ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2005, p. 3991–3996 Vol. 49, No. 10
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.10.3991–3996.2005

MINIREVIEW

From the Th1/Th2 Paradigm towards a Toll-Like Receptor/T-Helper Bias

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THE INNATE IMMUNE RESPONSE AND TOLL-LIKE RECEPTORS

An efficient defense against invading pathogenic microorganisms is achieved through coordination of a complex network of both innate and acquired immune responses. The first step for the elimination of a pathogenic bacterium or virus is reliable detection, a complex task due to both variation and evolution of the pathogenic microorganisms. In order to achieve this goal, the innate immune system has developed a strategy of recognizing conserved structures of microbes which are not present in mammalian cells, called pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLRs) are the major class of signaling receptors, first described for *Drosophila* (26), which recognize PAMPs and signal the presence of an invading pathogen (24, 51). The specificity of TLR recognition has been established for several important PAMPs: lipoteichoic acid, bacterial lipoproteins, and zymosan are recognized by TLR2, double-stranded RNA by TLR3, lipopolysaccharide and heat shock proteins by TLR4, flagellin by TLR5, and CpG motifs of bacterial DNA by TLR9 (3). Additional microbial ligands for TLRs have been found, as reviewed elsewhere (3, 48).

Ligation of TLRs by PAMPs leads to induction of cytokine production, and at first glance, the pathways involved are rather similar and seem to be inherently redundant. Hirschfeld and colleagues were the first to suggest that differential cytokine patterns are released when various TLRs are engaged by lipopolysaccharides (LPS) from different species: stimulation with *Escherichia coli* LPS, a ligand for TLR4, led to release of large amounts of tumor necrosis factor (TNF), interleukin-1β (IL-1β), IL-12p40, and IP-10 (gamma-interferon-inducible protein 10), whereas *Porphyromonas gingivalis* LPS, a TLR2 ligand, induced moderate amounts of TNF and IL-1β and no production of IL-12p40 or IP-10 (16). These results were later confirmed and extended by the observation that the specific effect of TLR4 on IL-12p40 and IP-10 release is mediated through intermediary production of endogenous beta interferon (IFN-β) (50). These and additional studies reviewed below showed that TLRs not only enable the innate immune system to recognize specific PAMPs, but by inducing specific cytokine profiles, bring a certain degree of specificity to the innate immune system and influence the nature of the adaptive immune responses.

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DENDRITIC CELL INTERACTION WITH T CELLS: TLRs AS A BRIDGE BETWEEN INNATE AND ACQUIRED IMMUNITY

Recognition of PAMPs by specific receptors in the dendritic cell (DC) membrane is a crucial event in the activation of DC and initiation of adaptive immune responses (7). The capture of microbial antigens in the peripheral tissues and migration to the draining lymph nodes is the first step in the generation of adaptive immunity. Subsequent presentation of the antigen to naive T cells in the context of the major histocompatibility complex (MHC) will thereafter induce T-cell activation and differentiation. It has become apparent that both of these two steps in the initiation of adaptive immunity are under control of TLRs.

In response to microbial pathogens, $CD4^+$ T cells differentiate into Th1 or Th2 cells; each of these subsets is responsible for activating immune responses adapted to the type of infectious agent. On the one hand, Th1 cells produce IFN-γ and induce B cells to release antibodies of the immunoglobulin G2 isotype, which are responsible for phagocyte activation and antibody-dependent cellular cytotoxicity and important for defense against intracellular pathogens (19, 46). On the other hand, Th2 cells produce IL-4, IL-5, and IL-10 and induce production of immunoglobulin E antibodies, which are responsible for immunity against parasitic infections (19, 46). In addition, peripheral tolerance is under control of a subset of regulatory T cells, which control excessive inflammation by producing large amounts of IL-10 and transforming growth factor β. How the nature of infection determines the type of T-cell response is an area of great interest, and the mechanisms responsible for this regulation are only presently being unraveled.

