Abstract—The family of Toll-like receptors plays an essential role in the induction of the immune response. These receptors sense the presence of microbial ligands and activate the nuclear factor-κB transcription factor. We review the key studies that led from the formulation of the concept of pattern recognition receptors to the characterization of Toll-like receptors, insisting on the important role played by the model organism Drosophila melanogaster and on the increasing evidence connecting these receptors to cardiovascular disease. (Arterioscler Thromb Vasc Biol. 2011;31:1734-1738.)

Key Words: immune system • macrophages • receptors • viruses

Toll-like receptors (TLRs) owe their names to a famous Drosophila gene, Toll. The name comes from the vernacular German Toll, meaning super or fantastic. It was used in the early 1980s by C. Nüsslein-Volhard to qualify the phenotype of a new mutant discovered in her pioneering mutagenesis screen to dissect the genetic pathways controlling embryonic development in the fruit fly Drosophila melanogaster. The Toll gene is involved in the differentiation of the dorso-ventral axis of the embryo; some mutant alleles (loss-of-function) cause dorsalization of the embryo, whereas others (gain-of-function) cause a ventralization. It took several years to realize that this receptor also has important immune functions in adult flies and that its orthologues in mammals play a key role in innate immunity (Figure 1).

Innate immunity is the first-line host defense mechanism that operates to protect animals (vertebrates and invertebrates) from infectious microorganisms. As such, it plays a critical role in containing most infections. In addition, molecules induced during the innate immune response, including cytokines and costimulatory molecules, play a critical role in the induction of the adaptive immune response in mammals. C. Janeway was among the first to stress the importance of recognizing that the immune response to infectious microbes involves “not only specific antigen determinants, but also certain characteristics or patterns common on infectious agents but absent from the host.”

Pattern Recognition Receptors: From the Concept to the Discovery of TLRs

In his concluding lecture of the 1989 Cold Spring Harbor meeting on innate recognition, Janeway insisted on the fact that, in addition to the first signal delivered by the interaction of the antigen receptor with its specific ligand, lymphocytes require a second signal, coming from the antigen-presenting cells, to be activated. Because the pattern recognition receptors (PRRs) recognizing these microbial molecules on the antigen-presenting cells had to be limited in number and encoded in the germline (as opposed to the rearranging antigen receptors of the adaptive immune response), he postulated that these molecules should obey the following 3 rules: (1) they are absent from the host, allowing discrimination between self and nonself; (2) they are conserved among large numbers of microorganisms, allowing recognition of a wide array of microorganisms by a limited number of receptors; and (3) they are essential constituents of the microorganisms, thus preventing escape from recognition by the innate immune system through mutation. For example, the outer membrane of all Gram-negative bacteria is composed of lipopolysaccharides (LPS), whereas most viruses produce double-stranded RNA molecules in infected cells. Peptidoglycan, bacterial lipopeptides, and unmethylated CpG DNA motifs are other examples of microbial molecular patterns, sometimes called pathogen-associated molecular patterns, although they are not solely expressed by pathogens. Most of these molecules were known at the time to induce signaling cascades activating the transcription factor nuclear factor-κB (NF-κB) and leading to inflammation, but the nature of their receptors remained unknown. The fruitless search for PRRs led to an alternative model in the mid-1990s, heralded by P. Matzinger; this model postulated that danger signals, rather than nonself, was the main driving force for the immune system.
defense mechanisms in model organisms prone to genetic analysis, such as the fruit fly *Drosophila*, may shed light on the nature of the elusive PRRs. Infection of insects leads to the secretion in the hemolymph (insect blood) of a family of potent cationic antimicrobial peptides. Initially characterized by H. Boman and his colleagues in 1981 in the moth *Hyalophora cecropia* (which gave its name to cecropin, the first identified antimicrobial peptide), these peptides were subsequently shown to also participate to host-defense in plants and mammals (eg, defensins, cathelicidins). The analysis of the inducible promoters of the genes encoding these peptides, performed in parallel on *cecropin* by Boman and his collaborators, and *diptericin*, a novel peptide identified in dipteran insects (the group to which *Drosophila* belongs) by J. Hoffmann et al, revealed the importance of DNA motifs strongly similar to the binding sites for the mammalian transcription factors of the NF-κB family. This striking result, which was the first indication that evolutionary conserved mechanisms may be involved in the control of innate immunity in mammals and insects and that *Drosophila* may be a useful model to define molecularly the PRRs, led to a Human Frontier Science Program–sponsored collaboration involving in particular the laboratories of Hoffmann at Strasbourg and Janeway at Yale.

