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*J Immunol* 2012; 188:5207-5209; doi: 10.4049/jimmunol.1201050

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How Discovery of Toll-Mediated Innate Immunity in Drosophila Impacted Our Understanding of TLR Signaling (and Vice Versa)

Stefanie N. Vogel

I

was delighted when The Journal of Immunology invited me to write a Pillars of Immunology article on one of my favorite papers, the 1996 publication titled, “The Dor-soventral Regulatory Gene Cassette spätzle/Toll/cactus Controls the Potent Antifungal Response in Drosophila Adults” by Bruno Lemaitre et al. (1). In this seminal publication, the authors deftly combined genetic, biochemical, and molecular approaches to reveal that the Toll signaling pathway, originally recognized to regulate dorsoventral patterning in Dro-
sophila embryos by activating an NF-κB–like Rel protein (recently reviewed in Ref. 2), was also responsible for tran-
scriptional activation of the gene that encodes Drosomysin, an antifungal peptide. Using adult flies with gain-of-function or loss-of-function mutations in genes shown previously to contribute to induction of both the antifungal peptide Droso-
mycin and antibacterial peptides, Lemaitre et al. found the following: (1) The embryonic signaling pathway comprising gene products between sos and cact, and not upstream or downstream genes—that is, genes constituting the Toll signaling “cassette”—was necessary for the antifungal response in adult flies; (2) although this same pathway contributes par-
tially to the induction of antibacterial gene expression (includ-
ing genes encoding Cecropins, Attacin, and insect Defensins), an additional pathway regulated by a distinct locus, imd, was also necessary for antibacterial peptide gene expression; (3) expression of other antibacterial peptide genes, Dipterocin and Droso-
cin, is regulated by the imd signaling pathway, apparently without cross-talk from the Toll signaling pathway; and (4) finally, and most importantly, adult flies with mutations in the Toll signaling pathway succumb to infection with the fungus Aspergillus fumigatus, but not the bacterium Escherichia coli, and conversely, flies with defects in imd failed to survive E. coli infection owing to uncontrolled bacterial replication, yet re-
sisted infection with A. fumigatus. Flies with mutations in both pathways failed to survive fungal or bacterial challenge. The image of the dead adult fly with fungal hyphae extending from its body (Fig. 5 in Ref. 1) is undoubtedly the most widely reproduced figure shown in innate immunity lectures today! The implications of this paper were far-reaching in that it impacted the discovery of mammalian TLRs by providing the first direct evidence that resistance to infection was the consequence of inducible expression of antimicrobial genes secondary to activation of the Toll NF-κB–like signaling pathway. The significance of this work was acknowledged recently by naming the senior author, Jules Hoffmann, as coreipient of one half of the 2011 Nobel Prize in Physiology or Medicine.

In a 2004 review, Lemaitre (3) provided a timeline of key observations in both insect and mammalian immunology that resulted in a convergence of ideas, leading first to their landmark publication and ultimately to the discovery of mammalian TLRs. Notably, the identification of inducible antibacterial peptides in insects (4) and mammals (5), coupled with the finding that genes encoding antibacterial Attacin peptides contained NF-κB–like binding sites in their pro-
motors (6), pointed to a signaling pathway in insects that was homologous to known immune signaling pathways leading to NF-κB translocation in mammals. However, in 1991, Gay and Keith (7) and Schneider et al. (8) independently reported a significant degree of homology between the cytoplasmic domains of Drosophila Toll protein and the human IL-1R. Gay and Keith found that this homology ex-
tended for 135 aa with identical or conservative substitutions at 45% of positions (now known as the Toll/IL-1R/resistance [TIR] domain). Consistent with this finding, Heguy et al. (9) described a 50-aa subregion within the TIR domain that was essential for IL-1–mediated activity. When coupled with the 1988 observation by van der Meer et al. (10) that a low dose of IL-1 protected granulocytopenic mice from lethal Gram-
negative infection, it is clear that the idea of TIR domain-
mediated “nonspecific” host immunity was actually already established for mammals in the early 1990s.

That NF-κB translocation to the nucleus was observed in IL-1–treated cells and paralleled the signal transduction events mediated by Drosophila Toll upon activation by its endog-
enous ligand, spätzle, provided further support for the idea that parallel signaling pathways leading to NF-κB trans-
location might regulate induction of innate immune response genes in both insects and higher species. Between 1993 and 1995, multiple studies in Drosophila provided evidence for an additional, nondevelopmental role for Toll in insect immu-
nity (11–15), culminating in a series of papers by Lemaitre
et al. (16, 17), including their 1996 Cell paper showing that mutations in Toll and imd in vivo resulted in increased susceptibility to infection in Drosophila (1).

