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Toll-like receptors and the host defense against microbial pathogens: bringing specificity to the innate-immune system

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Abstract: Toll-like receptors (TLRs) have been identified as a major class of pattern-recognition receptors. Recognition of pathogen-associated molecular patterns (PAMPs) by TLRs, alone or in heterodimerization with other TLR or non-TLR receptors, induces signals responsible for the activation of genes important for an effective host defense, especially proinflammatory cytokines. Although a certain degree of redundancy exists between signals induced by the various TLRs, recent studies have identified intracellular pathways specific for individual TLRs. This leads to the release of cytokine profiles specific for particular PAMPs, and thus, TLRs confer a certain degree of specificity to the innate-immune response. In addition to the activation of the innate-immune response, TLR-mediated recognition represents a link between the innate- and acquired-immune systems, by inducing the maturation of dendritic cells and directing the T helper responses. Alternatively, recent data have also suggested TLR-mediated escape mechanisms used by certain pathogenic microorganisms, especially through TLR2 induction of anti-inflammatory cytokines. Finally, the crucial role of TLRs for the host defense against infections has been strengthened recently by the description of patients partially defective in the TLR-activation pathways. *J. Leukoc. Biol.* 75: 749–755; 2004.

Key Words: *pathogen-associated molecular patterns · acquired-immune system · dendritic cells · pattern-recognition receptors · cytokines*

THE INNATE-IMMUNE RESPONSE AND TOLL-LIKE RECEPTORS (TLRs)

The host defense against pathogenic microorganisms comprises innate and acquired immunity. These two relatively distinct sets of responses are sequentially activated during the infection and ultimately ensure the elimination of the microbial pathogen. Whereas the innate-immune system is activated within minutes after the invasion of the host and is responsible for the defense during the initial hours and days of the infection, acquired immunity requires at least 7–10 days before a proper cellular or humoral response occurs. Although the innate-immune system, comprising both cellular [e.g., monocytes, neutrophils, natural killer (NK) cell] and humoral (e.g., complement, lysozyme) components, is very effective in dealing with the vast majority of the infections, it has been long believed to be nonspecific to the invading pathogen. The secondary activation of specific acquired immunity mediated by T- and B-lymphocytes would overcome this shortcoming and eliminate the pathogen. Although this scenario is correct to a certain extent, the dogma of the nonspecific nature of the innate-immune responses has been recently challenged by the discovery of a novel class of receptors, the TLRs, which have been proven to be crucial for recognition of microbes by the innate-immune system and for bridging the innate- and acquired-immune responses.

Toll has been first described initially as a type I transmembrane receptor, with an important role in the dorso-ventral development of the Drosophila embryo [1]. In addition to that, it had become apparent that the absence of Toll in genetically deficient Drosophila also results in a severely impaired defense against fungi and Gram-positive bacteria [1]. Whereas the extracellular domain of Toll contains leucine-rich repeats, the intracellular tail of the receptor was shown to display a striking homology with the intracellular domain of the interleukin-1 receptor (IL-1R) type I, being designated as the Toll/IL-1R (TIR) domain. The initial data suggested that Toll is an important component of Drosophila antimicrobial defense and that mammalian homologues might have similar functions. Indeed, 10 different mammalian TLRs have been identified in humans [2].

During the last few years, extensive research in this field has identified TLRs as a major class of signaling receptors, recognizing conserved bacterial structures called pathogen-associated molecular patterns (PAMPs) [3, 4]. The specificity of TLR recognition for several important PAMPs has been identified, including recognition of peptidoglycan (PGN), bacterial lipoproteins, and zymosan by TLR2; double-stranded RNA by TLR3; lipopolysaccharide (LPS) and heat-shock proteins

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(HSPs) by TLR4; flagellin by TLR5; and CpG motifs of bacterial DNA by TLR9 [5]. A multitude of studies have reported additional microbial ligands for TLRs, as summarized in other reviews [2, 5]. In addition, an increasing number of reports suggest recognition of endogenous ligands such as HSPs, fibronectin, and hyaluronic acid oligosaccharides by TLRs and modulation of autoimmune processes [6]. The scope of the present review is to focus on the consequences of TLR microbial interaction for the host defense against infections and to review the mechanisms activated by TLRs during infections with live microorganisms.

