

Toll-Like Receptors and Their Function in Innate and Adaptive Immunity

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Abstract

Over the past 3 years our knowledge about how we sense the microbial world has been fundamentally changed. It has been known for decades that microbial products, such as lipopolysaccharide, lipoproteins, or peptidoglycan, have a profound activity on human cells. Whereas the structure of many different pathogenic microbial compounds has been extensively studied and characterized, the molecular basis of their recognition by the cells of the innate immune system remained elusive for a long time. It was Charles Janeway [Cold Spring Harb Symp Quant Biol 1989;54/1:1–13] who developed the idea of microbial structures forming pathogen-associated molecular patterns that would be recognized by pattern recognition receptors. The discovery of the family of Toll receptors in species as diverse as *Drosophila* and humans, and the recognition of their role in distinguishing molecular patterns that are common to microorganisms have led to a renewed appreciation of the innate immune system. Moreover, it is now clear that the activation of the innate immune system through mammalian Toll-like receptors has also an instructive role for the responses of the adaptive immune response and, thus, may influence allergic diseases such as asthma.

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Drosophila Toll Receptors

Flies do not have an adaptive immune system and rely entirely on their innate immune system to combat and contain microbial infections. The Toll protein has been identified as a crucial member of this innate response. Toll is a type I transmembrane receptor [1] initially identified as an essential receptor controlling the dorsoventral polarization during the embryonic development of the fly larvae [2, 3]. A group of 12 proteins is required for the Toll signalling cascade: spätzle, the endogenous ligand for Toll, is generated through a proteolytic cleavage from a serine protease cascade including the proteins gastrulation-defective, snake, and easter. The activation of this cascade is facilitated through the proteins windbeutel, pipe and nudel [4]. Engagement of Toll by spätzle leads to receptor dimerization, and recruitment of the adaptor proteins tube [5], and the activation of the serine-threonine kinase pelle [6]. Tube and pelle then interact with each other via their death domains [7, 8]. Through an unknown mechanism activated pelle leads to the phosphorylation and subsequent degradation of the inhibitor cactus [9], freeing the transcription factor dorsal, a *Drosophila* Rel/nuclear factor (NF)-kB protein, and allowing it to translocate into the nucleus to activate specific target genes essential for the establishment of the dorsoventral axis of the embryo.

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What led to the discovery that this pathway is also involved in the immune response of the adult fly? For long, researchers had studied the induction of antimicrobial peptides in *Drosophila*, such as drosomycin, diptericin and cecropin [10], in response to infectious challenge. These genes contain κ B elements in their promoter region [11]. However, dorsal mutants showed normal injury-induced expression of diptericin [12], indicating that a different transcription factor had to be involved. One year later, Lemaitre et al. [13] reported that the *spätzle/Toll/cactus* gene cassette was required for an antifungal response of *Drosophila*, demonstrating that the Toll receptor not only had a pivotal role during embryogenesis but also for the immune response of the fruit fly.

Despite its similarity, the Toll pathway leading to the expression of antimicrobial peptides also shows some remarkable differences to the developmental Toll pathway. In addition to the adaptor protein tube, the Toll pathway also requires the adaptor protein dMyD88 [14]. Also, the transcription factor driving the expression of drosomycin in the adult fly is another Rel protein known as Dif [15–17]. The proteolytic processing of spätzle is also required for the antimicrobial response, although not accomplished by the same protease cascade. Moreover, two different pathways leading to the cleavage of spätzle have been discovered, one activated by fungal infection, the other by Gram-positive bacteria. For the fungal pathway, one essential member is a blood serine protease named persephone [18]. However, persephone does not contain any known pattern recognition binding domain and, thus, is unlikely to be the ‘starting’ receptor of the fungi-induced toll activation. Werner et al. [19] reported 2 years ago on a family of *Drosophila* peptidoglycan recognition proteins (PGRP) that were able to interact with Gram-positive bacteria [20]. One of these, PGRP-SA, has now been shown to be inactivated by the mutation *semmelweiss*, blocking Toll activation by Gram-positive bacteria but not by fungi [21]. To date, nine Toll proteins have been identified in *Drosophila*, from which only two, Toll and Toll 5, are able to induce drosomycin in transfected cells [22]. Interestingly, none of these Toll proteins is required for responses against Gram-negative bacteria. This function has been originally accredited to another *Drosophila* Toll homologue, called 18-Wheeler [23, 24]. However, the role of 18-Wheeler has been reevaluated and has been shown to be dispensable in adult flies [25]. The response to Gram-negative bacteria is facilitated by the Imd pathway [26]. This pathway does not share any member with the Toll pathway. Just recently, the receptor starting this pathway has been characterized as another protein of the PGRP family,

