Drosophila innate immunity: an evolutionary perspective

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In response to microbial infections, *Drosophila* mounts a multifaceted immune response involving humoral reactions that culminate in the destruction of invading organisms by lytic peptides. These defense mechanisms are activated *via* two distinct signaling pathways. One of these, the Toll pathway, controls resistance to fungal and Gram-positive bacterial infections, whereas the Imd pathway is responsible for defense against Gram-negative bacterial infections. Current evidence indicates that recognition of infectious nonself agents results from interactions between microbial wall components and extracellular pattern recognition proteins. We discuss here evolutionary perspectives on our present understanding of the antimicrobial defenses of *Drosophila*.

Microorganisms represent a constant threat to all metazoans. Consequently, the development of powerful mechanisms to counter invading microorganisms was prerequisite for the evolution of the various animal phyla over billions of years. These mechanisms involve recognition of the invader, that is, the discrimination between self and infectious nonself as well as effector systems that efficiently target the microorganisms while respecting self-cells. Innate immunity—the paramount antimicrobial response of metazoans—depends on germ line–encoded receptors that recognize repeated patterns of molecular structures on the surface of microorganisms; these patterns are absent from eukaryotic cells[1,2]. One outcome of this recognition, which is probably common to all animals, is the induction of genes encoding cationic antimicrobial peptides that act by damaging the microbial cell membranes[3,4]. Adaptive immune responses have appeared in the ancestors of cartilaginous fish. In contrast to the innate immune system, adaptive immunity uses a large repertoire of receptors (immunoglobulins and T cell receptors) that are encoded by rearranging genes to recognize an enormous variety of microbial (although not exclusively) antigens. Importantly, all animal forms derived from these ancestral fish—that is, all gnathostome vertebrates—have retained innate immune mechanisms. It is now understood that these innate reactions trigger the adaptive immune responses and orient the effector mechanisms of these responses[5,6].

An estimated five to ten million species of metazoans have to cope with microorganisms by relying solely on innate immunity. Approximately 45,000 extant vertebrate species make use of both the innate and the adaptive arms in their immune defenses. Whereas adaptive immunity has attracted practically all the attention of immunologists over the last few decades, innate immunity has only recently re-emerged as a subject of interest. *Drosophila* can be largely credited for rejuvenating this interest. Having no adaptive immune response and being, like most invertebrates, highly resistant to microbial infections, *Drosophila* is particularly well suited to the study of innate immunity. The powerful tool of molecular genetics, together with the fully sequenced genome, mean *Drosophila* is probably the best model available to date with which to investigate the minutiae of prototypical innate immunity. We will review here our present knowledge of *Drosophila* immune defense and highlight its similarities to the evolving picture of mammalian innate immunity.

The general picture of *Drosophila* host defense

The *Drosophila* host defense is a multifaceted process. The epithelial surfaces of the body serve as first-line defenses against microorganisms. The epidermis—the cells of the digestive and genital tracts—of the tracheae and of the Malpighian tubules all produce antimicrobial peptides, which inhibit microbial growth[7–9]. Microorganisms that have succeeded in entering the general body cavity (called the hemocoele; *Drosophila* lacks an organized blood vessel system) are countered by both cellular and humoral defenses (**Fig. 1**). The cellular defenses consist essentially of phagocytosis by macrophage-like cells, called the plasmatocytes (**Fig. 1**). Larger invading microorganisms are encapsulated by a specialized flattened cell type, called the lamellocytes[10,11].

The hallmark of the humoral reactions is the systemic antimicrobial response. It corresponds to the challenge-induced synthesis by the fat body—a functional equivalent of the mammalian liver—of antimicrobial peptides that are secreted into the hemolymph, where their combined concentrations can reach 300 μM in infected flies[12,13] (**Table 1**). The humoral reactions also involve several proteolytic cascades (**Fig. 1**). Of paramount importance among these is the melanization cascade, which locally generates quinones and toxic oxygen intermediates and culminates in the production of melanin at wound sites or around microorganisms[14,15]. *Drosophila* and *Anopheles* have the equivalent of a complement-like cascade that may contribute to opsonization of microorganisms[16,17]. Hemolymph zymogen cascades also play a crucial role in activating the synthesis of antimicrobial peptides in the fat body, as we explain below. However, whether and how hemolymph coagulation participates in the host defense remains to be established.

