Toll and Toll-like receptors in Drosophila

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Abstract
The Drosophila Toll receptor controls the immune response to Gram-positive bacteria and fungi by activating a signalling pathway partially conserved throughout evolution. The Drosophila genome encodes eight additional Toll-related receptors, most of which appear to carry out developmental rather than immune functions. One exception may be Toll-9, which shares structural and functional similarities with mammalian TLRs.

Introduction
Innate immunity represents a rapid and efficient response that multicellular organisms mount to defend themselves against infection. This first-line host defence system is conserved throughout evolution from invertebrates to vertebrates. In mammals, it plays an active role in the onset of the specific adaptive response. Activation of innate immunity relies on germ-line-encoded receptors that recognize conserved microbial molecular patterns and are known as pattern recognition receptors (PRRs) [1]. The genetic tools developed in Drosophila, together with the fact that its immune system is devoid of the adaptive component, make this organism a powerful model to study the innate immune response. The fly’s immune response is characterized by the synthesis of anti-microbial peptides by the fat body, an equivalent of the mammalian liver. These peptides can be used as efficient markers of the immune response, as their expression is significantly up-regulated following infection [2]. In 1996, it was shown that the Toll receptor controls the induction of one of these peptides, Drosomycin, following fungal infection. By contrast, induction of the anti-bacterial peptide Diptericin in response to Gram-negative bacterial infections involves a Toll-independent pathway [2]. In mammals, work in the past 5 years has established Toll-like receptors (TLRs) as primordial receptors involved in the innate immune response mediating host response to distinct microbial patterns, from bacterial cell-wall lipopolysaccharide (LPS) (TLR4) to viral double-stranded RNA (TLR3) (reviewed in [3]).

The Drosophila Toll pathway
The Drosophila Toll receptor was initially discovered in the early 1980s through a mutagenesis screen for genes involved in dorso-ventral patterning of the Drosophila embryo. The gene was cloned in 1988, and shown to encode a transmembrane receptor, characterized by the presence of an intracytoplasmic domain that shows striking similarities to that of the interleukin-1 receptor (IL-1R). It is therefore referred to as the TIR (Toll/IL-1R receptor) domain. Unlike that of the IL-1R, the ectodomain of Toll is not composed of immunoglobulin-like motifs, but rather of leucine-rich repeats (LRRs), flanked by characteristic cysteine-rich motifs (Figure 1) (reviewed in [4]).

A complex of signalling adapters assembles around the TIR domain of Toll. DmMyD88, which contains both a TIR domain and a death domain (DD), plays a central role in this complex, and interacts with the DD-containing kinase Pelle, the homologue of IRAK (‘IL-1R-associated kinase’) [5–8]. Toll signalling requires another adapter-like molecule, Tube, which interacts with both DmMyD88 and Pelle. Interestingly, TLR2 and TLR4 also require an additional adaptor, namely Mal/TIR domain-containing adapter protein (TIRAP), to activate nuclear factor κB (NF-κB) [9,10]. Despite these homologies, there are some differences between the Toll receptor complex and that of TLRs; indeed, whereas in Drosophila, the DD domain of DmMyD88 acts as dominant-negative, in the mammalian system, it is the TIR domain that has this property. This may be correlated with the fact that Tube contains a DD, whereas Mal/TIRAP contains a TIR domain. In addition, DmMyD88 contains a 150-amino-acid C-terminal extension, which is absent from the mammalian factor MyD88, and is mandatory for proper signalling through Toll in Drosophila cells [11].

Activation of the receptor complex leads to degradation of the inhibitory κB (IκB) homologue Cactus, enabling nuclear translocation of the NF-κB-like transcription factor Dif, which promotes expression of the drosomycin gene [12]. How Pelle signals to Cactus is still a matter of controversy. A TRAF-6 (‘tumour necrosis factor receptor-associated factor-6’)-like molecule has been identified in Drosophila, but so far there is no genetic evidence that it is involved in the Toll pathway. Furthermore, the Drosophila IKKβ and IKKγ homologues (where IKK represents ‘IκB kinase complex’) are not involved in the regulation of Cactus degradation, pointing to major differences in the components linking the Toll–TLR receptor complex to the nucleus in flies and mammals [2].

Key words: bacterial infection, fruit fly, innate immunity, signal transduction.