TLRs influence several steps of DC activation and T-cell differentiation. First, TLRs are crucial for the uptake of microorganisms by DC. Several DC subsets are present in the circulation and tissues of mammalian organisms, and each has its own constellation of pattern recognition receptors, including TLRs, C-type lectins, mannose receptors, and scavenger

receptors. The best-studied DC subsets are the classical myeloid (mDC) and plasmacytoid DC, which express specific TLR expression profiles. In humans, freshly isolated mDC express TLR1, TLR2, TLR3, TLR5, TLR6, and TLR8, whereas plasmacytoid DC express TLR7 and TLR9; in contrast, both mouse DC subsets express TLR1, TLR2, TLR4, TLR6, TLR8, and TLR9, whereas TLR3 is expressed only on mDC (18). The uptake of microorganisms by DC through TLRs induces the upregulation of costimulatory and MHC molecules, a switch in the chemokine expression, and migration to the draining lymph nodes (7).

Second, in addition to their effects on antigen uptake and DC migration, an even greater impact of TLRs on the initiation of the adaptive immune responses is exerted at the level of DC/T-cell interaction. The activation of T cells by antigen-presenting cells such as DC is the result of three distinct signals: (i) signal 1 derives from ligation of T-cell receptors by specific pathogen peptides presented in the context of MHC class II molecules; (ii) signal 2 consists of the activation of costimulatory molecules (CD28 interaction with CD80/86 and CD40 interaction with CD40 ligand); and (iii) signal 3 is a polarizing signal given by specific cytokines, with IL-12 driving a Th1-type response, whereas IL-4 and IL-10 activate mainly Th2-type responses. The recognition and uptake of microorganisms through TLRs lead to the increased expression of the costimulatory molecules on the surface of DC cell membrane, which are the major signaling route for DC maturation (28). Because presentation of an antigen in the absence of costimulatory signals leads to anergy instead of activation of T cells, the stimulation of CD80 and CD86 by TLR-mediated signals is a crucial step in the activation of adaptive immunity (20). In this way, TLRs participate in the translation of the nonspecific information contained in conserved PAMPs into antigen-specific information and clonal expansion of T cells.

After activation of T cells, a crucial step in the additional tailoring of adaptive immunity is the differentiation into either Th1 or Th2 cells. Circumstances such as the density of the peptides presented, types of costimulatory molecules expressed, and state of DC activation influence whether the T cells differentiate into either Th1 or Th2 phenotypes (10). However, the most important signal responsible for Th differentiation is the type of cytokine profile present at the time of T-cell stimulation, with either a Th1-inducing profile represented by IL-12 family members and alpha interferon or a Th2-inducing profile represented by IL-4 and IL-10. In the last few years, it has become apparent that the induction of a specific cytokine profile upon recognition of microbial pathogens greatly depends on recognition by specific TLRs.

TLRs AND Th1/Th2 DIFFERENTIATION

Release of cytokines upon recognition of microorganisms is one of the most important effects of TLR activation. There is strong evidence for an important role of TLRs in driving Th1 responses through stimulation of IL-12p70 and IFN-α release from DC. In this respect, activation of TLR4 by LPS and TLR9 by CpG DNA induces strong Th1 responses through IL-12p70 release (8), and stimulation of alpha interferon by TLR3, TLR4, TLR7, and TLR9 has been seen as an important driving force of TLR-mediated Th1 responses (35). Likewise, flagellin induces Th1 cytokines through stimulation of TLR5 (1). In contrast, the absence of MyD88 resulted in a Th2-biased response (22). These data suggested that TLRs control Th1 differentiation, whereas the absence of TLR-mediated signals generates Th2 responses (43).

