origin of the complement and coagulation cascades.

Reciprocal Links Between Adaptive and Innate Immunity

The adaptive immune system appeared ~450 million years ago when a transposon that carried the forerunners of the recombinase activating genes, RAG-1 and RAG-2, was inserted into the germ line of early jawed vertebrates (35). The ability to mount an adaptive immune response allowed organisms to remember the pathogens that they had already encountered, and natural selection made the adaptive immune response a virtually universal characteristic of vertebrates. However, this did not lead to discarding the previous form of host defense, the innate immune system. Indeed, this earlier form of host defense has been coopted to serve a second function, stimulating and orienting the primary adaptive immune response by controlling the expression of costimulatory molecules.

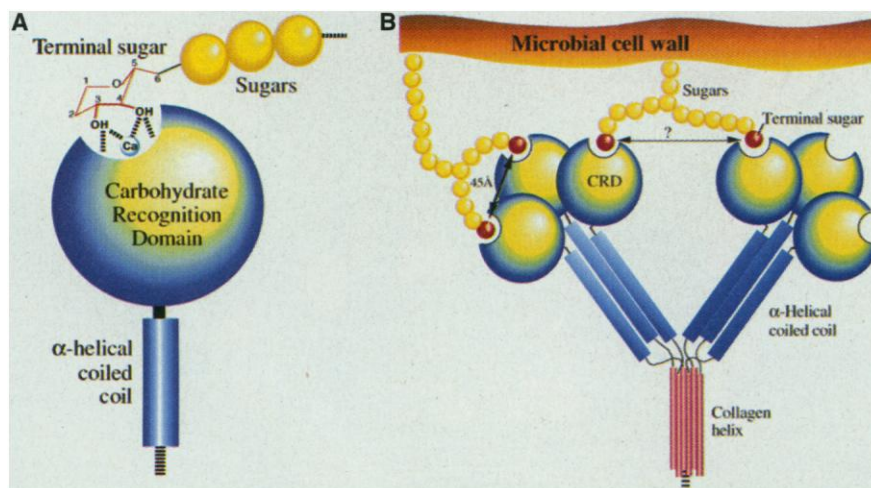


Fig. 3. Carbohydrate pattern recognition by MBP. (A) Atomic pattern of hydroxyls equivalent to equatorial C3- and C4-OH groups that is recognized by an individual carbohydrate recognition domain (CRD) (30). Such a pattern is present in mannose and fucose but not, for example, in sialic acid. (B) Molecular pattern recognized by MBP oligomers. The distances between CRDs within a trimer and between CRDs of different trimers are important parameters that would allow for high-affinity binding of carbohydrate chains with the correct minimum length to span these distances. Carbohydrates bound to MBP are shown in red, the remaining oligosaccharide chains are shown in yellow.

It had been surmised for a decade that cells of the innate immune system bear receptors for conserved molecular patterns associated with microbial pathogens. According to this model, when the protein antigens derived from pathogens are processed and presented as peptides that serve as the stimulus for specific T cell receptors, PRRs on the antigen-presenting cells also induce the synthesis of costimulatory molecules, cytokines, and chemokines. These activated antigen-presenting cells serve to attract and activate the antigen-specific T cells that are essential to all adaptive immune responses (1, 2, 21). It was known that the substances that can induce costimulation include bacterial LPS, synthetic double-stranded RNA, glycans, and mannans. Furthermore, experimental evidence indicated that the processed antigen ligand for the T cell had to be on the same cell as the costimulatory molecule. This is obviously of crucial importance for maintaining self-tolerance; bystander presentation of costimulatory molecules would mean that tolerance would be lost whenever an infection occurred.

To validate this model, it was necessary to identify receptors for microbial patterns that, upon binding pathogen ligands, initiate signaling cascades leading to the production of costimulatory molecules and cytokines. Molecules such as MBP do not qualify for this role, because they activate proteolytic cascades or promote phagocytosis but are not known to induce costimulation. The breakthrough came with the identification of a human homolog of Toll, initially cloned as a cDNA and later named hTLR4 (for human Toll-like receptor) (36). It turns out that an LPS-binding and signaling receptor complex is assembled when hTLR4 interacts with LPS bound to CD14, a peripheral membrane protein held to the cell surface by a glycosylphosphoinositol tail. The presence of LBP further increases signaling. The hTLR4 protein has a leucine-rich repeat sequence in its extracellular domain that interacts with CD14 complexed with LPS. TLR4 then transduces the LPS signal across the membrane because destructive mutations of this gene lead to an LPS-unresponsive state in mice, which are also deficient in the clearance of Gram-negative bacteria (37). It has since become apparent that humans, like flies, have numerous Toll-like receptors.

