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Toll gates for pathogen selection

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Two classes of innate immune receptors discriminate between different microbial pathogens to mediate host defence by mammalian macrophages.

All species need an immediate, systemic reply to the microbial pathogens in their environment. This reply — known as the innate immune response — is characterized by the *de novo* production of mediators that either kill the pathogen directly or induce phagocytic cells to ingest and kill it. In the fruit fly *Drosophila melanogaster*, distinct cell-surface receptors belonging to the Toll family mediate separate anti-bacterial and anti-fungal responses in the same type of cell[1]. They do this by inducing genes that encode anti-microbial peptides.

Does this model describe the situation in mammalian systems? On page 39 of this supplement, Underhill *et al.*[2] describe the ligand specificity of two members of the mammalian Toll-like receptor family, TLR2 and TLR4. These data show that the situation is indeed similar to that in *Drosophila* — that is, two distinct Toll-like receptors, both expressed in macrophages, discriminate between different microbial pathogens or their products. Whereas TLR4 is the main protein involved in recognizing Gram-negative bacteria and lipopolysaccharide (a glycolipid constituent of the bacterial outer membrane), TLR2 is the key in responses to other types of microbial pathogen, such as yeast and Gram-positive bacteria.

The innate immune response is often driven through recognition, by the host cell, of surface components of the microbial pathogen. Monocytes and macrophages are central to the intensity and specificity of this response. Most — if not all — microbial pathogens are thought to activate the innate immune system using mechanisms with common features. For example, many macrophages contain a surface protein called CD14, which binds ligands such as lipopolysaccharide and triggers an innate immune response[3]. But CD14 is not thought to participate directly in signalling. Rather, one or more of the mammalian Toll-like receptors acts in concert with CD14 to discriminate between microbial pathogens or their products[1] and initiate transmembrane signalling.

Mammalian cells may express as many as ten distinct Toll-like receptors[4]. All span the cell membrane, with repeating leucine-rich repeats in the external domain and a common sequence motif — the Toll-homology domain — in the cytoplasmic tail. Members of the interleukin-1 receptor family, which is another essential element of the innate immune system, also contain a Toll-homology domain in their cytoplasmic tails. Most attention has been paid to the TLR2 and TLR4 proteins, and the importance of TLR4 in responses to Gram-negative bacteria and lipopolysaccharide was first suggested by powerful genetic data. Beutler and colleagues[5] established that TLR4 is encoded by the lipopolysaccharide (*Lps*) gene, and that it controls sensitivity to this molecule. Akira and colleagues[6] also showed that mice in which the *TLR4* gene has been deleted have the same defects as mice with mutations in *Lps*.

But some reports indicate that lipopolysaccharide acts via TLR2. When TLR2 is expressed in cell lines that do not normally produce it, these cells become able to respond to lipopolysaccharide. Moreover, in at least one case, lipopolysaccharide-induced activation of a monocytic cell line is blocked by an anti-TLR2 monoclonal antibody[7]. So TLR2 has been considered a lipopolysaccharide receptor. But do these findings accurately reflect the physiological pathways of innate immunity?

These uncertainties can now be put aside thanks to the data from Underhill *et al.*[2] and to a report by Takeuchi *et al.*[8] in this month's *Immunity*. These authors found that mice lacking TLR2 respond to lipopolysaccharide in the same way as do wild-type animals. They characterized responsiveness to lipopolysaccharide in both whole animals and macrophages. When the authors looked at macrophages from their TLR2-deficient mice, they found them to be less responsive than those from wild-type mice to cell-wall preparations from several distinct Gram-positive bacteria. These data not only support the idea that TLR4 is the essential element of a lipopolysaccharide–receptor complex that determines subsequent cellular responses to lipopolysaccharide, but they also point to a key role for TLR2 in innate immune responses to other microbial pathogens and their products. And although the initial findings implicating TLR2 as a lipopolysaccharide receptor seem unlikely to be physiologically relevant, they should nonetheless be recognized for having opened a new chapter in innate immunity by showing that Toll-like receptors can activate cells in a ligand-specific way.

Where does this rapidly emerging field go now? Once again, studies in *Drosophila* may lead the way. For instance, Levashina *et al.*[9] have highlighted the importance of proteases in controlling expression of the *spaetzle/Toll/cactus* genes in an anti-fungal defence system. This group showed that the absence of a serine protease inhibitor (serpin) encoded by the *Spn43Ac* gene results in constitutive activation of an innate immune response in the fly. Their findings support two main ideas: first, that the protease system activated by exposure to a microbial pathogen (or a product derived from that pathogen) is an essential control point in innate immunity; and second, that the Toll protein is not itself a pattern-recognition receptor in *Drosophila*[10].

Given the parallels between the innate immune response in flies and in man, it is reasonable to look towards determining the ligand specificity of all the Toll-like receptors. In mammals, are the pathogen-activated protease cascades represented by proteins in the complement and coagulation pathways? Or will careful analyses reveal that cell-surface protease systems work in concert with CD14 and members of the Toll-like-receptor family to generate innate immune responses? Whatever the case, such studies should uncover in more detail the conserved ancestral features of the two systems of innate immunity that are now being worked out in the *Drosophila* and mammalian systems. ■

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1. Williams, M. J., Rodriguez, A., Kimbrell, D. A. & Eldon, E. D. *EMBO J.* **16,** 6120–6130 (1997).
2. Underhill, D. M. *et al. Nature* **401,** 811–815 (1999).
3. Ulevitch, R. J. & Tobias, P. S. *Annu. Rev. Immunol.* **13,** 437–457 (1995).
4. Rock, F. L., Hardiman, G., Timans, J. C., Kastelein, R. A. & Bazan, J. F. *Proc. Natl Acad. Sci. USA* **95,** 588–593 (1998).
5. Poltorak, A. *et al. Science* **282,** 2085–2088 (1998).
6. Hoshino, K. *et al. J. Immunol.* **12,** 3749–3752 (1999).
7. Brightbill, H. D. *et al. Science* **285,** 732–736 (1999).
8. Takeuchi, O. *et al. Immunity* **11,** 443–451 (1999).
9. Levashina, E. A. *et al. Science* **285,** 1917–1919 (1999).
10. Medzhitov, R. & Janeway, C. A. Jr *Proc. Natl Acad. Sci. USA* **95,** 429–430 (1998).