SPECIFICITY OF MICROBIAL RECOGNITION THROUGH TLRs

Despite the supposed nonspecificity of the innate-immune response, it has long been known that cytokine release upon stimulation with Gram-positive or Gram-negative bacteria shows important quantitative and qualitative differences [7–9]. This phenomenon now has been explained by the demonstration of the recognition of PGN and LPS, the main components of Gram-positive and Gram-negative bacteria by TLR2 and TLR4, respectively [10]. The TLR–PAMP interaction results in the recruitment of specific adaptor molecules such as MyD88 and Mal, which then bind the IL-1R-associated kinase (IRAK). The signal is thereafter transmitted through a chain of signaling molecules, which is apparently common to all TLRs, involving the tumor necrosis factor (TNF) receptor-associated factor-6 (TRAF6) and mitogen-activated protein kinases [11]. Thereafter, activation of nuclear factor (NF)-κB and activated protein-1 (AP-1) leads to transcription of genes involved in the activation of the innate host defense, notably proinflammatory cytokines (**Fig. 1**).

Ligation of TLR4 or TLR3 recruits an additional adaptor molecule called TIR domain-containing, adapter-inducing interferon-β (IFN-β; TRIF) [12, 13]. In addition to potentiating the secretion of the proinflammatory cytokines, TRIF mediates unique signals leading to secretion of IFN-β and indirect up-regulation of IFN-dependent genes such as IFN-inducible protein 10 (IP-10) and inducible nitric oxide synthase (iNOS; **Fig. 2**). Moreover, a recent study described TRAM as an adaptor molecule specifically recruited to TLR4 [14]. Conceptually, it is likely that recruitment of specific adaptor molecules, such as TRIF and TRAM, confers specificity to the response activated by a certain TLR and therefore as a consequence of recognition of a particular PAMP. Our recent finding that NOD2, an intracellular molecule involved in the pathogenesis of Crohn's disease, specifically mediates cytokine induction by TLR2 but not TLR4 agonists indicates that it may be part of a TLR2-specific pathway [15] (Fig. 2). It is to be expected that more adaptor molecules conferring specificity to the intracellular pathways induced by the various TLRs will be described in the near future.

Large receptor complexes, which are formed among various TLRs or TLR and non-TLR moieties, confer a further degree of specificity. In this way, heterodimers of TLR2/TLR1 recognize triacetylated bacterial lipopeptides, whereas TLR2/TLR6 heterodimeres recognize diacetylated *Mycoplasma* lipopeptides [16], and similar heterodimerization is likely to occur for other PAMPs. As mentioned, several non-TLR receptor chains cooperate with TLRs for the recognition of PAMPs; examples are CD14 and CD11b/CD18 for recognition of LPS by TLR4 [17], CD14 for recognition of lipoteichoic acid by TLR4 [18], and dectin-1 for recognition of zymosan and *Candida albicans* by TLR2 [19, 20].

The resulting model for the recognition of PAMPs by TLRs is one in which a variety microbial pathogens, each containing several different PAMPs, interacts with a certain combination of TLR (and non-TLR) receptors on the cell membrane of the host cells. As the various TLRs or TLR complexes will trigger specific intracellular pathways, the signal resulting from the activation of a specific combination of TLRs will induce a response best suited for the invading pathogen.