PGRP-LC [27–29]. In contrast to the circulating PGRP-SA that initiates Toll activation by Gram-positive bacteria, PGRP-LC is a membrane-bound pattern recognition receptor (PRR). However, the steps that link PGRP-LC to Imd are not yet known. *Imd* encodes a death-domain-containing protein similar to the mammalian RIP [30]. Through the adaptor protein dFADD [31], DREDD [32, 33] and dTAK1 [34, 35], the *Drosophila* I- κ B kinase complex [36–38] becomes phosphorylated and Relish, the third member of the *Drosophila* NF- κ B family, is activated [36, 39]. Relish initiates the transcription of antibacterial target genes such as drosocin and diptericin.

In sharp contrast to the mammalian Toll-like receptors (TLRs) that are able to distinguish a very broad variety of different pathogen-associated molecular patterns (PAMPs), *Drosophila* employs two distinct pathways: one to combat Gram-positive and fungal infections, and a second for Gram-negative infections. In addition, the Toll pathway in flies is remarkably similar to the IL-1/NF- κ B pathway in mammals. Starting with the intracellular TIR domain that both Toll and IL-1R have in common [40, 41], it contains homologues of MyD88 [42], IRAK [43], I- κ B and NF- κ B. The Imd pathway contains one homologue of the IL-1/NF- κ B pathway as well (TAK1) [34], but also shows intriguing similarity to the mammalian TNF pathway, containing homologues of RIP, FADD and caspase-8 [44].

Mammalian TLRs and Their Ligands

A functional immune system requires that an appropriate response be mounted to any given infectious or immunologic challenge. The TLRs have evolved as crucial system for alerting the host to the presence of numerous infectious agents, as well as for the release of endogenous signs of cell damage. Infectious microbes display certain molecular patterns that usually are necessary for microbial virulence and, in some cases, viability. Many of these molecular patterns, such as lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria, seem to be particularly potent activators of mammalian cells. A common name for these molecular patterns is PAMPs. Furthermore, the mammalian receptors responsible for recognition of PAMPs, are called PRRs. Failure of the immune system’s PRR to recognize a pathogen’s PAMP could lead to a delayed and blunted immune response, resulting in unchecked replication or invasion by the microbe. Thus, this initial failure could prove highly detrimental.

The archetypical example of an incomplete recognition by the TLR system is a Gram-negative bacterial infection of the C3H/HeJ mouse, which can die by inoculation of as little as 2 colony-forming units of the Gram-negative *Salmonella typhimurium* [45]. Eventually, this inbred mouse strain was shown to harbor a point mutation (P712H) in the intracellular region of TLR4 [46–48], resulting in defective signal transduction in response to LPS and hypersusceptibility to Gram-negative infections. However, studies by Kirschning et al. [49] and Yang et al. [50] first suggested TLR2 as an LPS signal transducer. Experiments by Heine et al. [51] and Takeuchi et al. [52] using animals with mutant TLR2 indicated that TLR2 was not necessary for LPS signalling, but it was required for responses to Gram-positive bacteria, bacterial lipoproteins and mycobacteria [52–55]. Other reports confirmed these findings [56–61]. Furthermore, lipoteichoic acid was suggested as a TLR2 ligand [57, 62]. The signalling of enteric LPS via TLR2 was later associated with lipoprotein impurities in many LPS preps [63, 64]. Further evidence for TLR4 as a main component of the LPS signalling came from experiments suggesting TLR4 as able of sensing small differences in LPS/lipid A structure [65, 66]. Curiously, some types of LPS may still signal through TLR2. LPS isolated from *Porphyromonas gingivalis* [67], *Leptospira interrogans* [68], *Rhizobium* and *Legionella pneumophila* [69] appear to stimulate cells via TLR2. Originally thought to be a receptor only for LPS/lipid A, TLR4 now emerges as a molecule responsible for the signalling induced by a wide variety of molecules. These include microbial products such as respiratory syncytial virus protein F [70], various bacterial lipids [71], fungal components [72, 73], synthetic lipids [74], and the anti-cancer agent Taxol [75, 76]. Interestingly, several endogenous ligands for TLR4 have also been identified, such as heat shock proteins [77–80], fibronectin domains [81], fibrinogen [82], lung surfactant protein A [83], hyaluronan oligosaccharides [84], and beta-defensin [85]. The possibility of host proteins being able to activate the TLR signalling cascade may have implications for a variety of inflammatory states, including autoimmune diseases. Unfortunately, there are still many controversies in the literature regarding the ligands for TLR2 and TLR4. For example, both lipoteichoic acid and LPS/lipid A from *P. gingivalis* are also reported to interact with the TLR4, and not the TLR2 signalling system [52, 67, 86], and the role of endotoxin in signalling by heat shock proteins is still unclear [87, 88]. It is possible that the conflicting data may be the result of different protocols used to purify various ligands, or even different bacterial strains used as a