Because of space constraints, we will focus here on the induction of antimicrobial peptides in the *Drosophila* host defense, a field of research that has made significant progress over the last few years. For more details on cellular reactions in *Drosophila* see[10,11,18,19].

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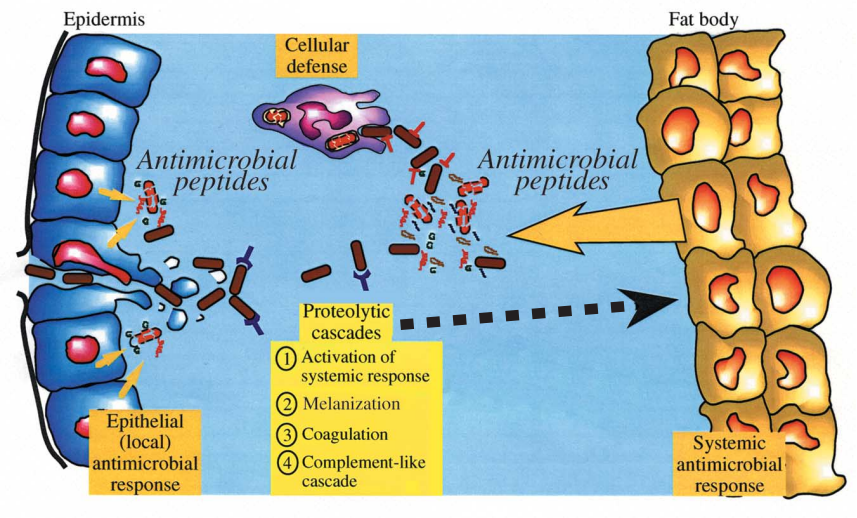

Figure 1. The antimicrobial defense of *Drosophila*. Note that this scheme is probably valid for all holometabolous insects. Bacteria are illustrated as brown rods; pattern recognition proteins as purple pincers; and putative opsonizing proteins as red T-shapes.

Two distinct signaling pathways to counter infections

In the mid-1990s we knew that *Drosophila* produces six distinct antimicrobial peptides with activity directed against various fungi, Gram-positive or Gram-negative bacteria (**Table 1**). The promoters of the genes encoding these peptides contain sequence motifs related to mammalian NF-κB response elements[20,21]. Experiments with transgenic fly lines established that these motifs are mandatory for immune-inducibility of the antimicrobial peptide genes[22–24]. At that time, independent studies had already pointed to significant similarities between the activation of the NF-κB–related transactivator Dorsal by the Toll pathway during dorsoventral patterning in the early *Drosophila* embryo and the cytokine-induced activation of NF-κB in immune-responsive mammalian cells[25]. These similarities prompted a genetic analysis of the immune induction of antimicrobial peptides in Toll pathway mutants of *Drosophila*.

In 1996, it was shown that induction of the antifungal peptide Drosomycin and, more generally, resistance to fungal infections did require a wild-type Toll transmembrane receptor and several other components of the embryonic Toll signaling pathway[26]. In contrast, induction of antibacterial peptides, such as Diptericin, was found to be largely Toll-independent, but required a wild-type copy of an unknown gene referred to as *immune deficiency* (*imd*)[27]. *imd* mutants appeared to have a compromised resistance to Gram-negative infections. The *imd* gene thus defined a second immune signaling pathway. These data set the framework for most of the subsequent studies on immune defenses in *Drosophila*.

The Toll pathway and fungal or Gram-positive infections

It is now understood that for antifungal and anti–Gram-positive bacterial defenses, *Drosophila* recruits most of the components of the Toll pathway (**Fig. 2**). These were initially identified through mutations that affect dorsoventral patterning in the early embryo[25,28–33]. However some significant discrepancies exist, as will be discussed below.

