Minireview

Fly immunity: great expectations

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Abstract

Preliminary analysis of the *Drosophila* genome sequence reveals important similarities and differences between the functioning of mammalian and invertebrate immune systems.

Immunology, like many other areas of biomedicine, has gained invaluable insights from studies of *Drosophila*. This may sound odd for those who think of the immune system in terms of antibodies and lymphocytes. Indeed, as an invertebrate, *Drosophila* does not have 'adaptive' immunity - the remarkably elaborate defense system that employs millions of antigen-specific receptors generated by T and B lymphocytes. All multicellular organisms, however, including humans and flies, have the innate capacity to recognise and destroy pathogens. Some key components of the innate immune system are quite ancient in origin, and must have evolved prior to the divergence of the plant and animal kingdoms some billion years ago. This degree of conservation makes the study of model organisms all the more valuable and the recent completion of the *Drosophila* genome sequence [1-3] is likely to dramatically accelerate the analyses of many aspects of immunity in both *Drosophila* and mammals.

The innate immune system uses a set of germline-encoded receptors, sometimes called pattern recognition receptors, that evolved to recognize conserved pathogen-associated molecules, such as bacterial lipopolysaccharide (LPS), peptidoglycan, and mannan [4]. Ligation of the pattern recognition receptors by microbial products leads to the activation of one of the three defense mechanisms in *Drosophila*: phagocytosis; proteolytic clotting cascades in the hemolymph; and induction of antimicrobial peptides [5,6]. Accordingly, three different types of pattern recognition receptors are thought to mediate these responses: phagocytic receptors are expressed on the surface of hemocytes; soluble receptors are secreted into the hemolymph; and, finally, receptors of the Toll family are expressed on the cells of the fat body (the insect analog of the mammalian liver), hemocytes, and surface epithelia. All three defense mechanisms have counterparts in the mammalian immune system - hemocytes are equivalent to macrophages, the clotting cascade is equivalent to the complement system, and antimicrobial peptides are found in humans as well as flies. This often allows the identification of homologous receptors and effector molecules between flies and humans.

Cloning by homology requires that we know enough about the function of a given protein family. In the case of pattern recognition, at least three types of protein domain are most commonly found to mediate the recognition of microbial structures: the carbohydrate recognition domain (CRD) of the C-type lectin family, the leucine-rich repeat (LRR) domain, and the scavenger receptor cysteine-rich (SRCR) domain. Pattern recognition receptors with CRD domains have been known for quite some time in the mammalian system, and some of them are fairly well characterized [7]. The macrophage mannose receptor and the mannan-binding lectin (MBL), for example, mediate the phagocytosis of microbial pathogens and the activation of the lectin pathway of complement, respectively. The basis for microbial recognition by these receptors is well understood, and has to do with the identity of amino acid residues at positions 185 and 187 (in the rat MBL) in the ligand-binding pocket of the CRD. Amino acids at these positions can distinguish between equatorial and axial orientation of the 3-OH group of the bound hexose. Thus Glu185 and Asn187 would form hydrogen bonds with the equatorial 3-OH group of the