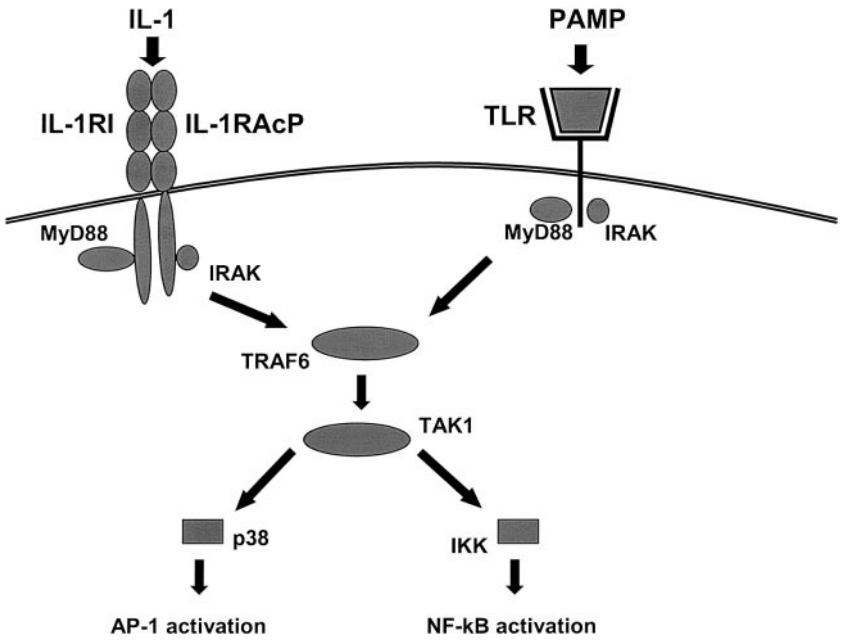

Fig. 1. The common pathway of intracellular signaling by IL-1R and TLRs. After the discovery of the human homologues of *Drosophilla* Toll, it had become apparent that intracellular domains of IL-1R type I and TLRs share a high degree of homology; they recruit identical adaptor molecules including MyD88 and IRAK and induce similar intracellular pathways leading to activation of nuclear factors NF-κB and AP-1 (with permission from ref. [11]). IL-1RacP, IL-1R accessory protein; TAK1, transforming growth factor-β-activated kinase 1; IKK, inhibitor of κB kinase.