Toll is a transmembrane receptor[28]. Its extracellular domain contains leucine-rich repeats and its intracytoplasmic region shows significant sequence similarity with the corresponding region of the interleukin 1 receptor (IL-1R) and is referred to as the Toll–IL-1R (TIR) domain. This domain interacts with several intracytoplasmic partners, which all have a death domain region (**Fig. 2**). Two of these are considered as adaptor proteins: a *Drosophila* homolog of MyD88, which, in addition to the death domain, has a TIR domain similar to that of Toll[34,35] and Tube[29]. The third death domain protein, Pelle[32], also has a serine-threonine kinase domain[36]. Mutants in any of these three proteins do not mount a wild-type antifungal response when challenged[26,35]. Upon activation (see below), the Toll receptor-adaptor complex signals to a latent transcriptional factor of the NF-κB–Rel family of inducible transactivators. This factor is complexed to the ankyrin-repeat inhibitor protein Cactus[30,31], and Toll signaling translates into dissociation of Cactus from the Rel protein[25]. The *Drosophila* genome encodes three Rel proteins, each with a common Rel-homology domain that is responsible for dimerization and DNA binding. These proteins are Dorsal—which was initially identified in screens for dorsoventral patterning in the embryo[37,38]— Dorsal-related immunity factor (DIF)[39] and Relish[40]. Of these three Rel proteins, DIF is the predominant transactivator in the antifungal and anti–Gram-positive bacterial defense in adults[41–43] (**Fig. 2**). Dorsal can substitute for DIF in larvae[41,43]. Cactus dissociation from DIF or Dorsal is mediated by its phosphorylation[44]. The kinase responsible for Cactus phosphorylation is at present unknown. The Pelle kinase does not directly phosphorylate Cactus and the Pelle substrate has not been identified so far[45]. DIF activates transcription of a large number of genes, probably several hundred, as suggested by genome-wide analysis of *Drosophila* immune responses[46,47]. Prominent among these genes are those encoding the antifungal peptides Drosomycin and Metchnikowin. We assume that the products of this large number of genes act in concert to fight off fungal and bacterial infections. Resistance to these infections is by no means explained solely by the induction of antimicrobial peptides.

The key player in the activation of Toll, both in embryonic development and in the immune response, is a cystine-knot cytokine–growth factor-like polypeptide, Spaetzle[25,26,33,48,49]. Spaetzle is cleaved to its active form as the end result of a proteolytic cascade, which has been determined in detail in the embryo[50]. A genetic analysis showed that the genes encoding the zymogens of the embryonic cascade (*easter, snake* and *gastrulation defective*) are dispensable for the induction of a Toll-dependent immune response by septic injury[26]. This raised the question of whether Toll activation in the host defense is mediated by a distinct proteolytic cascade or whether Toll interacts directly with microbial structural patterns. The question was partially answered by the analysis of mutants for the blood serine-protease inhibitor (serpin) Spn43Ac[51]. In these mutants, referred to as *necrotic* (*nec*), Spaetzle is predominantly present in its cleaved form and Drosomycin is constitutively expressed, that is, it is challenge-independent. Expression is abolished in *Spaetzle* and *Toll* loss-of-function mutants or by introducing the wild-type serpin Spn43Ac onto a *nec* mutant background.

These observations indicated that Toll-dependent expression of the *Drosomycin* gene is mediated *via* a serpin-controlled proteolytic cascade and that Toll is triggered—as in the embryo—by cleaved Spaetzle, rather than by directly interacting with structural microbial

Table 1. Inducible antimicrobial peptides of *Drosophila*

Antimicrobial peptide family	Main biological activities at physiological concentrations	Number of genes		Post-translational modifications	Concentration in the blood (systemic response)	Epithelia expressing various antimicrobial peptides
		Per genome	Expressed			
Diptericin	Antibacterial, Gram-negative	2	2	Two O-glycosylations, COOH-terminal amidation	0.5 μM	Midgut
Attacin	Antibacterial, Gram-negative	4	4			Midgut
Drosocin	Antibacterial, Gram-negative	1	1	O-glycosylation	40 μM	Calyx, oviduct, tracheae
Cecropin	Antibacterial, Gram-negative	4	4	COOH-terminal amidation	50 μM	Calyx, oviduct, seminal receptacle, spermathecae
Defensin	Antibacterial, Gram-positive	1	1		1 μM	Seminal receptacle, spermathecae, labellar glands
Metchnikowin	Antifungal	1	1		40 μM	Labellar glands
Drosomycin	Antifungal	7	2		100 μM	Labellar glands, seminal receptacle, spermathecae, tracheae, salivary glands

