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Of mice and man: TLR11 (finally) finds profilin

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Toll-like receptors (TLRs) are primordial pathogen-recognition proteins that function as sentinels for the innate immune system. One of the TLR mysteries relates to TLR11, a receptor present in mice, but not humans, and known to recognize uropathogenic *Escherichia coli*. The first defined ligand for TLR11 has now been described as a profilin-like protein from *Toxoplasma gondii*. This discovery potentially gives us important clues as to how a gene expressed in mice, but not humans, actually relates to human infectious diseases.

Introduction

Toll-like receptors (TLRs) [1] recognize components of various bacteria, viruses, fungi and parasites [2]. Yarovsky and colleagues demonstrated recently that a profilin-like molecule from *Toxoplasma gondii* activates TLR11, and that TLR11 has an important role during infection in mice [3]. In addition, TLR11 is also a receptor for human uropathogenic *Escherichia coli* [4]. However, although present in mice, the human *TLR11* gene contains several stop codons and does not code for a full-length protein. Nevertheless, we believe

that what we learn about TLR11 will provide us with important insights into the pathogenesis of a variety of important human infections.

TLRs

The TLR family is a group of pattern-recognition receptors that are evolutionary conserved germline-encoded transmembrane proteins [1]. These receptors are characterized by multiple leucine-rich repeats (LRRs) in the extracellular domain and a cytoplasmic Toll-interleukin-1 (IL-1) resistance (TIR) domain. TLRs are crucial components of the innate immune system because they recognize conserved motifs on pathogens, which are often referred to as pathogen (or microbe)-associated molecular patterns (PAMPs or MAMPs) [2]. For example, TLR4 (in conjunction with MD2 and CD14) recognizes endotoxin [lipopolysaccharide (LPS)] from Gram-negative bacteria and TLR2 is the receptor for bacterial lipopeptides and lipoproteins, whereas TLR3 and TLR7 can recognize viral RNA. Ligation of a TLR by its ligand typically results in the stimulation of proinflammatory activity that has the capacity to protect the host from microbial invasion. TLRs also have an essential role in enhancing the adaptive immune response through the upregulation of

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co-stimulatory molecules. Thirteen mammalian TLRs have been identified but only 10 (TLR1–10) are expressed in humans.

TLR11 identified as the receptor for uropathogenic *E. coli* and *T. gondii* profilin

The recent discoveries of the importance of TLR11 in uropathogenic *E. coli* [4] and *T. gondii* [3] infection have raised an exciting issue that does not appear to have been encountered in the pathogenesis field before. These reports, based heavily on experiments in knockout mice, appear to unequivocally identify TLR11 as being involved in two important human diseases. A clear-cut phenotype was observed. This was particularly surprising for uropathogenic *E. coli* because existing dogma suggests that either TLR2 (by recognition of cell wall components) or TLR4 (LPS) should be sufficient to fight this infection. Most intriguing was the observation that, although TLR11 is expressed in abundance in mice, the human *TLR11* gene appears to contain a stop codon that would prevent expression of the protein [4]. Thus, we have data on two human diseases that appear to have clear-cut roles in pathogenesis in mice, however, the gene product involved is not expressed in humans. Curious indeed.

Thanks to the current report of TLR11 function, some of the mystery of this gene appears to be lifting. *T. gondii* is an obligate intracellular protozoan parasite, which is an important cause of human infection [5]. The parasite has a complicated life cycle, in which the definitive host (in which the sexual cycle takes place) is the domestic cat. Many intermediate hosts exist, including the mouse, which can infect cats (the cats ingest bradyzoites present in the tissues of their murine prey). Humans get infected primarily through the contact of oocysts in feline feces. Previously, it was shown that both host resistance to *T. gondii* and parasite-induced IL-12 production by dendritic cells require the adaptor molecule MyD88, suggesting strongly the involvement of TLRs [6].