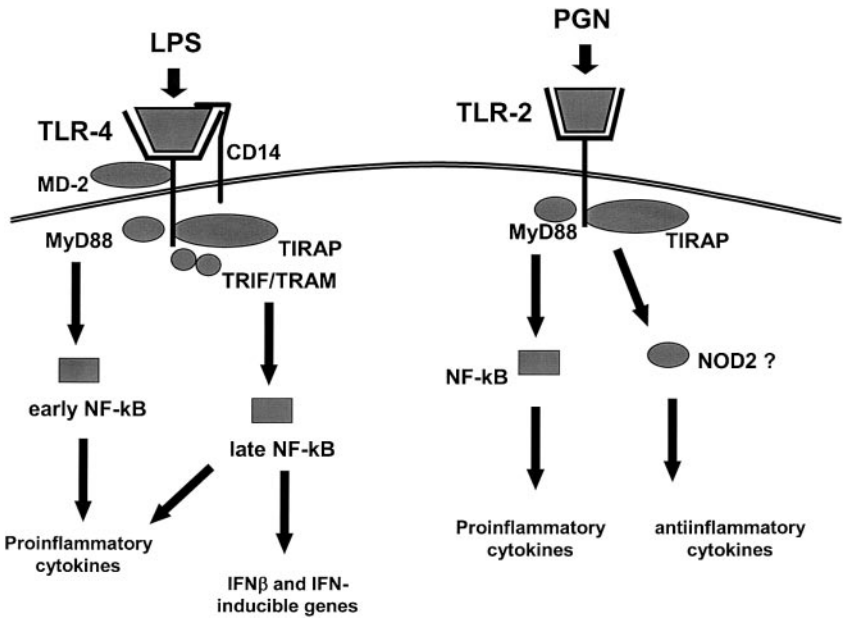

Fig. 2. Differential TLR-mediated pathways can bring specificity to innate immunity. In addition to the common MyD88 pathway, TLRs can also recruit specific adaptor molecules and activate differential intracellular pathways: Ligation of TLR4 recruits TRIF and transverse rectus abdominis musculocutaneous (TRAM), mediating unique signals leading to secretion of IFN-β and indirect up-regulation of IFN-dependent genes such as IP-10 and iNOS. We have also recently shown that nonobese diabetic (NOD)2, an intracellular molecule, specifically mediates cytokine induction by TLR2 but not TLR4 agonists, indicating that it may be part of a TLR2-specific pathway. TIRAP, TIR domain-containing adapter protein; MD-2, myeloid differentiation protein-2.

THE ROLE OF TLRs DURING INFECTION WITH LIVE MICROORGANISMS

In vitro studies strongly suggest that TLRs have a crucial role in the recognition of microbial pathogens and that signals mediated by TLRs are crucial for mounting an effective host defense. Several in vivo studies have investigated the role of the adaptor molecules MyD88 and IRAK4 in infections with live microorganisms as an initial screening for a role of TLRs in experimental models of infection. MyD88 is essential for the stimulation of proinflammatory cytokines such as TNF, IL-1β, IL-12, or IL-6, virtually by the entire range of TLR agonists. Based on these observations, it has been hypothesized that MyD88−/− mice would be highly susceptible for infections with Gram-negative and Gram-positive microorganisms. Indeed, MyD88 is essential to host defense against Gram-positive bacteria such as *Staphylococcus aureus* [21] and *Listeria monocytogenes* [22] but also *Mycobaterium avium* [23] and parasites such as *Toxoplasma gondii* [24], *Leishmania major* [25], and the intestinal nematode *Trichuris muris* [26]. The protective mechanisms triggered through MyD88 mainly include release of proinflammatory cytokines and of reactive nitrogen and oxygen intermediates [22, 24]. It is interesting that the phagocytic capacity of MyD88−/− cells remains intact [27]. In contrast with the microorganisms mentioned, *Mycobacterium tuberculosis* elicits host defense through largely MyD88-independent pathways [28], and prion pathogenesis is completely MyD88-independent [29]. No data about Gram-negative infection in MyD88−/− mice are available.

A similar phenotype is found in IRAK4−/− mice, which are deficient in cytokine production, resistant to endotoxic shock but highly susceptible to *S. aureus* infection [30]. IRAK4−/− mice display increased susceptibility to lymphocytic choriomeningitic virus infection, likely as a result of defective IFN-γ production [30].

TLR2 is the major receptor for PAMPs of Gram-positive bacteria, such as PGN and lipoteichoic acids [5], and TLR2 has been hypothesized to have a central role in the host defense against these microorganisms. Indeed, TLR2−/− mice are highly susceptible to infection with *S. aureus* [21, 31], *Streptococcus pneumoniae* [32, 33], *M. tuberculosis*, or *Mycobacterium bovis* [34, 35], but the mechanisms of protection induced by TLR2 ligation are unclear and seem to differ in the different infections. Defective cytokine stimulation in TLR2−/− mice has been implicated in infections with *S. aureus* and *M. tuberculosis* [32, 34], whereas increased levels of inflammation (despite similar cytokine levels) have been incriminated in experimental, pneumococcal infections [33]. TLR2 also mediates host defense against *T. gondii* by mediating cytokine and NO release [36].