Abbreviations used: PRR, pattern recognition receptor; TLR, Toll-like receptor; LPS, lipopolysaccharide; IL-1R, interleukin-1 receptor; TR, Toll/IL-1R, TIRAP, TIR-domain-containing adapter protein; LRR, leucine-rich repeat; DD, death domain; NF-κB, nuclear factor κB; IκB, inhibitory κB; Spz, Spätzle; PGRP, peptidoglycan recognition protein.

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The *Drosophila* family of Toll receptors

As mentioned above, the response to Gram-negative bacterial infections in *Drosophila* is under the control of a Toll-independent pathway. The discovery that TLR4 played a crucial role in the response to LPS derived from Gram-negative bacteria suggested that a Toll-related receptor would be involved in the response to Gram-negative bacterial infections in *Drosophila*. Indeed, the *Drosophila* Toll family counts nine members (Toll, 18-Wheeler and Toll-3 to -9), which makes it comparable in size with that of mammalian TLRs [13,14]. Overexpressing constitutively active versions of all the Tolls, be it alone or in combinations, does not mimic the effects of LPS treatment in the *Drosophila* macrophage-like S2 cells. Furthermore, inhibition of these receptors by RNA interference does not affect the cells’ response to LPS or bacteria. Finally, *18w* mutant flies were recently shown to resist infection by bacteria or fungi, much as wild-type flies, indicating that this receptor does not participate in host defence [15]. So far, mutants for the other *Toll* genes are not available. However, the fact that *DmMyD88* mutant flies resist infection by Gram-negative bacteria much like wild-type flies does not support the hypothesis that Toll receptors participate in the sensing of Gram-negative bacterial infections [6]. This raises the question of the role of the eight other members of the family. In fact, most *Tolls* are highly expressed during embryogenesis and metamorphosis [13]. *In situ* hybridization shows dynamic tissue- and stage-specific expression of these receptors, suggesting that they play a role in embryonic development [16]. In keeping with this possibility, phylogenetic analysis of TIR domains indicates that, with the exception of Toll-9 (see below), *Drosophila* Tolls are more closely related to each other than to mammalian TLRs [17]. This suggests that these two groups of receptors evolved independently, possibly to carry distinct functions: a developmental role in insects, and a role in host defence in mammals.

Detection of infectious non-self in *Drosophila*

The results discussed above leaves the question open as to the identity of PRRs in *Drosophila*. Although Toll mediates important receptor functions during infection in *Drosophila*, it is not a PRR. Rather, Toll is activated by the cytokine Spätzle (Spz). Upon infection, proteolytic cleavage of Spz yields a 12 kDa C-terminal fragment that is thought to bind to and activate Toll [18]. Recognition of Gram-positive bacteria involves the secreted peptidoglycan recognition protein (PGRP)-SA, which is thought to activate as-yet-unknown proteases [19]. In the case of fungal infections, an as-yet-unknown PRR activates the serine protease Persephone [20]. Thus, in *Drosophila*, non-self recognition occurs upstream of Toll, and activates a proteolytic cascade that generates an active ligand for Toll. PGRPs are highly conserved from...
insects to mammals, and the *Drosophila* genome contains 13 members of this family, either secreted or membrane-associated [21]. One of these, PGRP-LC, was recently shown to be an essential component of the receptor complex sensing Gram-negative bacterial infections [22–24]. Therefore individual members of this family may be activated by specific microbial patterns, in the same way as TLRs are in mammals.

Toll-9 is structurally related to TLRs, both in the TIR domain [17] and in the ectodomain, as it is the only *Drosophila* Toll receptor that does not have N-flanking cysteine-rich motifs (Figure 1). Interestingly, transfection of S2 cells with a constitutively active version of Toll-9 leads to activation of the *drosomycin* promoter, suggesting that it can signal to the IκB homologue Cactus (Figure 2; also see [14]). This activation is blocked by dominant-negative versions of DmMyD88 or Pelle, implying that Toll-9 uses the same signalling pathway as Toll does to promote *drosomycin* expression (Figure 2).

**Concluding remarks**

In conclusion, the Toll receptor plays a critical role in the onset of the immune response in *Drosophila*. However, it does not function like mammalian TLRs, and requires the cytokine Spz for its activation. Genetic screens in *Drosophila* have recently identified another evolutionary conserved family of receptors, the PGRPs, one of which functions upstream of Toll in the response to Gram-positive bacterial infections. *Drosophila* also expresses one TLR-like molecule, Toll-9. Identification of the stimuli to which it responds, and characterization of its function in *Drosophila*, could shed light on the ancestral function of TLRs.

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**References**


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