Toll-like receptors in the spotlight

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Evidence that the relatively new field of Toll-like receptors (TLRs) has come of age was apparent at a meeting held by the Biochemical Society at the Novartis Respiratory Centre in Horsham, UK, on 3 February 2003. The meeting covered a wide range of topics including how the innate response is tailored to the invading pathogen, how TLRs might have evolved in humans to recognize microbes, emerging complexities in signaling pathways, and new genetic- and genomic-based techniques to address questions in the area of innate host defense. In addition, as prime sensors of microbial products, TLRs could be useful drug targets.

TLRs are characterized by an ectodomain composed of leucine-rich repeats and a cytoplasmic tail called the Toll-IL-1 receptor homology domain (TIR), which is also found in members of the IL-1R family and in other cytoplasmic proteins such as MyD88 (ref. 1). MyD88 functions as an adapter, being ubiquitously linked to all TLRs, and couples the TLR to downstream signaling kinases. Another TIR-containing adapter protein, Mal (also called TIRAP), is essential for the production of tumor necrosis factor (TNF) and IL-6 *via* the MyD88-dependent TLR2 and TLR4 signaling pathway. There are also three other adapters with TIR domains (see below). Whether adapter usage will explain the specificity of gene expression induced by stimulation of individual TLRs is a central question. Differences between adapters may therefore reveal specificity of function. Indeed, structural differences between Mal and MyD88 and tyrosine phosphorylation of Mal might be important for the specific function of Mal in TLR4 signaling (Luke O'Neill, Dublin, Ireland).

Genetic experiments have established evidence for MyD88-independent pathways downstream of certain TLRs, in particular TLR3 and TLR4. A TIR domain–containing molecule, named TRIF (also called TICAM-1)[2,3], seems to be involved in the TLR MyD88-independent pathway activated by TLR3 and TLR4 and is particularly important for IRF-3 activation (Shizuo Akira, Osaka, Japan). Two other TIR domain–containing proteins have been identified, MyD88-4 (also called RIVIG-2) and MyD88-5 (also called Drp or SARM) (Luke O'Neill and Fernando Bazan, Palo Alto, CA, USA). Their functions are not yet known, but they may also confer receptor specificity for functional effects. In addition, mutant mice generated by random mutagenesis of germline cells have shown interesting immunological phenotypes (Bruce Beutler, La Jolla, CA, USA). Monocytes from one such mutant exhibit normal MyD88-dependent signaling pathways yet have very strong defects in responses mediated by MyD88-independent signaling, such as IRF-3 activation. Other random mutants revealed genes that could potentially encode unique coreceptors and universal adapters similar to MyD88 that might help determine TLR specificity.

How TLRs interact with their ligands is another crucial question. Bazan used a genomic approach to address this point, describing a model whereby lipopolysaccharide (LPS) is directly sensed by TLR4, but only when MD-2 masks the charged portions of the LPS molecule, with the lipid A moiety protruding and interacting directly with TLR4. There was, in fact, a general consensus that microbial products such as LPS and peptidoglycan bind directly to TLRs. This contrasts with the situation in the fly, where the ligand Spatzle, generated during infection, binds Toll. The closest human relative to Spatzle may be the protein Noggin, and Bazan pointed out that humans have many 'dead' Noggin genes. Both Bazan and Nick Gay (Cambridge, UK) propose that once the IL-1R system (which does not exist in the fly) evolved—which occurred after the evolution of TLRs—ancestral human Tolls were freed of evolutionary constraints and could evolve to recognize pathogen-associated molecular patterns (PAMPs) directly. The role of the TLR4-associated proteins in LPS binding and signaling was also discussed by Doug Golenbock (Worcester, MA, USA), who demonstrated that LPS-binding protein (LBP) was also required for LPS function *in vivo*, probably acting to deliver LPS to MD-2.

Prior exposure to LPS can desensitize immune cells to subsequent challenge with LPS, a phenomenon termed 'endotoxin tolerance'. This effect is not limited to the TLR4 agonist LPS, but also affects signaling initialized by agonists for other TLRs[4]. TLR4 signaling induces expression of a wider repertoire of genes than does TLR2 signaling, although microarray studies have shown that of 799 genes induced by LPS, only 48 displayed tolerance upon rechallenge (Stefanie Vogel, Baltimore, MD, USA). Some genes can also be 'heterotolerized' by a TLR2 agonist (that is, a TLR2 agonist will cause subsequent tolerance towards a TLR4 agonist). This may represent 'macrophage reprogramming' caused by different combinations of *trans*-activating and *trans*-repressing factors elicited as a consequence of the initial TLR stimulus. In tolerized cells, MyD88 is not recruited to TLR4, so the MyD88-independent pathway must induce genes that cannot be tolerized. It will be interesting to determine whether other newly characterized adapters such as Mal and TICAM can be recruited to TLR4 during tolerance.

Given that the field only really came into existence in 1998, the rapid progress in our understanding of TLRs has been remarkable. There is currently a strong belief that selective targeting of the TLRs will be a fruitful approach in designing new therapies to combat a variety of diseases. This is reinforced by the demonstration that vaccinia virus encodes A52R, a protein that blocks NF-κB activation in response to multiple TLRs[5]. These results underscore the potential for interfering with TLR signaling in the context of drug development in the context of drug development for conditions such as sepsis syndrome and inflammatory diseases.

1. Akira, S. *et al. Nat. Immunol.* **2**, 675–680 (2001).
2. Yamamoto, M. *et al. J. Immunol.* **169**, 6668–6672 (2002).
3. Osiumi, H. *et al. Nature Immunology* **4**, 161–167 (2002).
4. Dobrovolskaia, M. *et al. J. Immunol.* **170**, 508–519 (2003).
5. Harte, M.T. *et al. J. Exp. Med.* **197**, 343–351 (2003).