The most intensively studied TLR deficiency is that of TLR4, partly as a result of the availability of the natural TLR4-defective mutants, C3H/HeJ and ScCr mice. Even before the molecular nature of the LPS hyporesponsiveness in C3H/HeJ mice was discovered, it was known that C3H/HeJ mice are more susceptible to Gram-negative infections such as *Neisseria meningitidis* meningitis and *Escherichia coli* urinary tract infection [37, 38]. These earlier observations were confirmed later [39, 40] and were extended by the demonstration of increased susceptibility to Gram-negative infections such as *Haemophilus influenzae* pneumonia [41], *Salmonella* peritonitis and *Klebsiella pneumoniae* sepsis [42, 43] but also Gram-positive infections such as *S. pneumoniae* pneumonia [44]. In addition, TLR4 mediates recognition of the fungal pathogens *Aspergillus fumigatus* [45–47] and *C. albicans* [48, 49], and the host defense against the latter was impaired in TLR4-deficient mice [48]. A crucial defect in these infection models in TLR4−/− mice is the decreased neutrophil recruitment to the site of infection [39, 41, 48], which is a result of defective production of chemokines [41, 48] and decreased expression of chemokine receptors [50]. Host defense against *M. tuberculosis* is only marginally affected in TLR4−/− mice [34]. TLR4 is not involved in the host defense against experimental *Legionella pneumoniae* [51] or influenza virus infection [52], but

TLR4-deficient mice have an impaired resistance against respiratory syncitial virus as a result of defective IL-12 release and NK-cell function [52, 53].

TLRs: THE BRIDGE BETWEEN INNATE AND ACQUIRED IMMUNITY

When the innate host defense mechanisms fail to eliminate the pathogenic microorganisms during the first days of an infection, the host will mount an additional immune response adapted specifically to the particular invading bacteria. This acquired-immune response is mediated by clonal expansion of T cell and B cell populations able to interact specifically with particular microorganisms. By enhancing microbicidal mechanisms of the cells of the innate-immune system, finally the pathogen is being eliminated. These processes are mediated by presentation of pathogen-derived peptides by professional antigen-presenting cells (APC) to T cells. Dendritic cells (DC) are the most effective APC, which function as sentinel at the frontline of host defense in tissues such as skin and mucosa and bridge the innate and acquired immunity [54]. Stimulation of immature DC by microbial stimuli induces production of proinflammatory cytokines such as TNF and IL-12, which can induce differentiation of T cells into T helper cell type 1 (Th1) cells. In addition, these stimuli induce up-regulation of costimulatory molecules such as CD40, CD80, and CD86 [54]. This process is called DC maturation, and it strongly potentiates the ability of DC to activate naive T cells. DC migrate to the lymphoid organs, where presentation of antigen and T cell proliferation finally take place [55].

It has become apparent that TLRs play a crucial role in these processes, and they form the bridge between the microbial recognition by the innate-immune system, DC maturation, and T cell proliferation [56]. Subsets of human DC express TLRs on their surface, which respond differently to microbial antigens [57]. A variety of microbial PAMPs are able to induce cytokine release and DC maturation: LPS through TLR4, CpG through TLR9, bacterial lipopeptides through TLR2 [56, 58]. The stimulation of specific TLRs results in the release of IL-10 or IL-12, leading to skewing of the T cell response toward Th1 or Th2 cytokines [59]. Thus, TLR2-mediated signals seem to preferentially induce a Th2 profile, whereas TLR4 activation mainly leads to a Th1 response [60]. In addition, release of IL-6 by DC relieves the suppression of effector T cells by regulatory T cells [61]. Thus, through specific TLR stimulation, DC can process the information leading to the polarization of the acquired-immune response.

It has also been demonstrated that at least two distinct, intracellular signaling pathways regulate DC maturation by different TLRs: One pathway induced mainly by TLR9 is strictly dependent on MyD88, whereas another pathway induced primarily by TLR4 can induce DC maturation through a MyD88-independent mechanism [56]. It has been suggested that these two pathways converge at the level of TRAF6 [62].

