Abstract
The pro-inflammatory cytokine interleukin-1 (IL-1) signals via the Type-I IL-1 receptor (IL-1RI), inducing an increase in the expression of many genes with roles in immunity and inflammation. The signalling pathways involve two adapter proteins, MyD88 and Tollip, which via two IL-1 receptor-associated kinases (IRAK and IRAK-2) activate transcription factors such as nuclear factor-κB and protein kinases such as p38 mitogen-activated protein kinase. A role for the low-molecular-mass G-proteins Rac, Ras and Rap in these processes has also been indicated. IL-1RI is the founder of a diverse superfamily of receptors, which all share a cytosolic domain, termed the Toll/IL-1 receptor (TIR) domain. The superfamily can be divided broadly into three subgroups. The first of these is most similar to IL-1RI and includes the receptor for IL-18 and the Th2 cell regulator T1/ST2. The second subgroup is most similar to the *Drosophila melanagaster* protein Toll and includes Toll-like receptor 2 (TLR2), which is required for host defence against Gram-positive bacteria and fungi, and TLR4, which is required for lipopolysaccharide responsiveness, and thus is involved in host defence against Gram-negative bacteria. There are also a number of TLRs in plants and insects, all involved in host defence. The third subgroup contains non-receptor proteins which possess a TIR domain and are cytosolic. MyD88 is a member, and it presumably complexes with IL-1RI via a TIR–TIR interaction. The other two members are proteins encoded by the vaccinia virus, A46R and A52R, which block TIR-dependent signalling. This receptor superfamily therefore appears to play a central role in inflammation and host defence against infection, pointing to the TIR domain as a critical molecular player in the innate immune response.

Introduction
The pro-inflammatory cytokine interleukin-1 (IL-1) has been studied for many years, because of the role it plays in inflammation and the pathogenesis of inflammatory diseases such as rheumatoid arthritis. Its many effects include induction of fever, the acute-phase response, leucocyte adhesion and migration, enhanced proteoglycan breakdown in joints and an overall increase in immune reactivity which occurs via the induction of many different cytokines. Its effects on innate immunity are therefore clear, and IL-1 can be considered a link between innate and adaptive immunity. It is strongly induced by bacterial products such as lipopolysaccharide (LPS), and acts either directly or indirectly (via the induction of other cytokines) on antigen-presenting cells and T- and B-lymphocytes. A key challenge concerning IL-1 has been the attempt to uncover its mechanism of action. The Type-I IL-1 receptor (IL-1RI) was first described in 1988 [1]. However, the signalling pathway activated proved elusive with, at times, controversial signalling mechanisms being implicated in its effects [2,3]. An important breakthrough came in 1989 with the description of the activation of the transcription factor nuclear factor-κB (NF-κB) by IL-1 [4]. NF-κB has since become the focus of much attention because of its virtual ubiquity as a regulator of inflammatory and immune gene expression. Most genes involved in these processes possess NF-κB-binding sites in their 5′-flanking regions. The pathway leading to NF-κB activation by IL-1 has now been described in detail and involves a number of novel proteins. These are documented below. Other important signals activated include p38 mitogen-activated protein kinase (MAP kinase) and Jun N-terminal kinase (JNK; reviewed in [5]).

Apart from progress on IL-1 signalling the advent of genomics has led to a number of proteins being described which have sequence similarities with IL-1RI or indeed IL-1 itself.
This apparent expansion in the ‘IL-1 system’ illustrates the difficulties that will confront investigators interested in IL-1. Current approaches that aim to annotate novel gene sequences rely in the first instance on an examination of sequence similarities with genes whose proteins have known functions. The IL-1 ligand and receptor system is therefore proving fruitful in this regard as there are now at least five gene sequences which appear to encode proteins similar to IL-1 (currently named IL-1β, IL-1α, IL-18, IL-1H1 [6,7]) and over 30 that encode proteins with sequence similarity to the cytosolic portion of IL-1RI (reviewed in [5]). Many of these, including the potentially novel ligands, have not yet had a function ascribed to them. The challenge will now be to test these predictions in biological systems. The new receptor superfamily is defined by the Toll/IL-1 receptor (TIR) domain. Two of the receptors in the superfamily, termed Toll-like receptor 2 (TLR2) and TLR4, are critical for the host response to Gram-positive and Gram-negative bacteria, respectively. What was previously thought of as a non-specific response – the innate response to these pathogens – therefore appears to have some specificity. The defining of this new superfamily further emphasizes the importance of a system, first apparent in the case of IL-1, for innate immunity and inflammation.