source of material. However, as we learned from the story of LPS, the presence of small amounts of contaminating compounds can play a critical role in determining the signalling pathways induced by specific ligands. Thus working with reagents of high purity is paramount to understanding ligand specificity.

All known TLRs, with the exception of TLR10, have upon submission of this manuscript been involved in signalling to specific compounds (table 1). These include TLR1 (together with TLR2) in the response to triacylated bacterial lipoproteins [89, 90], TLR3 for responses to poly(I:C; double-stranded RNA) [91], TLR5 for responses to flagellin and flagellated bacteria [92], TLR6 (together with TLR2) for responses to diacylated bacterial lipoproteins [93] and soluble factor from group B streptococci [94], TLR7 and TLR8 in mediating cellular stimulation induced by imidazoquinolones (small antiviral compounds) [95, 96] and TLR9 as a main signalling molecule for CpG bacterial DNA [97]. There is an amazingly broad ligand specificity for many of the TLRs. How such different ligands as proteins and lipids can induce signalling via the same TLR is unclear. Indications on direct interaction with TLRs and their ligands exist, but the evidence has so far been limited [65, 66, 98, 99]. One explanation may be that molecules in receptor complexes other than the TLRs (such as CD14 or MD-2) would play major roles as binding proteins, while the TLR interaction is of lower affinity, and may require conformational changes in ligand-binding protein. Further studies are necessary to verify these hypotheses.

The family of TLRs is a sophisticated and highly specialized system that can identify a variety of microbial and endogenous ligands, and activate the immune system to the presence of danger. Obviously, the body must be able to respond differently to various challenges, and the specificity in the response via TLRs appears increasingly complex. Currently, ten members of the TLR family (TLR1–10) are described, and we expect more receptors in this class to be described. The specificity of the TLR system appears at several levels: first, the individual TLR that is the essential signal transducer for a particular ligand; second, the ability of other receptors (TLRs as well as other surface and soluble proteins) to form a receptor complex to enhance the signal; third, the interaction of intracellular adapter molecules, kinases and regulatory proteins with the TLR, and finally, the activation of a specific set of transcription factors by specific receptor complexes. Essential signal transducers for the TLR family will be mentioned below. The hypothesis that specific sets of TLRs can cluster to induce responses to specific ligands

Table 1. Ligands for mammalian TLRs

<i>TLR1 (via TLR2)</i> Bacterial products [170] Diacylated lipoproteins [89, 90]
<i>TLR2</i> G+ bacteria [52–54, 57, 171–173] LPS from <i>L. interrogans</i> [68], <i>P. gingivalis</i> LPS [67], <i>L. pneumophila</i> , <i>Rhizobium</i> [69] Spirochetes [54, 174] Mycobacteria [54, 55, 175] Tri- and diacylated bacterial lipoproteins [54, 56, 58, 59, 135, 171] Lipoteichoic acid [57, 62] Peptidoglycan [52, 53, 57] Zymosan [171] Mycobacterial lipoarabinomannan [176] Other bacterial glycolipids [71, 177, 178] BCG cell walls [179] LPS-deficient mutant of <i>Neisseria meningitidis</i> [180, 181] Modulin (PSM) [182] Glycoproteins, <i>Prevotella intermedia</i> [183] GPI anchors, <i>Trypanosoma cruzi</i> [184] Soluble tuberculosis factor, PIM (mannosylated phosphatidyl inositol) [55, 185] Heat shock protein 60 [186] Necrotic cells [187] Measles virus/protein H [188] Bacterial fimbriae/synthetic peptides [189] Polymannuronic acid [190] Group B streptococci-soluble factor [94] <i>Aspergillus fumigatus</i> [191] V antigen, <i>Yersinia enterocolitica</i> [192]
<i>TLR3</i> Viral double-stranded RNA/poly(I:C) [91]
<i>TLR4</i> G bacterial [46, 52] <i>Mycobacterium tuberculosis</i> [55, 193] BCG cell walls [179] LPS/lipid A [46–48, 63, 65, 66] Acyclic lipid A agonist [74] Other bacterial glycolipids [71, 177, 178] Taxol [75, 76, 194] Respiratory syncytial virus/protein F [70, 195] Heat shock protein 60 [77, 186] Heat shock protein 70 [196] Fibronectin A domain [81] Fibrinogen [82] Saturated fatty acids [197] Cryptococcus [73] <i>A. fumigatus</i> [72] Lipoteichoic acid from <i>Treponema</i> [178] Polymannuronic acid [190] Minimally modified LDL [198] Surfactant protein A [83] Beta-defensins [85] Heparan sulfate [199] Murine retroviruses [200]