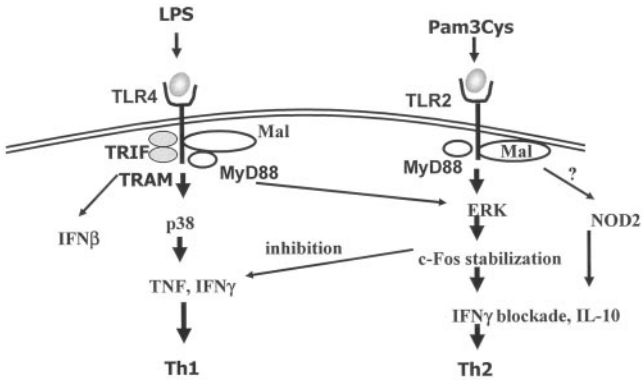

FIG. 1. Differential TLR-mediated pathways can bring specificity to innate immunity. In addition to the common MyD88 pathway, TLRs can also recruit specific adaptor molecules and activate differential intracellular pathways: ligation of TLR4 recruits TRIF and TRAM, mediating unique signals leading to secretion of IFN-β and indirect upregulation of interferon-dependent genes, such as IP-10 and inducible nitric oxide synthase. We have also recently shown that NOD2, an intracellular molecule, specifically mediates cytokine induction by TLR2, but not TLR4 agonists, indicating that it may be part of a TLR2-specific pathway.

However, recent data suggest that ligation of particular TLRs results in the skewing of Th responses towards either Th1 or Th2 cytokine profiles (37) rather than a model in which the absence of TLR-mediated signals is the driving force of Th2 differentiation. Although in certain circumstances low-dose inhaled LPS can promote Th2 responses and allergic inflammation (13), LPS stimulation of TLR4 induces DC maturation and strong Th1-type responses through release of IL-12 (39). Several other TLRs, such as TLR5, TLR9, or TLR3, also induce a Th1-type profile through intermediary release of IL-12 or alpha interferon (see above). In contrast, TLR2 stimulation induces the release of only small amounts of IL-12p70 (4), and TLR2-mediated signals preferentially induce a Th2 profile in DC (39). In support of this observation are the data demonstrating that, whereas interaction of *E. coli* LPS with TLR4 induces production of IL-12p70, the recognition of *P. gingivalis* LPS by TLR2 is unable to induce IL-12p70 release and favors a Th2-type response (16, 36). Consistent with a preferential role of TLR2 in the activation of Th2 responses, the TLR2 ligand Pam3Cys activates mainly Th2-type responses (11), whereas interaction of *Yersinia enterocolitica* V antigen or the phosphatidylserine from *Schistosoma mansoni* with TLR2 results in IL-10 release and induction of T-regulatory cells favoring a Th2 bias (45, 52). These data demonstrate that signals mediated by various TLRs can induce specific cytokine profiles, with TLR4, TLR5, TLR9, and TLR3 inducing a Th1-type response and TLR2 inducing a skewing towards Th2 development (Fig. 1).

Important insights into the molecular mechanisms responsible for mediating the Th1/Th2 cytokine profiles by TLRs have also been recently gained. *E. coli* LPS stimulates Th1

responses through IL-12p70 production, and the latter depends on the phosphorylation of p38 and c-Jun N-terminal kinase (1). In contrast, the engagement of TLR2 by the bacterial lipopeptide Pam3Cys and the classic schistosome egg antigens enhances extracellular signal-regulated kinase activation, resulting in the stabilization of the transcription factor c-Fos, a suppressor of IL-12, yielding a Th2 bias (1, 11). In conclusion, through specific TLR stimulation, DC will process information leading to the polarization of the acquired immune response. By driving specific Th1 or Th2 differentiation, TLRs are therefore a crucial link between innate and acquired immunity.