The TIR superfamily

Figure 1 describes the currently known members of the TIR superfamily. The superfamily is very diverse, but all members share the common feature of being involved in host defence or inflammation. Broadly speaking, it can be divided into three subgroups. All have TIR domains, but are grouped according to other similarities. The TIR domain spans over 200 amino acids and has three particular regions (or boxes) that are strongly conserved [5,8,9]. The first subgroup all have extracellular immunoglobulin domains and are defined by IL-1RI, the signalling receptor for IL-1. This receptor can bind the two IL-1 agonists, IL-1α and IL-1β, and also the IL-1 receptor antagonist, IL-1Ra. Also in this subgroup is the IL-18 receptor (IL-18R) [10]. IL-18 is structurally very similar to the IL-1 ligands. It is a critical cytokine for Th1 cell activation. These are the only two receptors in this subgroup with known ligands. Orphan receptors include T1/ST2, which has recently been shown to play a key role in Th2 cell function [11], most probably by acting as a co-stimulating receptor. The remaining orphan receptors have no known ligand or function.

In this subgroup there are also receptor accessory proteins. Both IL-1RI and IL-18R require accessory proteins, which do not appear to bind ligand but which are required for signalling. These are termed IL-1 receptor accessory protein (IL-1RACP) [12] and ‘accessory protein-like’ (AcPL) [13] respectively. Other accessory-protein-like proteins have been described in this subgroup, and include ‘IL-1 receptor accessory protein-like’ (IL-1RAPL) [14], which appears to be expressed mainly in brain, where it may have a role in learning and memory. The precise function of the accessory proteins is at present unknown although they are required for signalling.

The second subgroup was defined originally by the *Drosophila melanogaster* protein, Toll, which was first described in the establishment of dorsoventral polarity. The similarity in sequence between Toll and IL-1RI [15] was unexpected as IL-1 has no known role in development. A further parallel between IL-1 and Toll signalling was that Toll activated a transcription factor termed Dorsal. This protein is a member of the Rel family of transcription factors, including the NF-κB subunits p50 and p65, which are activated by IL-1 during inflammation (see below). The parallel was not that surprising as in effect the same signalling apparatus was being used, although for different end-points. Subsequently however, it was shown that Toll was also critical for defence against fungal infection in the adult fly [16], where it activates another Rel family member termed Dif [17]. The ligand for Toll in both development and host defence is a protein termed Spatzle, which is generated from a pro-form by a serine protease. In the adult fly this is kept in check by a serpin termed Spn43Ac and is activated during infection [18]. Apart from Toll, the fly also contains other TLRs, including 18-Wheeler, which is required for anti-bacterial defences [19].

Most importantly, 10 human TLRs have been described, termed TLR1–10 [9,20]. Of these, only two have a known function. TLR2 is required for responses to Gram-positive bacteria and fungi, responding to such products as peptidoglycan and lipoteichoic acid [21–23]. Upon binding, TLR2 internalizes into the phagosome, and this internalization is critical for signalling [23]. TLR4 on the other hand is required for responses to LPS [24]. This was discovered in the effort to determine the molecular basis for the LPS hyporesponsiveness of the C3H/HeJ mouse. The *Tlr4* gene in this
Innate Immunity

mouse is mutated such that a proline is converted into a histidine in the TIR domain [24]. This mutation occurs in the conserved Box 2 of the TIR domain and renders TLR4 inactive. In humans, extracellular mutations have been described which interestingly also result in hyporesponsiveness to LPS [25].