patterns[51]. The identity of one of the receptors for these patterns during Gram-positive bacterial infection has now been determined in *Drosophila*. *semmelweis* (*seml*), an ethyl-methyl-sulfonate (EMS)-induced mutation in a protein encoding a peptidoglycan-recognition protein, does indeed abolish Toll-dependent activation of the response to this type of infection[52] (**Fig. 2**). As a result, *seml* mutant flies have severely reduced survival rates when challenged with Gram-positive bacteria. The phenotype is rescued both by a wild-type copy of the gene and by transfer of wild-type hemolymph to mutant recipient flies. The latter results indicate that interaction of the microorganism with the cognate pattern recognition protein is likely to occur in the circulating hemolymph. The Toll-dependent activation of an antifungal response is not affected by the mutation in the peptidoglycan recognition protein. This points to the existence of a distinct extracellular pathway for activation of Toll by fungi[52]. This inference has been confirmed by a new EMS-generated mutation, *persephone*; in this mutant, fungal but not Gram-positive bacterial activation of Toll is compromised (P. Ligoxygakis, personal communication; **Fig. 2**).

In addition to Toll, *Drosophila* express eight related genes encoding transmembrane receptors. All are expressed during embryonic development and some are also expressed at later stages[53]. Toll and 18-Wheeler (Toll2) can act as homophilic adhesion molecules through their leucine-rich repeats, which have multiple roles in development[54–56]. The adapter DmMyD88 specifically interacts with Toll but does not bind most of the other members of the family[34,35]. The possible role of these other members of the Toll family in the host defense, if any, awaits further analysis.

The Imd pathway and Gram-negative infection

The Imd pathway governs defense reactions against Gram-negative bacteria (**Fig. 3**). This type of infection induces the transcription of a large number of genes, which encode the antibacterial peptides Diptericins, Cecropins, Drosocin and Attacins (**Table 1**). As for the Toll pathway, the immune induction of these genes relies on a member of the Rel family of inducible transactivators. In contrast to Toll signaling, the Rel protein in the Imd pathway, Relish, is not inhibited by Cactus, but carries its own inhibitory sequences in the form of several COOH-terminally located ankyrin repeat domains[40]. Activation of Relish requires a signal-induced endoproteolytic cleavage, which frees the Rel homology domain from the ankyrin repeats and allows for its nuclear translocation[57]. In parallel, this proteosome-independent cleavage sets free a Cactus-like COOH-terminal domain. How the proteolytic cleavage of Relish occurs has not yet been determined. A signalosome equivalent, comprising proteins with significant sequence similarities to the mammalian IκB kinase β (IKKβ) and to the structural protein IKKγ (also known as NEMO), is required for Relish activation and subsequent induction of the target genes. Mutants in both components of this signalosome equivalent (*ird5* and *kenny*) are highly prone to Gram-negative infections, but resist Gram-positive bacterial and fungal infections as well as wild-type flies[58,59]. Studies with cell cultures further showed that this signalosome equivalent can be activated by bacterial lipopolysaccharide (LPS) and that this activation results in phosphorylation and cleavage of Relish[60] (**Fig. 3**).

The upstream events that link Gram-negative infection to activation of the signalosome-equivalent and to phosphorylation and cleavage of Relish are not fully understood but significant progress has recently been made. The *imd* gene product is identified as a 25-kD protein containing a death domain with significant sequence similarity to that of mammalian tumor necrosis factor-α (TNF-α) receptor–interacting protein (RIP)[61]. *imd* acts upstream of a gene encoding a *Drosophila* caspase-8 homolog, DREDD, which is also required for resistance to Gram-negative infection[61–63]. Downstream of *imd*, mitogen-activated protein 3 (MAP3) kinase is also required for Relish-dependent gene expression; mutants that are deficient in this kinase show significantly lower resistance to Gram-negative bacteria and a compromised induction of antibacterial peptides[64]. The amino acid sequence of this kinase indicates that it is a homolog of mammalian transforming growth factor–activated kinase 1 (TAK1). The precise roles of DmTAK1 and DREDD are the object of intense research at present, DmTAK1 being a good candidate for activation of the signalosome-equivalent DmIKKβ-DmIKKγ. The RIP analog IMD is most likely a partner of an extensive receptor-adaptor complex, which detects Gram-negative infection (**Fig. 3**). The receptor has not yet been identified and it is unclear at present whether it interacts directly with bacterial structural patterns—and would therefore qualify as a genuine pattern recognition receptor—or whether it