How the ligand-TLR interactions lead to the activation of the differential intracellular pathways has not been fully elucidated. The TLR-PAMP interaction results in the recruitment of specific adaptor molecules, such as MyD88 and Mal, which then bind the IL-1 receptor-associated kinase. The signal is subsequently transmitted through a cascade of signaling molecules which seem to be common to all TLRs, involving TNF receptor-associated factor 6 and mitogen-activated protein kinases (2). Subsequently, activation of NF-κB and AP-1 leads to transcription of genes coding for mediation of the innate host defense, notably proinflammatory cytokines. However, ligation of either TLR4 or TLR3 recruits an additional adaptor molecule called TRIF (17, 56). In addition to potentiating the secretion of the proinflammatory cytokines, TRIF mediates unique signals leading to secretion of IFN-β and indirect upregulation of interferon-dependent genes, such as IP-10 and inducible nitric oxide synthase (Fig. 1). Another adaptor molecule that is specifically recruited to TLR4 is TRAM (57). In addition, we have recently reported that NOD2, an intracellular molecule involved in the pathogenesis of Crohn's disease, interacts with TLR2 pathways and amplifies especially the Th2-type cytokines, suggesting that NOD2 may be part of a TLR2-specific pathway (31) (Fig. 1). It is to be expected that more adaptor molecules with specificity for the various TLRs will be discovered, which will explain the nature of the intracellular signals induced by each of these receptors.

TLR/Th BIAS AND SUSCEPTIBILITY TO INFECTIONS

The in vitro data suggesting a bias in the TLR/Th cytokine profiles induced by the various bacterial PAMPs have been corroborated by experiments in vivo. It has been known for several years that defects in proinflammatory Th1 responses, leading to biased Th2 cytokine profiles, have deleterious effects for the outcome of bacterial and fungal infections (19). The crucial role played by TLR4 for the efficient release of proinflammatory Th1 cytokines, known to be important for host defense against infections, is strengthened by the large number of studies demonstrating increased susceptibility to infections in TLR4-deficient mice. It has been known for more than 30 years that C3H/HeJ mice, a strain hyporesponsive to LPS, are more susceptible than other mice to infections caused by gram-negative organisms, such as *Neisseria meningitidis* meningitis and *E. coli* urinary tract infection, due to defective cytokine release (44, 55). These mice were later described to have a loss-of-function mutation in the TLR4 gene (34). Subsequently, these earlier observations were confirmed (42, 47) and extended by the demonstration of increased susceptibility to infections caused by other gram-negative organisms, such as *Haemophilus influenzae* pneumonia (54), *Salmonella* peritonitis, and *Klebsiella pneumoniae* sepsis (9, 53). A crucial defect in the host response in TLR4$^{-/-}$ mice is the decreased neutrophil recruitment to the site of infection (30, 47, 54), which is due to both defective production of chemokines (30, 54) and decreased expression of chemokine receptors (14).

A different picture has emerged in mice deficient in TLR2. According to the in vitro data showing specific induction of Th2 responses by TLR2 (see above), one would hypothesize that TLR2$^{-/-}$ mice have a phenotypic Th1 profile, with improved outcome of disseminated infections requiring a strong cellular immunity. Indeed, in contrast to TLR4 knockout animals, TLR2$^{-/-}$ mice are less susceptible to lethal infections with *Yersinia enterocolitica* or *Candida albicans*, and their resistance is mediated by a stronger Th1 response due to diminished production of IL-10 during infection (32, 45). In the case of *Candida* infection, we have demonstrated that TLR2 signals induce proliferation and survival of CD4$^+$ CD25$^+$ T-regulatory cells, which are largely responsible for the increased IL-10 release induced by TLR2. This trend is reversed in TLR2$^{-/-}$ mice, resulting in improved survival during disseminated candidiasis (32). Similarly to *Yersinia* and *Candida*, another important fungal pathogen, *Aspergillus fumigatus*, evades immune recognition through TLR2-mediated IL-10 production during germination, whereas proinflammatory TLR4-mediated signals are lost (33).