It is worth noting that the original confusion over whether TLR2 or TLR4 was the receptor for LPS appears to have been resolved by the recent observation that repurification of LPS removes protein components responsible for signalling via TLR2, while leaving the ability to signal via TLR4 intact [26]. This provides a salutary tale for those

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Figure 1
The TIR domain superfamily

The major known members of the TIR superfamily are shown. The superfamily is defined by a 200-amino acid domain termed the TIR domain. It splits into three subgroups. Subgroup (a) contain immunoglobulin domains and include IL-1RI and IL-18R and their respective accessory proteins. SIGIRR (single-immunoglobulin IL-1 receptor-related) is also in this subgroup but has only one immunoglobulin domain [48] whereas IL-1RII has no TIR domain, acting as a decoy receptor [49,50]. Subgroup (b) all have leucine-rich repeats (LRRs) and include the TLRs. Subgroup (c) are non-receptor cytosolic proteins and include MyD88, which acts as an adapter for the superfamily, and two viral proteins, A46R and A52R, which inhibit TIR-dependent signalling. See text for details. TIGGIR-1, three immunoglobulin IL-1 receptor related-1.

The TIR superfamily

(a) Immunoglobulin domain sub-group

- IL-1RI
- IL-1RII
- B15R
- IL-1RACP
- IL-18R
- IL-18RACP
- T1/ST2
- IL-1Rrp2
- IL-1RAPL
- TIGGIR1
- SIGGIR

Mammals
Mammals
Pox viruses
Mammals
Mammals
Mammals
Mammals
Mammals
Mammals
Mammals
Mammals
Insects
Insects
Insects
Insects
Mammals
Mammals
Mammals
Mammals
Mammals
Plants
Plants
Plants
Mammals
Pox viruses
Pox viruses

(b) Leucine rich-repeat sub-group

dToll
cToll
trToll
18W
MstProx
Tehao
TLR1
TLR2
TLR3
TLR4
TLR5 - 10
N
L6
RPP1
RPP5

Mammals
Insects
Insects
Insects
Insects
Insects
Mammals
Mammals
Mammals
Mammals
Mammals
Plants
Plants
Plants
Mammals

(c) Adapter sub-group

- MyD88
- A46R
- A52R

Mammals
Pox viruses
Pox viruses
investigating microbial products of uncertain purity.

In addition to TLR4, proteins termed CD14 and MD2 are required for LPS responsiveness [27]. TLR4, however, appears to be the signalling component. Whether TLR4 (or indeed TLR2) are actual receptors for microbial products is yet to be proven definitively. If the situation is similar to the fly, a ligand for TLRs should be generated in response to bacterial products.

The role of the other TLRs remains unknown. TLR3 may have a role in dendritic-cell function, as it appears to be expressed exclusively in these cells [28]. An intriguing possibility is that different TLRs will respond to different pathogens, lending specificity to what was previously thought of as non-specific innate immunity.

Also in this subgroup are a number of plant proteins with roles in host defence. These include N protein from tobacco, which confers resistance to tobacco mosaic virus [29]. These proteins all have a TIR domain and leucine-rich repeats but intriguingly are cytosolic. Whether there will be mammalian counterparts that are also cytosolic is an interesting possibility.

The final subgroup currently comprises three members: MyD88, A46R and A52R. All are cytosolic, and rather than being receptors they are involved in signalling. MyD88 is a critical protein for signalling by IL-1RI, IL-18R, TLR2 and TLR4 [30,31] and, by extension, presumably the entire family. It has a TIR domain and also a death domain and is recruited to the receptor complex in response to IL-1, where it acts as an adapter. It occurs upstream of the IL-1 receptor-associated kinases IRAK and IRAK-2. A46R and A52R are viral proteins, encoded by pox viruses [32]. Based on alignments, both contain a TIR domain and when overexpressed interfere with IL-1R, TLR4 and IL-18 signal transduction, presumably by interfering with the assembly of signalling components [32]. This would appear to be part of the means by which pox viruses interfere with host defence.