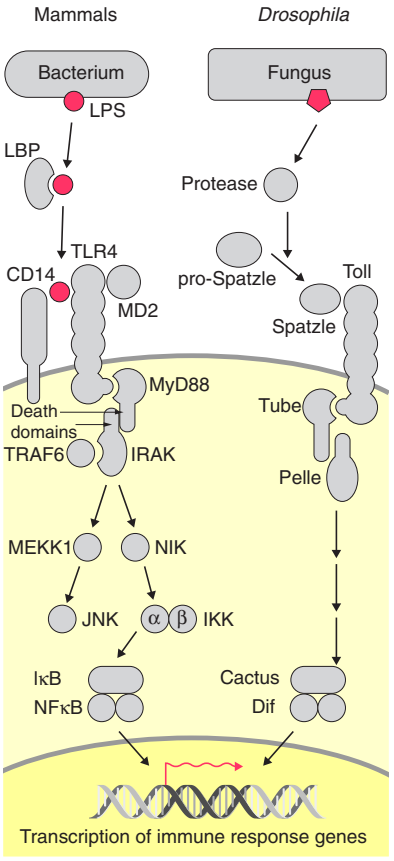

Figure 1
Signaling through mammalian and *Drosophila* Toll-family receptors (TLRs). The left-hand side of the figure shows signaling through mammalian TLR4 in response to bacterial lipopolysaccharide (LPS). LPS monomers from bacterial membranes bind to LPS-binding protein (LBP) which transfers the LPS monomer to CD14 in the membrane of phagocytes. CD14 and MD2 promote the binding of LPS to TLR4 which signals to the cell interior. Binding of LPS by TLR4 recruits the adaptor molecule MyD88 to the cytoplasmic domain of the receptor and MyD88 in turn binds to TRAF6, which binds the serine-threonine kinase IRAK. This complex is believed to activate the phosphorylation of the two subunits of the NFκB kinase (NIK) and cause them to form a heterodimer, IκB kinase (IKK). The IKK dimer then phosphorylates IκB, causing it to dissociate from NFκB which is thereby released to migrate to the nucleus and bind to DNA, activating the transcription of genes encoding inflammatory mediators. The right-hand side of the figure shows signaling through *Drosophila* Toll in response to fungal components. Components of the fungal cell wall are believed to activate a protease which cleaves the precursor pro-Spätzle to Spätzle, the ligand for Toll. In the *Drosophila* pathway, Tube and Pelle are homologous to MyD88 and TRAF6, and Cactus and Dif are homologous to IκB and NFκB. The intervening components in the *Drosophila* pathway are not known. MyD88 and TRAF6, and Tube and Pelle, bind through a motif known as the death domain which occurs widely in regulatory interactions including those that mediate apoptosis. Modified from *Immunity* by Anthony DeFranco, Richard Locksley and Miranda Robertson, to be published by New Science Press Ltd.

terminal mannose residue, which is characteristically exposed on the surface of microbial carbohydrates. A search of the *Drosophila* genomic sequence with the MBL CRD sequence readily identifies a large number of proteins with the CRD, including two that have Glu-Pro-Asn residues in the positions corresponding to residues 185-187 in the mammalian MBL. These two novel C-type lectins are thus likely to function as pattern recognition receptors in the *Drosophila* immune system.

The structure and mechanism of microbial recognition by LRR and SRCR domains are not known, and we therefore cannot rely on a signature motif to identify candidate receptors among many proteins that contain these domains but may have functions unrelated to immune recognition. Even in these cases, however, the candidate pattern recognition receptors may be identified among the LRR and SRCR proteins using additional criteria, such as inducibility by microbial challenge. This is clearly feasible, and it is likely that all the pattern recognition receptors in *Drosophila* that can be identified on the basis of homology will soon be characterized. This, in turn, will certainly help to identify novel receptors involved in mammalian innate immunity. This approach has already paid off in the recent past with the identification of the human homologs of the *Drosophila* Toll receptor [8,9]. The first human Toll homolog, in fact, turned out to be the receptor that mediates LPS responsiveness [10,11]. As LPS is the major molecular marker of gram-negative bacteria, Toll-mediated recognition is critical for the detection of microbial infection. On the other hand, excessive amounts of LPS, caused, for example, by gram-negative bacteria, leads to endotoxic shock - a condition responsible for 20,000 deaths annually in the US alone.

Assuming that most pattern recognition receptors belong to one of the known protein families, we can hope, for the first time, to ask some fundamental questions: how many pattern recognition specificities are required to protect a multicellular organism from infection? Did the repertoire of innate immune receptors change with the evolution of adaptive immunity in vertebrates? It seems likely that at least some receptors may be unique to arthropods: there are no known mammalian homologs of the gram-negative bacteria binding proteins (GNBP) for example, whereas the *Drosophila* genome contains three genes of the GNBP family. On the other hand, one of the best studied human pattern recognition receptors, CD14, does not seem to have a *Drosophila* homolog, although recognition of LPS by mammalian cells critically depends on both CD14 and the Toll-like receptor 4 (TLR4; Figure 1) [10-12]. Furthermore, the ectodomain of human TLR4 is associated with another protein, called MD2, which is also involved in LPS recognition [13] but does not appear to have a fly homolog. *Drosophila* cells appear to recognize LPS through an unknown member of the Toll family, of which there are eight in the *Drosophila* genome. It is quite interesting then, that *Drosophila* and humans have evolved

different mechanisms for LPS recognition, both of which, however, are mediated somehow by Toll receptors. Characterization of the mechanism of LPS recognition in *Drosophila* would thus be of considerable interest.