Table 1 (continued)

<i>TLR5</i> Flagellated bacteria, flagellin [92]
<i>TLR6 (via TLR2)</i> Diacylated lipoprotein (MALP-2) [93, 201] Peptidoglycan, G+ bacteria, zymosan [100] Modulin (PSM) [122, 182] Soluble tuberculosis factor [122] Group B streptococci-soluble [94] Necrotic cells [187]
<i>TLR7</i> Imiquimod, R848/resiquimod (imidazoquinolines) [95]
<i>TLR8 (human)</i> R848/resiquimod [96]
<i>TLR9</i> CpG bacterial DNA [97, 99, 202]

was proposed by Ozinsky et al. [100] using vectors coding for dominant negative or constitutively activated TLRs. Later, this theory has been supported by studies of knock-out mice. For example, TLR1 is not necessary, but enhancing the stimulatory response of triacylated lipoproteins via TLR2 [89, 90]; in contrast, TLR6 is necessary for a response of diacylated lipoproteins via TLR2 [93]. The small molecule MD-2 is required for LPS responses via TLR4 [101–103] and may work in aggregates for assembly of receptor complexes [104]. Furthermore, the role of CD14 in binding a variety of microbial compounds and enhancing activity for ligands towards cellular activation is well established [105].

Toll Receptor Signalling

For intracellular mediators of TLR signalling, several players are now directing responses via different TLRs. Several of the molecules, such as MyD88 [42, 106, 107], IRAK [108, 109] and TRAF-6 [110], are known from studies of IL-1 receptor signalling. MyD88 is the common adapter protein for all the TLRs, and is interacting directly with the intracellular domain of the TLRs through homophilic TIR/TIR domain interaction [111]. The structure of the TIR domain of TLR1 and TLR2 has been solved [112], providing the first thoughts as to why the signalling of the mutated TLR4 in the C3H/HeJ [46] mice is impaired. The NH₂ terminus of MyD88 contains a death domain, which is required to recruit IRAKs to the

receptor complex [42, 113]. Four different members of this family have been isolated: IRAK-1 [108, 109], IRAK-2 [43], IRAK-4 [114] and IRAK-M, which is preferentially expressed in myeloid cells [115]. There is some redundancy in the function of IRAK-1, since responses in IRAK-1-deficient mice are not completely abolished [116, 117]. Interestingly, both IRAK-2 and IRAK-M lack kinase activity but are still able to compensate for IRAK-1 deficiency [115, 118]. Whereas the role of the kinase domain of IRAK-1, IRAK-2, and IRAK-M is unclear, IRAK-4 requires its kinase activity in order to activate NF- κ B and acts apparently upstream of IRAK-1 [114]. The importance of IRAK-4 is even more visible in IRAK-4-deficient mice, which are severely impaired in their IL-1 and TLR signalling [119]. Surprisingly, IRAK-M-deficient mice show an increased inflammatory response upon challenge with various TLR ligands, indicating that IRAK-M is involved in the negative regulation of the IRAK complex [120]. An additional protein also involved in the negative regulation at the level of the IRAKs is the Toll-interacting protein (Tollip) [121–123], which is complexed to IRAK-1 and recruits IRAK to the TIR domain independently of MyD88. Then, the death domain both of MyD88 and IRAK-1 interact, leading to progressive autophosphorylation of the N-terminus of IRAK-1. Subsequently, IRAK-1 will be released from the complex and can now associate with TRAF-6 [110]. The essential role of TRAF-6 in the signalling of IL-1 and TLR ligands has been shown using TRAF-6-deficient mice [124, 125]. The final steps leading to the activation of NF- κ B and MAP kinases involve two activator complexes, TRIKA1 and TRIKA2 [126, 127]. TRIKA1 contains an ubiquitination enzyme complex, which adds a unique ubiquitin chain onto TRAF-6 [126]. This polyubiquitination is required for the activation of transforming growth factor- β -activating kinase (TAK1) [128, 129], which together with its two associated proteins, TAB1 [130] and TAB2 [131], forms TRIKA2 [127]. TAK1 is the kinase that phosphorylates and activates I- κ B kinase and MKK6 and, thus, is leading to the activation of the NF- κ B and JNK/p38 pathway, respectively [127].