The TIR domain is therefore present in a large number of proteins, acting as a switch in the process of cellular activation during innate immunity and inflammation. Given its occurrence in plants this signalling system is likely to be ancient in terms of evolution, occurring in the common unicellular ancestor of plants and animals. It has been suggested that the structure of the TIR domain may be similar to a bacterial protein, CheY, which is important for the chemotactic response in certain bacteria [9]. In addition, a possible homologue has been found in the bacterium Streptomyces coelicor [33]. This would place the TIR domain as one of the earliest signalling domains to have been described, perhaps attesting to its molecular efficiency as a relay system for cellular responses.

**Signal transduction**

Figure 2 presents a current model for IL-1 signal transduction. As stated above, the area of IL-1 signalling has proved difficult and at times controversial. There is now a consensus, however, on the major pathway activated by IL-1, which culminates in the activation of the transcription factor NF-κB. A number of proteins are involved. In essence, upon binding IL-1, IL-1RI and IL-1RAcP form a signalling complex which recruits MyD88. IRAK and IRAK-2 are then recruited followed by another adapter, TRAF6 (tumour necrosis factor receptor-associated factor 6) [34–36]. This then leads to activation of the inhibitory κB (IκB) kinase (IKK) complex, resulting in phosphorylation of IκB, releasing the NF-κB dimer allowing it to translocate to the nucleus [37]. The components linking TRAF6 to the IKK complex (which is a multiprotein complex) may involve a protein termed evolutionary conserved signalling intermediate in Toll pathway (ECSIT), which couples to the kinase MAP kinase kinase-1 (MEKK1) [38]; a role for NF-κB-inducing kinase having been ruled out [37]. A role for the kinase TAK-1 (transforming growth factor-β-activated kinase) has also been indicated [39]. The precise role of any of these proteins (other than IKK) is not known. Recently, an additional component has been described, Toll-interacting protein (Tollip), which appears to regulate the recruitment of IRAK to the receptor complex, allowing it to interact with MyD88 via its death domain [40]. This leads to hyperphosphorylation of IRAK, which then dissociates from the complex and is degraded. The importance of this dissociation is not clear nor are the actual dynamics of the protein–protein interactions involved. In any event, MyD88, IRAK and TRAF6 are all required for NF-κB activation by IL-1, as mice deficient in any of these are unresponsive [41–43]. MyD88 and IRAK are also required for IL-18 responsiveness [41,44]. This implies that the TIR domain will activate common signalling proteins.

It is likely that additional proteins will be involved in these processes. We have recently provided evidence for a role for the low-molecular-
mass G-protein Rac in the NF-κB pathway [44]. Intriguingly, we could find no evidence for a role for Rac in the pathway to the IKKs. Instead, it was involved in the ability of the p65 subunit of NF-κB to transactivate gene expression once bound to the NF-κB promoter [45]. This second pathway is therefore required subsequent to the release of NF-κB from IκB. Preliminary evidence indicates that Rac is recruited to the IL-1R receptor complex and may interact with MyD88 (C. A. Jeffries and L. O’Neill, unpublished work).

The second area of investigation in IL-1 signalling concerns MAP kinases. IL-1 activates all three of the best-characterized mammalian MAP kinase cascades: those involving p42/p44 and p38 MAP kinases and JNK (reviewed in [5]).

The consequences of their activation are at present not fully understood. Given that transcription factors are substrates for these kinases they may be involved in changes in gene expression. In addition, p38 MAP kinase has been shown to be involved in the stabilization of mRNAs that contain AU-rich repeats [46]. It is likely that p38 phosphorylates a substrate that binds such repeats, leading to stabilization of the mRNA. Many immune-system and inflammatory genes have such repeats, making this process another important point of control.