THE USE OF TLRs AS ESCAPE MECHANISM FROM HOST DEFENSE

An aspect of TLR biology, which has only recently become apparent, is the hijacking of the TLR signaling by certain pathogens to evade the recognition and elimination by the immune system. Several studies to date suggest that TLR2-dependent mechanisms induced by certain microorganisms contribute to evasion or inhibition of the immune response. These effects on host defense are based on the initial observation that TLR2-induced signals in DC preferentially induce a Th2 cytokine pattern [60], which is known to have down-modulatory activity on cellular immunity. Subsequently, it was demonstrated that the *M. tuberculosis* 19-kD protein inhibits IFN-γ-regulated human leukocyte antigen-DR and Fc receptor for immunoglobulin G-1 expression on human macrophages through TLR2-dependent mechanisms [63]. The results of these in vitro studies were corroborated by similar data in in vivo experimental infections. *Yersinia enterocolitica* and *C. albicans* have been shown to exploit TLR2-mediated IL-10 release to induce immunosuppression [64, 65]. In the case of *Candida* infection, this effect is attained through generation of CD4+CD25+ regulatory cells [65]. Lack of TLR2 in knockout mice renders them more resistant to lethal *Yersinia* and *Candida* infections [64, 65]. Similarly, *A. fumigatus* also evades immune recognition during germination through TLR2-mediated IL-10 production, whereas proinflammatory TLR4-mediated signals are lost [47]. Another example of deleterious TLR2 activation is that of mycobacteria and human immunodeficiency virus type 1 (HIV-1) coinfection, in which HIV-1 expression is induced by mycobateria through TLR2 signaling [66]. All these data suggest that several microorganisms, among which the fungal pathogens *C. albicans* and *A. fumigatus* are prominent, use TLR2-mediated induction of anti-inflammatory cytokines to down-modulate the microbicidal functions of leukocytes and evade the host defense (**Fig. 3**).

TLRs IN HUMAN INFECTIOUS DISEASES

Given the results in experimental infections, one might assume that TLRs have a crucial role of these receptors in human diseases as well. Several lines of evidence have confirmed this assumption. Infusion of endotoxin into human volunteers modulates the expression of TLRs in humans [67]. A higher expression of TLR2 and TLR1, known to mediate cell activation by lipoproteins from *Mycobacterium leprae*, has been found in patients with localized tuberculoid lepra, whereas these receptors were far less expressed in those with disseminated lepromatous disease [68]. A polymorphism of TLR2 gene (Arg677Trp), which is unable to mediate mycobacterial signaling [69], has also been associated with lepromatous leprosy [70]. These data suggest that the intensity of the immune response to this pathogen is proportional to the expression of TLR2 and TLR1. This hypothesis is also in line with the description of hyporesponsiveness to vaccination with *Borrelia burgdorferi* outer-surface lipoprotein in humans with decreased cell-surface expression of TLR1 [71].