The idea of the existence of cellular receptors that could sense pathogens and deliver “danger” signals to cells was put forward in 1989 by the late Charles Janeway (18). He proposed that such receptors on mammalian APCs would not only recognize conserved pathogenic structures but also signal intracellularly, leading to the transcriptional upregulation of costimulatory molecules based, in part, on the observation that “a whole variety of microbial substances could stimulate the antigen-presenting cells to express what we believed to be costimulatory activity” (19). In 1997, Ruslan Medzhitov, while working with Dr. Janeway, cloned “hToll” (later identified as human TLR4) (20). Because these investigators did not know the nature of the ligand for hToll, or if there existed a spätzle homolog in mammals, they engineered a construct that encoded the CD4 ectodomain with the TLR4 intracellular domain. This would have allowed them to cross-link the CD4 ectodomains with anti-CD4 Ab, if necessary. When this CD4–hToll fusion protein was overexpressed in mammalian cells, however, it constitutively activated NF-κB and resulted in the upregulation of costimulatory molecules, as originally predicted by Janeway. This work, published just 1 y after Lemaître et al. (1), represents the first direct link between TLR signaling in mammalian cells that leads to NF-κB activation, that in turn, upregulates the costimulatory molecules, and thereby facilitates the transition between innate and adaptive immune responses in mammals. The cloning of five human TLRs (named TLR1–5) soon followed (21) and, importantly, these authors mapped the locations of each on the human genome. Still, the ligands for these human TLRs were not known at the time.

In contrast to the events that led to identification of human TLRs, the cloning of murine TLR4 was preceded by more than 30 y of publications showing that the LPS-unresponsive C3H/HeJ mouse strain, which arose by spontaneous mutation sometime between 1960 and 1965, was highly susceptible to infection by Gram-negative bacteria (reviewed in Refs. 22 and 23). With the use of standard genetic mapping techniques, the Lps gene (Lpsd, for the allele encoding normal LPS responsiveness, and Lpsd, for the defective allele) was mapped to mouse Chromosome 4 and was inherited codominantly (reviewed in Ref. 22). Macrophages from C3H/HeJ mice failed to respond to highly purified LPS or lipid A to produce cytokines or become tumoricidal in vitro, but responded normally to “endotoxin-associated proteins” that were present in less rigorously purified LPS preparations (24–27). Hence, a relationship between sensitivity to a bacterial product (i.e., LPS or bacterial proteins) and resistance to infection in mammals was well established prior to 1980. In fact, in the mid-1970s, Coutinho and colleagues prepared an antiserum that was used to identify the C57BL/10ScCr strain as LPS unresponsive (i.e., because this reagent also failed to stain cells from the C57BL/10ScCr strain, this group assessed its LPS sensitivity and showed that this strain was LPS unresponsive) (reviewed in Ref. 23). These and other studies led to the hypothesis that Lpsd mice lacked a signaling receptor for LPS, even prior to the cloning of Drosophila Toll in 1988 (28).

Although many laboratories attempted to clone the Lps gene by various approaches (reviewed in Refs. 22 and 23), Bruce Beutler and colleagues (29) were successful using a positional cloning approach to clone the murine TLR4 gene. This achievement was followed by a landmark paper by Poltorak et al. (30), published in December 1998, showing that the C3H/HeJ mouse strain carried a point mutation in the TIR domain of Tlr4 and that Tlr4 was largely deleted in the C57BL/10ScCr strain. For this discovery, Dr. Beutler was Dr. Hoffman’s corecipient of the 2011 Nobel Prize that was also shared with Dr. Ralph Steinman for his pioneering work on dendritic cells.

An avalanche of studies in mammalian innate immunity soon ensued. Within a few months after Poltorak et al. (30) published their findings, independent confirmation was provided by Malo and colleagues (31), followed in short order by work from Shizuo Akira’s laboratory (32) showing that mice with a targeted mutation in TLR4 (i.e., TLR4−/−) mice were unresponsive to LPS and exhibited increased susceptibility to infection. Kawai et al. (33) soon reported that MyD88−/− mice were refractory to lethal challenge with LPS in vivo and that MyD88−/− macrophages responded poorly to LPS to express NF-κB–dependent cytokine genes. This observation was key because MyD88, the functional homolog of Drosophila MyD88 (dMyD88), serves as a critical adapter protein for both IL-1R and TLRs (reviewed in Ref. 34). However, although most assumed that the TLR4 ligand was bacterial LPS, Shimazu et al. (35) showed that MD-2, a protein that binds noncovalently to the extracellular domain of TLR4, serves as the actual “receptor” for LPS, whereas TLR4 serves as the signal-transducing component of a larger “TLR4 signaling complex.”

It is impossible in this venue to begin to detail the many other studies constituting the unprecedented intellectual explosion in the field of innate immunity that followed the 1996 work of Lemaître et al. Nonetheless, the first experimental linkage of Toll signaling to innate immunity in Drosophila by Lemaître et al. is certainly deserving of recognition as a Pillar of Immunology.

Acknowledgments
I thank Douglas Golenbock, Neal Silverman, Martin Flajnik, and Charles Dinarello for thoughtful discussions and insights during the preparation of this article.

Disclosures
The author has no financial conflicts of interest.

References