Studies on transgenic mice have indicated that MyD88 and IRAK are involved in JNK activation. We have also found evidence for a role for MyD88, IRAK and TRAF6 in p38 activation. Also of note, we have recently provided evidence for Ras as an important component on the pathway to p38 [47]. IL-1 activates Ras rapidly, followed by activation of the related G-protein Rap. Rap acts to antagonize the Ras signal, and may be important for the transient nature of p38 activation by IL-1. More recently we have positioned Ras on the pathway: it occurs downstream of TRAF6 but upstream of MAP kinase kinase (MKK)3 or MKK6, the kinases upstream of p38 (E. M. Paalsson and L. O’Neill, unpublished work). The kinase upstream of MKK3 or MKK6 is not yet known, but Ras may lie upstream of it in an analogous manner to the position of Ras on the classical p42/p44 MAP kinase pathway, where it occurs upstream of Raf. Figure 1 presents a model for IL-1 signal transduction incorporating these elements.

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**Figure 2**

**IL-1 signal transduction**

IL-1 activates four protein-kinase cascades in cells. One of these culminates in the activation of the transcription factor NF-κB whereas the other three involve MAP kinases. Early events in all cascades involve MyD88, IRAK-1, IRAK-2, TRAF6 and possibly the kinases TAK1 (transforming growth factor-β-activated kinase) and TAB1 (TAK1-binding protein 1). A role for Ras in the activation of p38 MAP kinase has also been shown, while Rac has been shown to participate in a pathway leading to p65-mediated transactivation of gene expression by NF-κB. See text for further details.
Conclusions
The key role that IL-1 plays in host defence and inflammation has been proven over the past 15 years. The emergence of TLRs has opened up new avenues of research for those interested in the host response to pathogen-derived factors which trigger innate immunity. Recent years have seen a great expansion in proteins related to the IL-1 receptor, novel sequences which may encode IL-1-like ligands and novel signal-transduction pathways. The challenge now, as is evident in many areas of biological research, is to move from molecular taxonomy to the provision of a detailed quantitative understanding of this complex system, so critical to how the host responds to injury, whether caused by trauma, infection or autoimmunity.

References
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Toll-like receptor family and signalling pathway

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Abstract

Toll is a Drosophila gene essential for ontogenesis and anti-microbial resistance. Several orthologues of Toll have been identified and cloned in vertebrates, namely Toll-like receptors (TLRs). Human TLRs are a growing family of molecules involved in innate immunity. TLRs are characterized structurally by a cytoplasmic Toll/interleukin-1 receptor (TIR) domain and by extracellular leucine-rich repeats. TLRs characterized so far activate the MyD88/interleukin-1 receptor-associated kinase (IRAK) signalling pathway. Genetic, gene-transfer and dominant-negative approaches have involved Toll family members (TLR2 and TLR4) in Gram-positive and Gram-negative bacteria recognition and signalling. Accumulating evidence suggests that TLR2 is also involved in signalling-receptor complexes that recognize components of yeast and mycobacteria. However, the definitive roles of other TLRs are still lacking. A systematic approach has been used to determine whether different human leucocyte populations selectively or specifically express TLR mRNA. Based on expression pattern, TLR can be classified as ubiquitous (TLR1), restricted (TLR2, TLR4 and TLR5) and specific (TLR3). Expression and regulation of distinct but overlapping ligand-recognition patterns may underlie the existence of a large, seemingly redundant TLR family. Alternatively, the expression of a TLR in a single cell type may indicate a specific role for this molecule in a restricted setting.

Introduction

Toll was originally identified as a Drosophila gene required for ontogenesis and anti-microbial resistance [1,2]. Genetic analysis revealed that this gene controls dorsoventral polarization in the fruit fly as well as immunity against fungal infection. The recognition of sequence similarity between the cytoplasmic portion of Toll and that of signalling interleukin-1 (IL-1) receptor (IL-1R) components (the Toll/IL-1R module, or TIR module) represented the merging point of Drosophila work with more conventional cytokine/innate-immunity research [3]. A human homologue of Toll, the Toll-like receptor (TLR), has been identified [4]. Subsequently, several TLRs have been identified and cloned (TLR1–6) and many more are expected to be discovered [5,6]. Genetic, gene-transfer and dominant-negative approaches have involved distinct TLR family members (namely TLR2 and TLR4) in Gram-positive and Gram-negative bacteria recognition and signalling. Accumulating evidence suggests that TLR2 is also involved in signalling-receptor...