The shift from a Th1 towards a Th2 profile induced by TLR2 signals seems also to be involved during mycobacterial and human immunodeficiency virus type 1 coinfection, in which human immunodeficiency virus type 1 expression is induced by mycobacteria through TLR2 signaling (6). Subsequently, it has been demonstrated that the ligation of TLR2 by *Mycobacterium tuberculosis* 19-kilodalton protein inhibits IFN-γ-regulated HLA-DR and FcγR1 expression on human macrophages (15). Similarly, *Mycobacterium avium* inhibits IFN-γ signaling through TLR2-dependent STAT1beta expression (5).

All the data presented above suggest that ligation of TLR2 by pathogenic microorganisms such as fungi and mycobacteria induce a Th2 anti-inflammatory bias, either through release of IL-10 or through inhibition of IFN-γ signaling. This leads to downmodulation of the microbicidal functions of leukocytes and evasion from host defense (Fig. 2).

However, not all of the effects mediated by TLR2 are deleterious. TLR2 is the major receptor for PAMPs of gram-positive bacteria, such as lipopeptides and lipoteichoic acids (3), and TLR2 has a central role in host defense against these microorganisms. Indeed, TLR2$^{-/-}$ mice are more susceptible to infection with *Staphylococcus aureus* (25, 49) or *Streptococcus pneumoniae* (12, 23) than are wild-type mice, although the mechanisms responsible are unclear. Defective cytokine stimulation in TLR2$^{-/-}$ mice has been implicated in infection with *S. aureus* (12, 40), whereas increased levels of inflammation (despite normal cytokine levels) have been incriminated in experimental pneumococcal infections (23). Similarly, TLR2$^{-/-}$ mice show an increased long-term mortality during *M. tuberculosis* infection due to uncontrolled inflammation and injury of the lungs (38). The latter studies demonstrate that, in certain infections, the anti-inflammatory TLR2 signals have important protective effects against organ injury.

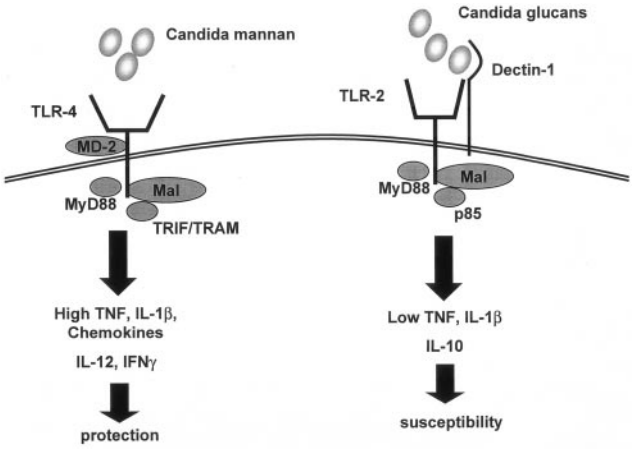

FIG. 2. TLR-mediated signals as escape mechanisms from host defense. Recent literature suggests that certain microorganisms not only are recognized by TLRs for the activation of host defense but also activate alternative TLR pathways with inhibitory effects on innate immunity. Thus, whereas interaction 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 host defense and increased susceptibility to infection. Similar mechanisms have been suggested for other microorganisms, such as *Y. enterocolitica* and *A. fumigatus*.

Thus, activation of TLR2 pathways induces Th2 responses which are important for resolution of inflammation and return to homeostasis. However, when the infection is overwhelming or when the TLR2 pathways are activated too early, the induction of Th2-type responses through TLR2 can be deleterious to the host by suppressing the innate immune response.

MODULATION OF TLR PATHWAYS AS POTENTIAL THERAPEUTIC STRATEGIES

The discovery of specific effects of each of the TLRs has important therapeutic consequences. Because certain TLRs have mainly proinflammatory effects, whereas others are involved in dampening the inflammatory reactions, there may exist the possibility of modulating either of these components of the immune response without affecting the other.