Yarovinsky *et al.* [3] identified profilin as a TLR11 activating ligand. Profilin is a molecule that is produced by *T. gondii*, and is present in abundance in soluble antigen preparations that are produced using parasite cultures. Also, they provided substantial data that this recognition event is important in mouse host defense, although not all differences observed in MyD88-knockout mice could be seen in TLR11-knockout mice. Profilins are a class of small actin-binding proteins present only in eukaryotic cells, which have a regulatory role in the polymerization of actin. Database searches revealed that the identified *T. gondii* profilin shares significant homology only with profilin genes from other apicomplexan protozoa, such as the malaria parasite. Therefore, one might speculate that TLR11 might be involved in the recognition of these parasites.

Perhaps the most appealing part of the discovery that *T. gondii* profilin is a ligand for TLR11 is that the context of the discovery makes sense. In other words, although there is still no evidence for a Toll receptor involved in the recognition of *T. gondii* during human infection and humans do not express TLR11, one can imagine that TLR11 might have a crucial role in human disease indirectly because of the role that the mouse has in

transmitting the disease to cats, who can then infect humans. Indeed, the role of TLR11 in mice, and any polymorphisms that might be discovered in the future, should be of interest to anyone who is interested in the biology of zoonoses. Nevertheless, although one can imagine the evolutionary pressures that gave rise to TLR11 in a progenitor animal, it remains unclear why it was downgraded to a non-coding gene in humans.

In considering this scenario, the first thought was that TLR11 actually does exist in the human genome and that the initial reports were incorrect. Several groups, including our own, have repeated the genomic analysis and have confirmed the initial reports that remnants of the human TLR11 do exist, however, the predicted mRNA has at least one clear-cut stop codon that insures that the protein is not expressed. Alternatively, it is tempting to suggest that human TLR5 performs the same function as mouse TLR11 because they both bind bacterial proteins [7,8]. Under such a model, one might predict a TLR gene tree, in which TLR5 and TLR11 are the result of a gene duplication in a mouse or human progenitor. Unfortunately, reconstructed TLR phylogenies are unreliable and the placement of TLR11 is highly dependent on the sequences included and the method used. The case might be helped when more genomes are complete; we found TLR11 in the rat genome (~71% identical) but not in the genomes of chimp, fugu, dog, frog and cat, which remain unfinished. A recently published paper has proposed a molecular tree model of all vertebrate TLRs, which includes a separate TLR11 family [9]. Most members of this family, which includes the TLR11–13 and TLR21–23 subfamilies, have representatives from mice, fish and frogs.

The context of what we previously knew about the role of TLR11 in disease was not as easy to grasp when all we knew was about its role in uropathogenic *E. coli* infection [4]. Zhang *et al.* demonstrated that uropathogenic strains of *E. coli* activate cells that are (over)expressing TLR11, a response which is not seen with non-pathogenic strains of *E. coli* or any other known TLR ligand. During *in vivo* infection in mice, TLR11 appears to protect the kidneys from ascending infection. However, the specific component of these uropathogenic *E. coli* that is recognized by TLR11 has not been identified yet.

It is important to keep in mind that the term, 'uropathogenic' was applied to *E. coli* because it identifies a microorganism that is cultured recurrently from the human urogenital tract. No literature exists on common mouse urinary pathogens in the wild, and hence it is difficult to conjecture rationally why such a clear phenotype attributable to a mouse-specific gene with a human pathogen should exist.

Concluding remarks

Despite these problems, it seems likely that we will soon identify the true ligand for TLR11 from uropathogenic *E. coli*. All the tools for this discovery exist. These include transformed cell lines, isogenic mutants in uropathogenic *E. coli*, knockout mice and effective small inhibitory (si)RNAs that can be used to aid in the biochemical identification of the components of the bacteria that activate the receptor. At that point, it will be possible to

determine if the ligand is active immunologically in humans, and which TLR (if any) is responsible for its recognition. Only then can the mystery of the mouse gene that seems to have a role in a human infectious disease be cleared up. Most importantly, it is probable that this type of discovery will provide special insight into the pathogenesis of additional infections that are likely to be identified as engaging TLR11 in the mouse. This is especially true of parasitic infections, to which the use of TLRs appears to be important [10,11] but to which no specific TLR has been assigned.

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