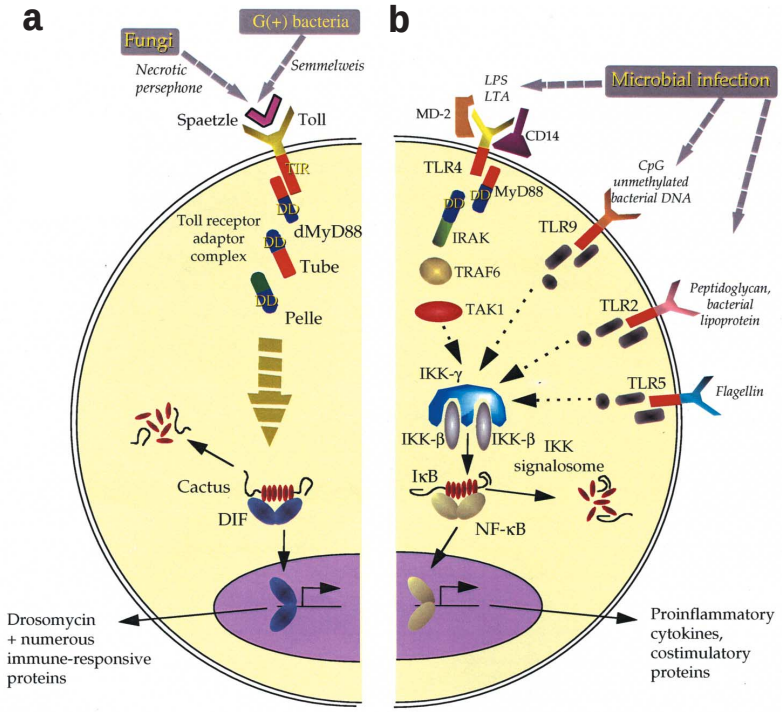

Figure 2. Toll pathways in *Drosophila* and mammals. (**a**) Present view of Toll-dependent induction of immune genes in fungal and Gram-positive (G(+)) bacterial infections in *Drosophila*. These microorganisms are sensed by circulating pattern recognition proteins, a process that is followed by proteolytic cleavage of the polypeptide Spaetzle; Spaetzle activates Toll, which leads to degradation of Cactus and nuclear translocation of the Rel protein DIF. Note that this scheme is valid for the systemic response by the fat body cells. (**b**) TLR signaling of microbial infection in mammalian innate immunity[73–75,77,78].

is activated by the end product of a proteolytic cascade, as in the case of Toll. For several reasons, which are beyond the scope of this review, we favor the second hypothesis in the case of the systemic antimicrobial response.

Septic injury activates both signaling pathways

The survival phenotypes of flies with mutations in components of either the Toll or the Imd pathway are generally clear-cut. However, when the various antimicrobial peptides are considered, a more complex picture emerges. Injuries generated with a sterile needle are sufficient to activate both pathways, albeit at a low level, as illustrated by the discrete expression and fast kinetics of all antimicrobial peptide genes. Septic injury with either Gram-positive or Gram-negative bacteria induces transcription of all antimicrobial peptide genes. However, the levels of expression are significantly higher for genes encoding peptides with activities against Gram-negative bacteria when such bacteria are used to challenge the flies. Natural infections generated by coating flies with fungal spores are followed by a slow, but strong, induction of antifungal peptides, while the genes encoding antibacterial peptides remain essentially silent[65]. In contrast, injections of fungal spores induce the whole panoply of antimicrobial peptides.

Some of these results are not easy to reconcile with our concept that two clearly distinct pathways regulate antimicrobial defenses in *Drosophila*. Microorganisms carry numerous structural patterns on their cell walls and our present view is that, when introduced by septic injury, a given pathogen can concomitantly activate the Toll and the Imd pathways by distinct patterns. We cannot exclude the idea that a cross-talk exists between the two pathways and occurs *via* the transcription factors. Heterodimerization between the various Rel proteins occurs in *Drosophila* cell lines and could variably affect expression of the antimicrobial peptide genes[66]. In addition, some of the antimicrobial peptide genes, for example *Drosomycin*, have both Toll- and Imd-responsive regions in their promoters; under extreme experimental conditions they could conceivably be activated by either or both pathway(s).

The possibility that a third, distinct, pathway may account for these discrepancies is ruled out by the observation that double mutant flies with mutations for both the Toll and the Imd pathway fail to express any antimicrobial peptides and show a strongly compromised resistance to any type of microbial infection[7–9,26] (S. Rutschmann, personal communication).