First, it is important to recognize that modulation of TLR-induced pathways has been long used in a therapeutic fashion. Several drugs used successfully in the treatment of infections are potent TLR agonists. Imiquimod is a strong TLR7 and TLR8 activator which induces IFN-α, and through this effect the drug exerts both potent antiviral and anticancer effects. The *Streptomyces nodosus*-derived amphotericin B is also a TLR2 ligand (41), which is very likely the cause of its febrile side effects. It would not be surprising if many more antibiotics, of which many are biological products, show interaction with the TLR receptors. Of note, the inhibitory effects of glucocorticoid hormones on inflammation involve the inhibition of several intracellular pathways induced by TLRs, including activation of mitogen-activated protein kinases and translocation of nuclear factors NF-κB and AP-1 (29).

Second, the discovery of TLRs will probably lead to a revolution in vaccine design. Adjuvant activity is crucial for the effectiveness of vaccines, but little has been known of how this is achieved. The discoveries of the last 10 years have demonstrated that adjuvant activity is determined by the proper maturation of dendritic cells and by the success of providing the proper costimulatory signals in the process of antigen presentation (35). We now know which cocktails of cytokines are released during a Th1 or Th2 response and how these will in turn stimulate a cellular or a humoral immune response. These processes are under full control of TLRs, and practically all adjuvants (with maybe the exception of alum) have TLR-stimulating activities. The discovery that several TLRs, such as TLR4, TLR5, or TLR9, have mainly Th1-inducing effects, whereas TLR2 induces a Th2-biased response, will have fundamental consequences on how the vaccines will be designed. For the first time, we will be able to control the development of either cellular or humoral responses during vaccination by adding a specific TLR agonist. Specific TLR2, TLR4, and TLR9 agonists have already been designed, have proved successful in animal models, and are currently being tested in vaccine trials (35).

Third, blockade of TLR pathways by specific agonists will undoubtedly find a place in the therapeutic arsenal. E5564, a specific TLR4 pathway inhibitor, is currently in trials as an antisepsis drug. As TLR2 has proved to be deleterious in several models of infection, anti-TLR2 antibodies could represent a viable adjuvant therapy, alongside antibiotics.

Finally, TLR-modulatory strategies would also likely be beneficial not only in infections but also in autoimmune diseases and cancer. *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) is a cocktail of TLR stimuli which has been advocated for almost 100 years as an anticancer treatment for evoking a strong immune response. BCG is currently used by instillation in the treatment of urothelial carcinomas. At the opposite side of the spectrum, TLRs mediate certain autoimmune diseases (21, 27), and it is very likely that anti-TLR therapies for autoimmune diseases will soon emerge.

CONCLUSIONS: FROM THE Th1/Th2 PARADIGM TOWARDS A TLR/Th BIAS

TLRs are a major class of pathogen recognition receptors: they recognize PAMPs from various classes of microorganisms, leading to the production of cytokines and activation of the microbicidal mechanisms of leukocytes, and they induce maturation and activation of DC, thereby providing a bridge between innate and acquired immunity. The results of in vitro experiments, as well as of in vivo infection models, provide support for the notion of a functional skewing of the cytokine profiles released by specific TLRs. Thus, whereas signals mediated by TLR4 induce strong proinflammatory cytokine profiles from monocytes, dendritic cells, and Th1 cells, TLR2 ligation induces the release of a Th2 cytokine profile. This represents a step forward in understanding the Th1/Th2 paradigm, in which TLRs govern diversion of pro- versus anti-inflammatory pathways from the moment a microorganism interacts with the leukocytes at the level of the cell membrane. The differential pathways induced by membrane-bound TLR4 and TLR2 represent the extension of the Th1/Th2 concept at the receptor level. This has important consequences for both our understanding of the innate immune system and the pos-

sible therapeutic use of strategies designed to target specific TLRs.

ACKNOWLEDGMENT

M.G.N. was supported by a VIDI grant of The Netherlands Organization for Scientific Research.

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