However, not all signalling of the TLRs goes through MyD88. When Akira and coworkers [132] investigated the responses of MyD88-deficient mice to LPS, it was evident that these animals were resistant to endotoxic shock because they could not produce any cytokines. Unexpectedly, activation of NF- κ B and MAP kinases still occurred, albeit with delayed kinetics and also activation of the IFN-regulatory factor 3 was unimpaired [133]. This so-called MyD88-independent pathway was also em-

ployed by a TLR3 ligand, poly(I:C), but not by CpG or MALP-2, indicating that this pathway was specific for only some TLRs [91, 134, 135]. This new adaptor protein, responsible for the MyD88-independent pathway, was thought to be discovered, when two groups independently identified another TIR domain-containing protein, named TIRAP [136] or Mal [137]. Both groups could show that TIRAP/Mal functioned downstream of TLR4, but was not involved in TLR9-, IL-1- or IL-18-mediated responses. In addition, other data by Shinobu et al. [138] indicated that TIRAP/Mal is involved in the activation of IRF-3 by LPS. However, after independent generation of TIRAP/Mal-deficient mice, the characterization of the results revealed some unexpected findings: whereas the responses with respect to IL-1, IL-18 and ligands of TLR3, TLR5, TLR7, and TLR9 were normal as expected, the responses to TLR4- as well as TLR2-dependent ligands were severely impaired [139, 140]. This indicates that both MyD88 and TIRAP/Mal are essential components for TLR4- and TLR2-induced signalling. Even more surprising, MyD88-TIRAP/Mal doubly-deficient mice clearly showed that both molecules cannot compensate for one another, and that TIRAP/Mal is not responsible for the induction of the MyD88-independent pathway leading to the activation of IRF-3 and IRF3-dependent genes, since upregulation of CD80/CD86 and induction of IP-10, IRG-1, and GARG-16 still occurred in these cells [140].

This induction is mainly due to a TLR3/TLR4-specific activation of an IRF3-dependent antiviral gene program [141] that leads to the expression of IFN- β . In an auto-crine loop, IFN- β induces the phosphorylation of STAT-1 and the subsequent induction of STAT-1-dependent genes [142]. This activation is also independent of PKR, another target downstream of TIRAP/Mal [136]. Just recently, another TIR domain-containing adaptor has been identified, the TIR domain-containing adaptor-inducing IFN- β (TRIF) [143]. TRIF is able to activate NF- κ B similar to MyD88 and TIRAP/Mal, but in contrast, only TRIF can activate the IFN- β promoter. A dominant-negative form TRIF inhibits NF- κ B activation through TLR2, TLR3, TLR4, TLR7 and IFN- β activation through TLR3. These data clearly indicate not only a crucial role for TRIF in the TLR3 signalling pathway, but also its involvement in other TLR signalling pathways. One obvious question is whether TRIF could also be responsible for the induction of IRF-3 in the TLR4 pathway. As tempting as this hypothesis may be, the data do not yet support it, and thus further investigations will be required.