At the time, the only known NF-κB gene in *Drosophila* was *dorsal*, which encodes the transcription factor activated by Toll on the ventral side of the embryo. However, induction of *diptericin* was not affected in *dorsal* mutant flies or in other mutants of the Toll pathway. A different picture emerged when a new marker was included in this analysis, the gene encoding the antifungal peptide *Drosomycin*, discovered in 1994. Indeed, genetic analysis revealed that the induction of the *drosomycin* gene is controlled by a transcription factor closely related to Dorsal, Dorsal-related immunity factor, whose activity is induced by the Toll pathway. A third *Drosophila* NF-κB family member, Relish, controls expression of *diptericin* on activation by the immune deficiency pathway (reviewed by Hoffmann and Reichhart).

*Toll* mutant flies, which fail to induce expression of Drosomycin, are highly susceptible to fungal infections and...
succumb rapidly to a challenge with the opportunistic fungus Aspergillus fumigatus. When these results were discussed in the framework of the collaboration between the Strasbourg and Yale laboratories, they prompted the search for orthologues of Toll in mammals, and the first human Toll was described in 1997. The scope of that report was limited to the induction of cytokines and the costimulatory molecule CD80, which are required to activate naïve T cells, by a constitutively active mutant receptor. The crucial data showing that TLR activation can occur at the cell terminus and the C terminus of the ectodomain. Although the N terminus and the central region of the ectodomain. The double-stranded RNA helix interacts with 2 distinct sites near the N terminus and the C terminus of the ectodomain. Finally, in the case of TLR3, the double-stranded RNA helix interacts with 2 distinct sites near the N terminus and the C terminus of the ectodomain. Although the emerging picture is that TLRs interact with different ligands in highly divergent ways, in all cases ligand interaction between different members of the family. In the case of TLR1 and TLR2, the acyl chains of lipopeptides bind to hydrophobic pockets located on the convex side of the horseshoe in the 2 subunits, thus cross-linking them. In the case of TLR4, the ligand is not LPS itself but a complex of LPS and MD2, which associates with the concave surface of TLR4 ectodomain. Interactions involving the Toll/interleukin-1R domain adapters MyD88 and DmMyD88, the DD kinases IRAK and Pelle, the inhibitors IxB and Cactus, and the Rel family transcription factors NF-xB and Dif. Mammalian TLRs are activated on direct binding of microbial molecular patterns, whereas Drosophila Toll is activated by the cytokine Spaetzle. Detection of microbial molecular patterns or virulence factors by different sets of sensors in Drosophila activates a proteolytic cascade, leading to Spaetzle activation. See the text for details. PGN indicates peptidoglycan; dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; Dif, Dorsal-related immunity factor.

Structure and Function of TLRs
We now know that TLR activation can occur at the cell surface or in endosomes. At the plasma membrane, TLR2 associates with TLR6 or TLR1 to sense either di- or triacylated lipoproteins, respectively. TLR5 and TLR11 (the latter present in mice only) sense microbial proteins. TLR5 is a receptor for flagellin, recognizing a peptide motif essential for the buildup of the bacterial flagellum, thus explaining why it is difficult for the bacteria to change this sequence to avoid recognition. In the endosomal compartment, other TLRs sense internalized nucleic acids: TLR3 interacts with double-stranded RNA, TLR7 and 8 recognize single-stranded RNAs enriched in U residues, and TLR9 detects unmethylated CpG DNA motifs (reviewed by Kawai and Akira) (Figure 2). These TLRs play essential roles in the sensing of viral infections, as illustrated by the development of herpes simplex virus-1 encephalitis in children with TLR3 deficiency. Some TLRs need coreceptors to accommodate their ligands, such as the lipid-binding molecule MD2 and the leucine-rich repeat protein CD14 for TLR4 and the scavenger receptor CD36 for TLR2. The TLRs are therefore bona fide PRRs, interacting with a diverse set of microbial ligands and signaling to trigger innate immunity. How TLRs can accommodate a range of ligands, from nucleic acids to proteins and lipids, has long been a mystery. Indeed, TLRs all share the same domain organization, with an ectodomain composed of leucine-rich repeats and a 150-amino-acid intracytoplasmic domain homologous to that of the interleukin-1 receptor (Toll/interleukin-1 receptor domain). Like all leucine-rich repeat proteins, the ectodomain of TLRs forms characteristic horseshoe-shaped structures. Interestingly, recent structural analysis of TLRs complexed with their ligands revealed a remarkable versatility of ligand interaction between different members of the family. In the case of TLR1 and TLR2, the acyl chains of lipopeptides bind to hydrophobic pockets located on the convex side of the horseshoe in the 2 subunits, thus cross-linking them. In the case of TLR4, the ligand is not LPS itself but a complex of LPS and MD2, which associates with the concave surface provided by the N terminus and the central region of the TLR4 ectodomain. Finally, in the case of TLR3, the double-stranded RNA helix interacts with 2 distinct sites near the N terminus and the C terminus of the ectodomain. Although the emerging picture is that TLRs interact with different ligands in highly divergent ways, in all cases ligand binding induces...
the C termini of the ectodomains to move close to each other, thus initiating signaling and triggering inflammation (reviewed by Jin and Lee).\textsuperscript{14}