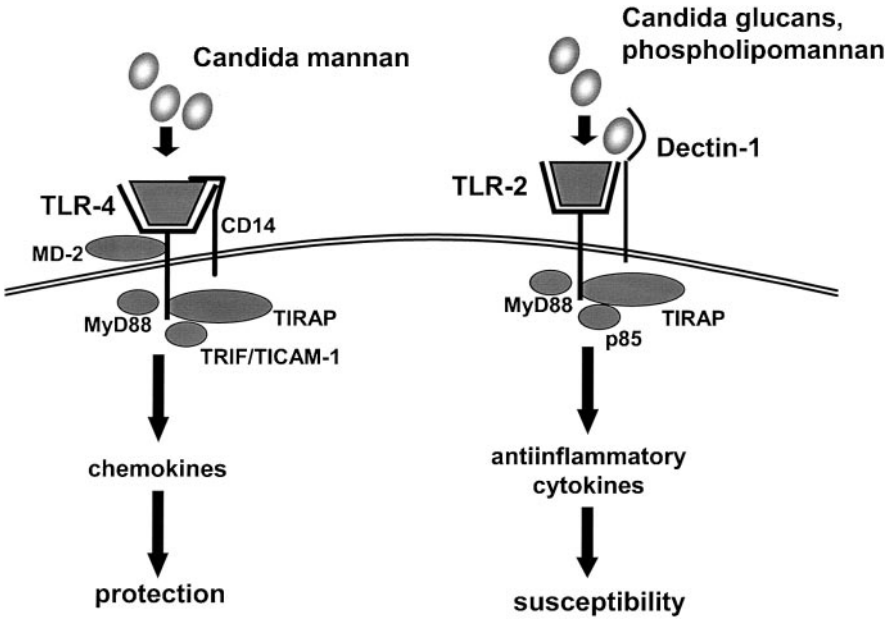

Fig. 3. TLR-mediated signals as escape mechanisms from host defense. Recent literature suggests that certain microorganisms are recognized by TLRs for the activation of host defense but also activate alternative TLR pathways with inhibitory effects on innate immunity. Thus, whereas interaction of *Candida* mannan with TLR4 induces release of chemokines, leukocyte recruitment, and protection, interaction of *Candida* glucans and phospholipomannan with TLR2 primarily mediates release of the anti-inflammatory cytokine IL-10, resulting in inhibition of the host defense and increased susceptibility to infection. Similar mechanisms have been suggested for other microorganisms such as *Y. enterocolitica* and *A. fumigatus*. TICAM-1, TIR-containing adapter molecule-1.

The role of a TLR4 polymorphism, the Asp299Gly mutation, on the susceptibility to infections is controversial. Whereas some studies have suggested an increased susceptibility to Gram-negative infections or Gram-negative septic shock [72, 73], others have been unable to find a role of this polymorphism in meningococcal disease [74], polymicrobial sepsis [75], and urogenital tract *Candida* and *Chlamydia* infection [76, 77]. Controversy also surrounds the functional consequences of this mutation: Whereas initial studies suggested hyporesponsiveness to LPS in individuals bearing this mutation [78], recent studies have failed to confirm this [79, 80]. We have also been unable to find a defective cytokine production in cells from volunteers bearing the Asp299Gly polymorphism after stimulation with exogenous (*E. coli* LPS, *N. meningitidis* LPS, *A. fumigatus*, *Cryptococcus neoformans*) and endogenous (human recombinant HSP-60) TLR4 ligands (C. van der Graaf, submitted). Another TLR2 polymorphism, the Arg753Gln mutation, has been found in two patients with *Staphylococcal* sepsis [81], but no studies have been published to confirm the role of this polymorphism.

Probably the most solid proof of the central role of TLR-mediated signals in human infections has been provided recently by the description of recurrent bacterial infections, especially caused by pyogenic bacteria, in patients with IRAK-4 deficiency [82, 83]. This deficiency resulted in defective response to LPS, IL-1, and IL-18 in vitro as well as in a skin blister model of aseptic inflammation [83]. As these patients do not exhibit other infections, it is likely that IRAK-4-independent pathways induce alternative, protective signals. Other partial defects in the TLR pathways are likely to be found in the next few years. However, a complete deficiency of one of the major TLR pathways is unlikely to be found, as it is probably not compatible with survival.

CONCLUSIONS AND FUTURE DIRECTIONS

The results of in vitro experiments as well as of in vivo infection models and from various groups of patients provide support for the notion that TLRs are a major class of pathogen-recognition receptors: they recognize PAMPs from the various classes of the microorganisms, leading to production of cytokines and activation of the microbicidal mechanisms of leukocytes; they induce maturation of DC and activate them, thereby providing a bridge between innate and acquired immunity; and they modulate the function of T regulatory cells. In addition, initial data on the differential pathways induced intracellularly by the different TLRs, such as the recruitment of TRIF by TLR3 and TLR4, suggest a specificity of the signals triggered by the various TLRs. As the gene-transcription profile of various TLR agonists, as measured by microarray techniques, exhibits a relatively high degree of redundancy between different TLRs, this raises the question of how extended this specificity is. Finally, an emerging field of investigation not addressed in this review is that of TLR recognition of endogenous ligands and the role of these receptors in noninfectious and autoimmune inflammatory processes.

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