The epithelial antimicrobial response

We have so far focused on the systemic antimicrobial response; this response is impressive because of the variety and the quantity of peptides produced by the fat body in response to infection. As noted earlier, the various barrier epithelia of *Drosophila* also produce antimicrobial peptides when exposed to microorganisms[7–9]. However, compared to those produced by the fat body cells, the spectrum of peptides produced by a given epithelium seems relatively restricted (**Table 1**). The epithelial antimicrobial response has been observed in other insects[67] and is commonly found in mammals, including humans. This contrasts with the systemic response defined here that, to date, has only been clearly demonstrated in holometabolous insects (which undergo complete metamorphosis). These data suggest that the epithelial response is the true ancestral antimicrobial defense. We were surprised when it was shown that the epithelial response of *Drosophila* is compromised only in Imd pathway mutants and not in Toll mutants[7–9]. We have repeatedly made the case that the gene encoding the antifungal peptide Drosomycin is a prototypical target gene of Toll in the systemic response. However, in the epithelial response, Drosomycin is inducible in Toll loss-of-function mutants[7]. This tissue-dependent difference is intriguing. Studies underway have now localized proximal Imd-responsive regions distinct from more distal Toll-responsive sequences in the Drosomycin promoter. Analysis of the control of immune gene expression in barrier epithelia is one of the challenges in the field. We anticipate that it will also contribute to our understanding of the epithelial (that is, midgut) responses of vector insects to parasites.

Evolution of the Toll and Imd pathways

Except for reports of Toll homologs in several insect species[68,69], an IKKβ homolog in an oyster[70] and Rel homologs in dipteran insects[71,72], we have no molecular information on innate immune signaling in other invertebrates. In contrast, a wealth of information has been generated for mammalian systems over the last few years. In particular, the discovery of the role of Toll in the *Drosophila* host defense has paved the way for the search of homologs in mammalian innate immune responses (**Fig. 2**). This culminated in the discovery of a ten-member family of Toll-like receptors (TLRs), which sense a large spectrum of microbial patterns that activate NF-κB[73–75]. One essential outcome of NF-κB activation is the transcription of genes encoding proinflammatory cytokines and costimulatory molecules involved in activation of adaptive immune responses. TLR-dependent NF-κB activation can also lead to expression of antimicrobial peptides.

The tantalizing structural and functional similarities between infection-induced Toll activation of *Drosophila* Rel proteins and TLR-dependent activation of NF-κB in mammals are interpreted as pointing to a common ancestry. This view holds that a signaling mechanism, which puts defense genes under the control of inducible transactivators of the Rel family was already present in *Urbilateria*. This view also assumes that activation of the latent transactivators was entrusted to Toll transmembrane receptors capable of detecting the presence of microorganisms.

With regard to sensing microorganisms, our present understanding of Toll activation in *Drosophila* and TLR activation in mammalian cells points to some differences. In *Drosophila*, detection of fungal and Gram-positive infection occurs by circulating pattern recognition receptors that lead, *via* zymogen cascades, to proteolytic cleavage of a cytokine-like polypeptide interacting with Toll. In contrast, mammalian TLRs appear to interact directly with microbial patterns, albeit in association with coreceptors or associated proteins, as illustrated in great detail for LPS sensing by the TLR4 complex (which involves the LPS-binding protein (LBP) and the CD14 and MD2 coreceptors)[73]. In addition, although *Drosophila* express nine distinct Toll genes, only one (Toll) is strictly required for defense against fungi and Gram-positive bacteria[53]. At present there are no data pointing to a role of Tolls in the response to Gram-negative infection. This contrasts with the extensive roles played by the various mammalian TLRs, which recognize peptidoglycan and bacterial lipopeptides (TLR2), double-stranded RNA (TLR3)[76], LPS and lipoteichoic acid (TLR4), flagellin (TLR5), mycoplasmal lipopeptides (TLR6) and CpG DNA (TLR9)[73–75,77]. The broad spectrum of recognition of microbial patterns by the mammalian TLR family member is most probably reflected in *Drosophila* by a significant number of circulating recognition proteins (such as the *semmelweis* peptidoglycan recognition protein) that all activate Toll *via* cleaved Spaetzle as a result of activating multiple proteolytic cascades. Although this is not fully substantiated at present, the *Drosophila* genome contains a plethora of genes that are good candidates for circulating recognition proteins, proteases and protease inhibitors.