The induction of antimicrobial peptides is by far the best understood defense mechanism in *Drosophila* [6]. The genes encoding these peptides are induced by the NF-κB signaling pathway which, in turn, is activated by Toll receptors in response to infection [6]. This has been clearly established for Toll-induced activation of the antifungal peptide drosomycin [14], and it is likely that other antimicrobial peptide responses are mediated by other members of the *Drosophila* Toll family. Upon fungal infection, the Toll ligand Spätzle is activated by proteolytic processing by an as yet unidentified protease. Ligation of Toll leads to the activation of a signaling pathway that involves the adaptor protein Tube, the protein kinase Pelle, and the Rel/NF-κB family of transcription factors [15]. A homologous pathway is used by the mammalian Toll and IL-1 receptor, except that the role of the Tube protein appears to be substituted by another adaptor called MyD88, which interacts with and recruits the Pelle homolog IRAK [16,17]. Additional components in the mammalian Toll pathway have been identified in the past few years. These include another adaptor, TRAF6, that functions downstream of IRAK, and the IκB kinases IKK1 and IKK2 [15]. The *Drosophila* genome contains three TRAF-like proteins and two IKK-like kinases. At least some of these are presumably involved in the Toll signaling pathway(s). Surprisingly, however, there seems to be only one Pelle and one Tube protein in *Drosophila*, which either means that all of the *Drosophila* Tolls signal through the same signaling intermediates, or, that some Tolls activate different signaling pathways. If all the Tolls signal through the same intermediates, and yet activate distinct target genes, it would imply that there are additional, currently unrecognized mechanisms that determine signaling specificity. If at least some Tolls can signal through distinct signaling pathways, it would mean that these other pathway(s) may also exist in mammals and our current view of the Toll signaling in both flies and humans is greatly oversimplified. Whichever is the case, the implications would be quite interesting for the understanding of mammalian Toll and IL-1 receptor signaling.

One notable difference between *Drosophila* and human Toll signaling pathways was thought to be at the level of the adaptor proteins, because Tube and MyD88 are not homologs of each other and yet perform a similar function - the recruitment of the kinases Pelle and IRAK, respectively. Given the homology of all of the other components in the pathway, this difference was somewhat bothersome. It is satisfying then to find that the *Drosophila* genome does in fact contain a MyD88 homolog, which presumably functions in the Toll pathway. This, of course, raises questions about the functional relationship between Tube and MyD88. Moreover, this finding probably tells us that there might be a mammalian homolog of Tube, which has not yet been found.

The few examples discussed here demonstrate that knowledge of the *Drosophila* genome sequence will have tremendous implications for studies of immunity in both *Drosophila* and mammals. Rapid identification of novel genes by homology will certainly boost research in both fields. It is worth mentioning, however, that cloning by homology is biased by what is already known, and the more traditional genetic screens will still remain as valuable as they were prior to genome sequencing.

As mentioned above, what is not in the *Drosophila* genome sequence can be as significant as what is there. It is scarcely surprising that there are no *Drosophila* homologs of the major histocompatibility complex molecules or RAG proteins (involved in the rearrangement of T cell receptor and immunoglobulin genes), since these are essential molecules of the adaptive immune system absent from *Drosophila*. Some important mediators of mammalian innate immunity, however, such as the inflammatory cytokines (for example, IL-1 and TNF-α), or acute phase response proteins (for example, C-reactive proteins) also do not seem to have *Drosophila* homologs. This means that, although innate immunity is ancient in origin, some components of the mammalian innate immune system evolved relatively late in evolution, presumably after the divergence of the invertebrate and vertebrate subphylae. Conversely, some innate immune receptors found in *Drosophila* (such as GNBP) may not exist in mammals. These examples illustrate that although some components of the innate immune system (such as Toll receptors) are conserved and universal to all metazoans, others are found only in certain types of host organisms.

Even mere comparison of the gene products involved in human and fly immunity can be a rich source of information and will surely help direct new research projects in both fields. In addition to the acceleration of gene discovery and functional characterization, a careful analysis of the *Drosophila* genome will allow a more profound exploration of the evolution of immunity than has been possible before.

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