TLR4 and other TLRs have a cytoplasmic TIR (Toll-IL-1 receptor) homology domain (see above). This domain communicates with a similar domain on an adapter protein (MyD88) that interacts with TLR4 by means of a like:like interaction of TIR domains. The next interaction is between the adapter and a kinase, through their respective "death domains" (DD). The kinase in turn interacts with TNF receptor-associated factor-6 (TRAF-6) (38). After TRAF-6, two sequential kinase activation steps lead to phosphorylation of the inhibitory protein

I κ B and its dissociation from NF- κ B. The first kinase is a mitogen-activated kinase kinase kinase, or MAPKKK, known as NIK, for NF- κ B-inducing kinase. The target of this kinase is another kinase made up of two chains, called I κ B kinase α (IKK α) and I κ B kinase β (IKK β), that together form a heterodimer of IKK α :IKK β , which phosphorylates I κ B. NF- κ B translocates to the nucleus to activate genes with κ B binding sites in their promoters and enhancers such as the genes encoding IL-1 β , IL-6, IL-8, the p40 protein of IL-12, and the costimulatory molecules CD80 and CD86.

A simplified scheme of these cascades is presented in Fig. 2, together with the outlines of the signaling pathway that controls the synthesis of the antifungal peptide drosomycin in *Drosophila*. The parallels between the two systems are striking, both in terms of structures and functions. The signaling pathway in mammals contains several proteins whose counterparts have not yet been defined in the *Drosophila* immune response, but homologs of TRAFs and IKKs have now been cloned in several laboratories. Protein domains similar to those encountered in the insect and mammalian pathways are found in host defense in plants, the latter positionally cloned as proteins that confer resistance to various plant diseases (39). The shared modules include leucine-rich repeats, TIR domains, and serine-threonine kinases linked in multidomain proteins as in animals. It is a provocative thought that innate immunity in both plants and animals may have evolved from common ancestral modules that have been used to protect against infection for more than 1 billion years of evolution.

Innate Immunity and Human Disease

A central unifying theme emerging in the field is that the templates for innate immunity have been conserved from primitive life-forms to humans. It is clear that disruptions in innate immunity predispose humans to infection as illustrated by several examples. In the severely burned patient the disruption of the skin as not merely a barrier, but an organ adorned with antimicrobial peptides and first-line effector cells like macrophages, poses great risks of infection. In patients with cystic fibrosis, the alterations in salinity of the bronchial airway fluid appear to disable the function of antimicrobial peptides that are found in the respiratory epithelium, thereby leading to colonization and infection with organisms like *Staphylococci* and *Pseudomonas* (40). Mutations in genes that encode for complement proteins (40, 41) and MBP result in recurrent infections (42). As our understanding of the TOLL-LBP-CD14 pathway unfolds, new targets that modify these pathways may be effective lead compounds in the treatment of septic shock. Finally, the ability to produce large amounts of both insect and mammalian antimicrobial peptides may provide new classes of antibiotics.

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REVIEW

Bacterial Biofilms: A Common Cause of Persistent Infections

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Bacteria that attach to surfaces aggregate in a hydrated polymeric matrix of their own synthesis to form biofilms. Formation of these sessile communities and their inherent resistance to antimicrobial agents are at the root of many persistent and chronic bacterial infections. Studies of biofilms have revealed differentiated, structured groups of cells with community properties. Recent advances in our understanding of the genetic and molecular basis of bacterial community behavior point to therapeutic targets that may provide a means for the control of biofilm infections.

For quite some time we have known that bacteria can adhere to solid surfaces and form a slimy, slippery coat. These bacterial biofilms are prevalent on most wet surfaces in nature and can cause environmental problems. Perhaps because many biofilms are sufficiently thick to be visible to the naked eye, these microbial communities were among the first to be studied by the late-developing science of microbiology. Anton van Leeuwenhoek scraped the plaque biofilm from his teeth and observed the "animalculi" that produced this microbial community with his primitive micro-

scope. However, it was not until the 1970s that we began to appreciate that bacteria in the biofilm mode of existence, sessile bacteria, constitute a major component of the bacterial biomass in many environments (1), and it was not until the 1980s and 1990s that we began to appreciate that attached bacteria were organized in elaborate ways (2). For example, different bacterial species specifically attach to different surfaces or coaggregate with specific partners in the mouth (3). Often one species can coaggregate with multiple partners, which themselves can aggregate with other partners to form a dense bacterial plaque. Advances in light microscopy coupled with developments in microelectrode technology have led to an appreciation that bacterial biofilms consist of microcolonies on a surface, and that within these microcolonies the bacteria have developed into organized communities

with functional heterogeneity.

Because bacterial biofilms can cause environmental problems and studies of biofilms have required the development of new analytical tools, many recent advances have resulted from collaborations between microbial ecologists, environmental engineers, and mathematicians. These efforts have led to our current definition of a bacterial biofilm as a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface.

Biofilms constitute a protected mode of growth that allows survival in a hostile environment. The structures that form in biofilms contain channels in which nutrients can circulate (4), and cells in different regions of a biofilm exhibit different patterns of gene expression (5). The complexity of biofilm structure and metabolism has led to the analogy of biofilms to tissues of higher organisms (6). These sessile biofilm communities can give rise to nonsessile individuals, planktonic bacteria that can rapidly multiply and disperse. The common view is that planktonic bacteria must expose themselves to deleterious agents in their environment, be they phage or amoeba in nature, biocides in industrial settings, or potent antimicrobial agents in a clinical setting. In this light, it is not surprising that

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