The difficulty that the scientist faces with the hypothesis of an ancestral Toll pathway in innate immunity is precisely the fact that in *Drosophila* Toll signaling is used both in development and in host defense. Did *Drosophila* recruit the ancestral host defense circuitry for developmental purposes or *vice versa*, did the *Drosophila* host defense recruit existing embryonic control mechanisms? The answer to this apparent chicken-and-the-egg dilemma will undoubtedly come from the study of other more primitive invertebrates, such as *Tribolium*[68].

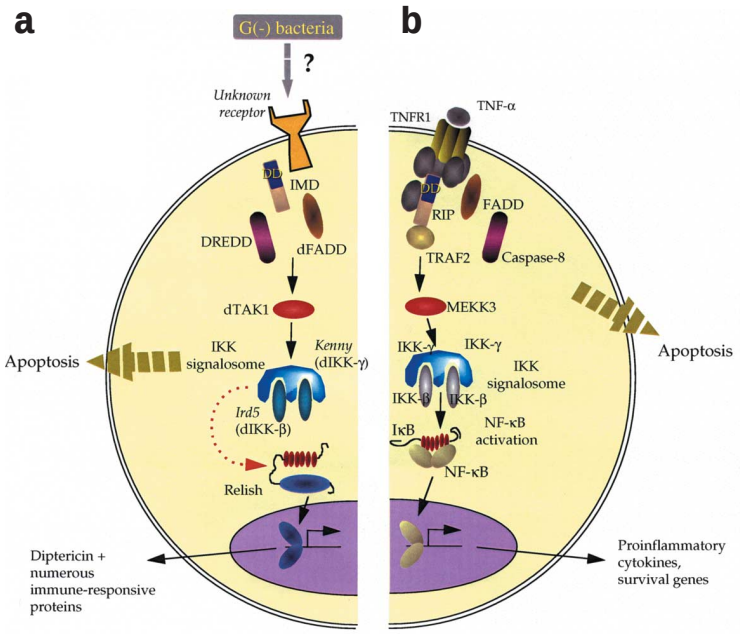

Figure 3. The Imd pathway of *Drosophila* and the TNF-α receptor pathway in mammals. (**a**) The Imd pathway regulating the *Drosophila* defense against Gram-negative (G(–)) infection. Note that the receptor sensing this infection has not yet been identified. This pathway can also promote apoptosis. (**b**) Outlines of the mammalian TNF-α receptor signaling pathway[79], which highlight compelling similarities with the Imd pathway.

The Toll pathway in *Drosophila* is unable to confer protection to Gram-negative infection. The Imd pathway, which comes into play here, is evocative in several aspects of the TNF-α receptor signaling pathway (**Fig. 3**). This is illustrated by the presence, in both systems, of signaling partners with striking similarities: RIP and IMD, FADD and DmFADD, caspase-8 and DREDD. In mammals, TNF-α signaling can lead to NF-κB activation or to apoptosis (**Fig. 3**). Remarkably, challenge-induced expression of antibacterial peptide genes in *Drosophila via* the Imd pathway is blocked by the anti-apoptotic viral protein p35. In addition, overexpression of *imd* in flies can promote apoptosis and in particular induce expression of the pro-apoptotic *Drosophila reaper* gene[61]. These results make the parallels between the Imd pathway and TNF-α signaling even more compelling. However, much more work is required before we will understand the precise significance of the connection of the Imd pathway to apoptosis. There is presently no direct evidence linking apoptosis to resistance to microorganisms in *Drosophila*, but this may simply reflect our present ignorance.

As for the Toll-TLR pathways, the parallels between the TNF-α and Imd signaling pathways also point to a common ancestry of host defense mechanisms, which predated the separation of the lineages giving rise to insects and vertebrates.

Antimicrobial peptides were first discovered in insects twenty years ago by Hans Boman and associates. It has been only five years since the first paper—which outlined the signaling pathways controlling the expression of these peptides during microbial infections in *Drosophila*—appeared. Through the intense efforts of many laboratories worldwide, the harvest of knowledge has been rich during these years. In view of the data available to date, we are progressing towards a unifying concept of an innate immune response that is common to all metazoans. To make this concept convincing, however, we are in great need of information on antimicrobial responses in other invertebrate groups more ancient than *Drosophila* (annelids or mollusks, for example) and highly evolved forms (such as sea urchins and agnathostomes). We are aware that such studies are presently underway and their results are anxiously awaited by the scientific community.

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