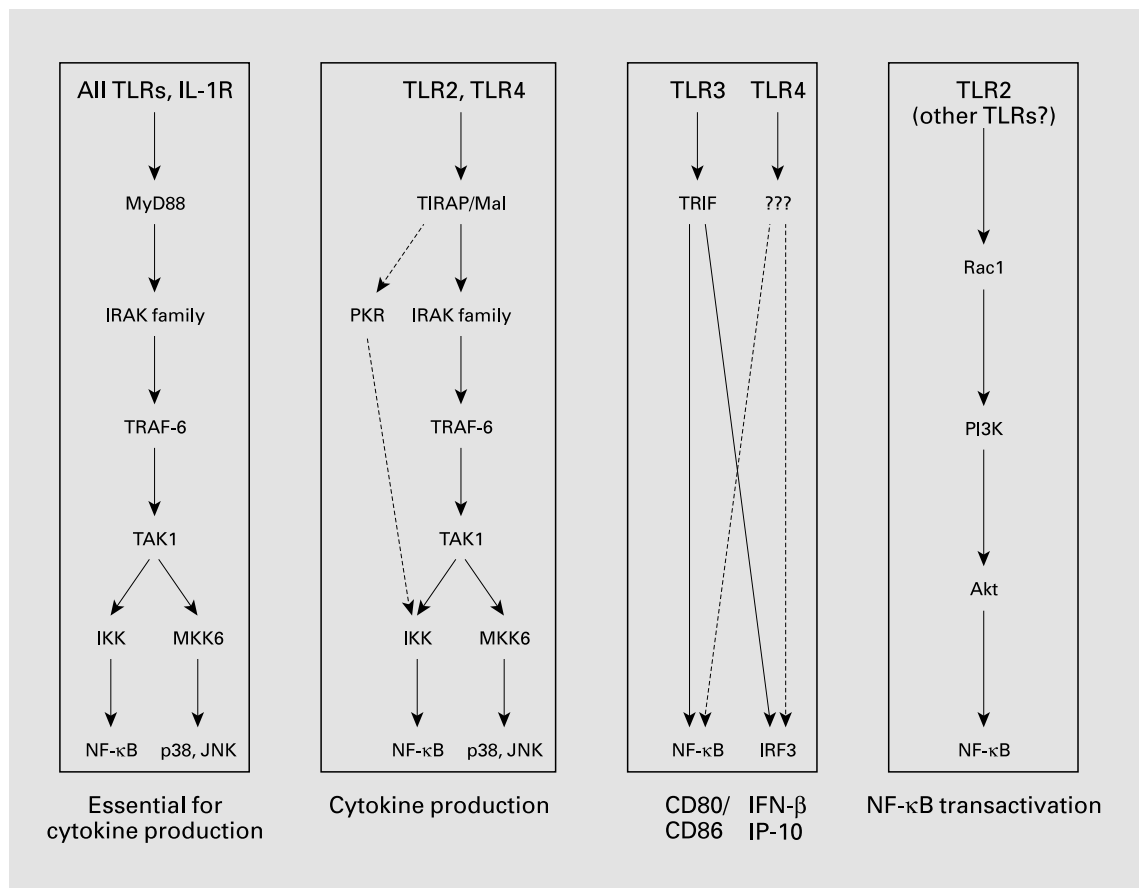


Fig. 1. TLRs engage different adaptor molecules: schematic representation of the major signalling pathways used by TLRs.

In addition to phosphorylation of I-κB that leads to translocation of the p65/p50 NF-κB heterodimer in the nucleus, p65 needs to be phosphorylated for its transactivation activity. At least for TLR2, it has been shown that this pathway involves the RhoGTPase Rac1, PI3K and Akt [144]. A PI3K binding motif can be found in TLR2, TLR1, and TLR6, but not in TLR3, TLR4, or TLR5, adding another level of distinct signalling molecules employed by different TLR ligands. On the other hand, RICK/Rip2/CARDIAK has been found to be involved in the signalling induced only by TLR2, TLR3, and TLR4, but not by TLR9 [145, 146]. To further increase the level of complexity, another pathway involved in the negative regulation of TLR signalling has been identified. CpG as well as LPS are able to induce SOCS1/JAB [147, 148]. Recent data indicate SOCS1/JAB as a negative regulator of signalling induced by LPS and other TLR ligands, possibly by binding directly to IRAK [149, 150]. If this negative regulation is linked to the recently described IRAK-

M-mediated downregulation of TLR signalling is currently unclear.

In summary, TLR signalling utilizes a number of different pathways, many of which are leading to the translocation of NF-κB into the nucleus (see fig. 1 for a schematic overview of the main pathways). Further work is required to elucidate the sophisticated molecular networks of signalling pathways that are involved in the response to different TLR ligands.

