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 immunological memory. Only quite recently has innate immunity gained renewed interest, particularly as it became apparent that (i) phagocytosis of invading microorganisms by blood cells, (ii) proteolytic cascades leading to localized blood clotting, melanin formation, and opsonization, 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...
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. Others contain a high content of a given amino acid, for instance His in histatins, Pro in bacteriocins and drosocin, and Gly in attacins and diptericin (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 potently antifungal, whereas the others (ccecropsins, diptericin, 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-κB) family of inducible transactivators. When κB-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 (IκB)-like protein, or is translocated into the nucleus to act as a transcription factor. The extracellular portion of this cascade comprises four serine pro-
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 integrated 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). Where-as there are many descriptions of mammalian LPS-binding proteins (23), two homologous LPS-binding proteins, bactericidal/permeability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBPs) 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 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 in-

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![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 Spaetzle 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.]
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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 pentultimate 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, which includes the neck domain, 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 proteasezymogens by LPS or β(1-3) glucan results in the formation of an insoluble coagulin gel that limits the infection. The upstream LPS-activatablezymogen 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.
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 glycosyl-phosphoinositol 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 destructive mutations of this gene lead to an LPS-unresponsive state in mice, which are...

References and Notes
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 microsco...