**Activation of Innate Immunity: Danger Versus Nonself Pattern**

Twenty years after the introduction of the PRR concept, the discovery and characterization of not only TLRs but also the Nod-like receptors, RIG-I-like receptors, C-type lectin receptors, peptidoglycan recognition proteins, and β-Glucan binding proteins have validated the concept and demonstrated that nonself recognition plays an important role in the activation of the innate immune response.\textsuperscript{15,16} However, the alternative “danger model” is still on the agenda. Indeed, the Toll pathway in *Drosophila* is not solely activated by microbial molecular patterns but can also be activated on sensing virulence factors. The circulating serine protease Persephone senses the presence of abnormal proteolytic activities secreted by invading fungi or bacteria and triggers the processing and activation of the Toll ligand Spätzle independently of the PRRs.\textsuperscript{17,18}

In mammals, several reports indicate that TLRs can be activated by endogenous molecules, generated as a result of cell death or tissue damage. These include oxidized lipoproteins, extracellular matrix components (eg, hyaluronic acid fragments, versican), or heat-shock proteins (Kawai and Akira\textsuperscript{12} and references therein). Of note, the interaction of TLRs with these endogenous agonists has not yet been structurally characterized. A complicating issue for these studies is that because of the exquisite sensitivity of TLRs, even the slightest contamination with microbial ligands (such as can occur for LPS and recombinant proteins), can bias the experiments.\textsuperscript{19} Self nucleic acids can also be released from dying cells and activate TLR7 and TLR9, thus triggering type I interferon production. Interferons then drive the expansion of B cell clones with specificity for nucleic acids and lead to autoimmune diseases, such as systemic lupus erythematosus.\textsuperscript{20}

The discovery that TLR signaling can be induced by endogenous molecules opens exciting perspectives for the treatment of acute and chronic inflammation and the associated diseases, in particular those affecting the cardiovascular system.

**TLRs in Cardiovascular Disease**

The association between bacterial infections and atherosclerosis prompted the search for biological functions of TLRs in blood vessels.\textsuperscript{21} Several TLRs, including TLR1, -2, and -4, are expressed by macrophages in atherosclerotic plaques in mice and humans.\textsuperscript{22,23} Studies using mutant mice further established that TLR4 has proatherogenic effects.\textsuperscript{24,25} Similarly, modulation of TLR2 activity affects atherosclerosis, with a mild reduction of the disease in low-density lipoprotein receptor (*Ldlr*) knockout mice in the absence of TLR2 and a strong increase of the atherosclerotic burden in *Ldlr* knockout mice treated with the triacylated lipid Pam3CSK4, which acts as a TLR2 ligand.\textsuperscript{26} TLR2 also plays a critical role at later stages during the conversion of atherosclerotic lesions into necrotic plaques. The macrophage apoptosis accompanying this process is triggered by reticulum endoplasmic stress and stimulation with atherogenic lipids, sensed by TLR2 and its coreceptor CD36.\textsuperscript{27}

Not all TLRs appear to affect atherosclerosis similarly. Indeed, TLR3, which signals through different adapters than do TLR2 and TLR4, appears to play a protective, rather than detrimental, role in the vessel wall of hypercholesterolemic ApoE/−/− mice.\textsuperscript{28} Thus, it is now well established that TLRs play complex roles in the onset and progression of atherosclerosis. Importantly, these receptors have also been connected to myocardial diseases,\textsuperscript{21} an area that certainly deserves further investigation in view of its clinical importance.

**Concluding Remarks**

The discovery of the critical role played by Toll receptors in innate immunity has been a fantastic adventure, and it illustrates the importance of invertebrate models to decipher critical biological pathways. We now know that several families of PRRs exist and play important roles in the onset of inflammation and the innate immune response, as once predicted by Janeway.\textsuperscript{2} Among these PRRs, the TLRs are the best studied, genetically and structurally. Interesting potential clinical and pharmacological developments are in sight.\textsuperscript{29,30} It is also becoming apparent that sensing virulence factors or tissue damage (ie, danger) can activate innate immunity by mechanisms that remain to be fully investigated, in particular in mammals. The *Drosophila* model undoubtedly holds promise not only for providing insights in this area but also for other complex topics involving the immune system, such as gut homeostasis, sterile inflammation, antiviral defenses, and of course the control of pathogen transmission to humans by insect vectors.

**Disclosures**

None.

**References**