TLRs and Adaptive Immune Responses

It is undisputed that the innate immune system is crucial for the first line of defense against invading pathogens. Without the immediate action of monocytes/macrophages and polymorphonuclear cells, we would never see the adaptive immune system at work. This initial wave of activation is facilitated through the recognition of micro-

bial PAMPs by their PRRs such as TLRs. But the role of the innate immune system is more than just holding the invading pathogens in check until the adaptive immune system kicks in. And this new role is crucially linked to TLRs: dendritic cells (DC) are playing a key role for linking the innate and the adaptive immune responses. As immature cells they are patrolling the peripheral tissues. Upon entry of invading pathogens, DCs recognize these pathogens through their TLRs [151]. Conveniently, they express the full repertoire of TLRs [152]. Following activation through TLRs a maturation program is induced in DCs, including high expression of MHC and the costimulatory molecules CD80 and CD86 [153]. Subsequently, DCs migrate to the lymph nodes to activate antigen-specific naïve T cells. Here, the cytokine milieu surrounding the Th0 cells is determining their fate: the production of IL-12 drives these cells into Th1 cells that are able to produce interferon- γ , whereas IL-4 leads to the differentiation into Th2 cells producing IL-4, IL-5, IL-10, and IL-13. It is exactly this set of cytokines that is also responsible for the development of allergic diseases like asthma, accounting for the regulation of antigen-specific IgE production, accumulation of eosinophils and activation of mast cells [154–156]. Interestingly, the incidence of allergic diseases and atopy in industrialized western countries has markedly increased over the last decades, and one hypothesis is that this increase is linked to the reduction of bacterial infections that occurs in a cleaner environment [157], a theory referred to as the ‘hygiene hypothesis’ [158, 159]. There is now growing evidence from a number of reports that bacterial infections in early childhood reduce the occurrence of atopy and allergic diseases [160–163]. Bacterial infections lead to the activation of multiple TLRs, and this activation is necessary to mount antigen-specific Th1 responses. This has been shown using MyD88-deficient mice. Upon immunization of these mice with ovalbumin together with complete Freund’s adjuvant, which contains mycobacterial lysate, the antigen-specific T cell proliferation as well as the production of IFN- γ and generation of ovalbumin-specific IgG2a antibodies were severely inhibited [164]. Moreover, these mice showed Th2 responses and IgE production by default. One hypothesis based on these results is that protection against microbial pathogens (containing TLR ligands) requires a Th1 response, whereas the efficient removal of eukaryotic pathogens such as parasitic worms needs a Th2 response. These pathogens as well as allergens may not contain any TLR ligand that could cause a shift from a Th2 to a Th1 response, which in the case of allergic diseases might be highly appreciated. However, recent data strongly indi-

cate that the effect on TLR-induced signals with respect to the Th1/Th2 shift may depend on the amount of the TLR stimuli present. In the absence of LPS signalling, Eisenbarth et al. [165] observed defective Th2 cell priming as measured by systemic antibody responses, lung inflammation and cytokine responses in mice expressing only a nonfunctional TLR4. A comparable study by Dabbagh et al. [166] also indicated a requirement for TLR4 signalling for an optimal Th2 response which might be independent of MyD88 [167].

The discovery that TLR signalling might be crucially involved in the establishment of Th1/Th2 responses, opens the field for the development of new strategies against allergic diseases such as asthma [168].

Conclusion

The field of innate immunity has gained an enormous amount of interest over the last 5 years. Coincident with this, the number of genomes that have been fully sequenced continues to grow, enabling researchers to identify and characterize the receptors and adaptors involved in their innate immune recognition. TLRs and their signalling pathways represent an ancient system of host defense. The TIR domain may be the archetype of innate immune defense against pathogenic invaders that has been evolved in eukaryotes. It is not only present in organisms from *Drosophila* to mammals, but also in plant resistance genes (R genes) that encode for defense proteins, many of which containing TIR domains [see 169 for review].

Much of the work over the last years has focused on defining ligands for the different TLRs and a number of ligands have been identified (table 1). However, the molecular basis for this recognition is not known for a single ligand, raising the possibility that additional receptors are involved in this process. Also, the characterization of TLR-dependent signalling for the instruction of adaptive immune responses has just started to be explored. Since TLRs play such a crucial role in innate and adaptive immune responses to distinct virulence factors, the development of selective inhibitors/activators may be a worthwhile endeavor to aid in the management of a number of infectious and immunologic diseases.

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