Control of adaptive immune responses by Toll-like receptors

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Recently, there has been considerable interest in how adaptive immune responses are controlled by the innate immune system. In particular, researchers have focused on how the differentiation of CD4 T cells is directed upon priming by dendritic cells. The identification of the Toll-like receptors as a family of pattern-recognition receptors involved in controlling dendritic cell activation has focused attention on these receptors as possible regulators of adaptive immune responses. However, recent studies have suggested that Toll-like receptors may only control the induction of Th1 responses and that a separate system of recognition regulates Th2 responses.

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Abbreviations

APC	antigen-presenting cell
DC	dendritic cell
LPS	lipopolysaccharide
PAMP	pathogen-associated molecular pattern
PRR	pattern-recognition receptor
TLR	Toll-like receptor

Introduction

Adaptive immune responses are initiated when T cells recognize foreign peptides bound to self-MHC molecules expressed on antigen-presenting cells (APCs). Over the years we have learned that T cell activation requires not only recognition of a foreign-peptide–MHC complex but also the expression of costimulatory molecules such as CD80 and CD86 on APCs. Consequently, expression of costimulatory molecules must be tightly regulated. Until recently this regulation was not well understood. Over a decade ago, Janeway hypothesized that regulation of costimulation must be controlled by receptors with specificity for microbial products, thereby linking innate recognition of non-self with induction of adaptive immunity [1]. The function of the recently identified Toll-like receptor (TLR) family appears to fit with this hypothesis, and recent evidence suggests that TLRs play an important role in controlling adaptive immune responses.

TLRs are evolutionarily conserved, germline encoded receptors that recognize specific molecular patterns associated with microbes. There are currently 10 known TLR family members and the number of known TLR ligands continues to grow. In many cases these ligands represent unique products of microbial metabolism, such as lipopolysaccharide (LPS) and peptidoglycan. Other ligands are highly conserved features of a particular class of microbe, such as hypomethylated CpG DNA motifs [2••], dsRNA [3••] or bacterial flagellin [4••]. Collectively, all these ligands have been termed pathogen-associated molecular patterns (PAMPs). Recognition of PAMPs by TLRs initiates a signaling pathway that leads to activation of NF-κB transcription factors and members of the MAP kinase family [5]. TLRs share a common intracellular domain that is similar to that of IL-1 receptors and therefore called the Toll/IL-1-receptor (TIR) domain. All TLRs signal through a TIR-domain-containing adaptor, MyD88. Mice lacking MyD88 are deficient in TLR signaling and generally do not respond to TLR ligands [6].

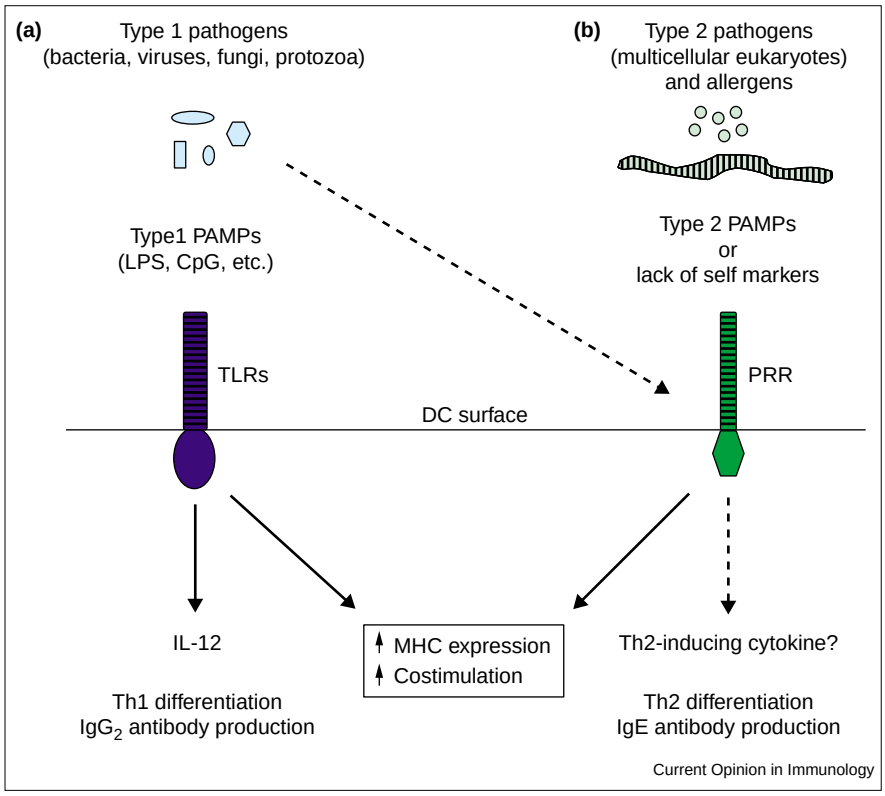
TLRs are expressed primarily on macrophages and dendritic cells (DCs) and control the activation of these APCs [5]. In DCs, TLR signaling triggers a maturation program that includes upregulation of MHC and costimulatory molecules and expression of pro-inflammatory cytokines such as TNFα, IL-1 and IL-6. This maturation of DCs significantly increases their ability to prime naïve T cells. In this way, TLRs link the recognition of pathogens with induction of adaptive immune responses. An important focus of recent research has been to understand whether this link between innate and adaptive immunity can affect what type of adaptive immune response is mounted. This review will discuss the role that TLRs play in the induction of adaptive immune responses.

TLRs and induction of adaptive immunity

DCs occupy a central position in the immune system as the cells responsible for priming of naïve T cells [7]. Priming of CD4 T cells is a critical event in the induction of an adaptive response, because the differentiation of these cells determines the nature of the adaptive response [8]. In response to viruses, intracellular pathogens and various bacteria, CD4 T cells differentiate into Th1 cells. This differentiation is dependent on the production of IL-12 by DCs. Th1 cells produce IFNγ and induce B cells to produce antibodies of the IgG_2 isotype (Type 1 responses). In contrast, infections with multicellular parasites, such as helminthes, generally lead to induction of Th2 cells, which produce IL-4, IL-5 and IL-13 and induce production of IgE antibodies (Type 2 responses).

Each of these antibody responses is most suited to eliminate the type of infection that leads to its induction. For instance, IgG_2 antibodies effectively mediate antibody-dependent cellular cytotoxicity (ADCC) against intracellular pathogens, whereas cross-linking of IgE bound to FcεR results in degranulation of mast cells, basophils and eosinophils. How the nature of infection determines the

Figure 1



Discrimination between pathogens by the innate immune system. Different adaptive immune responses are induced by Type 1 and Type 2 pathogens. (a) TLRs expressed on DCs recognize PAMPs expressed by Type 1 pathogens and induce DC maturation (including increased MHC expression and costimulation capacity) and IL-12 production. This response leads to Th1 differentiation by CD4 T cells and production of IgG_2 antibodies. In contrast, the induction of Th2 differentiation and IgE production by multicellular parasites and allergens appears to be independent of TLR signaling. (b) Instead, Type 2 pathogens may be targeted either by PRRs that recognize Type 2 PAMPs or by the lack of self markers on Type 2 pathogens. This recognition induces DC-maturation but does not lead to IL-12 production; however, it may induce expression of a currently unknown cytokine that induces Th2 differentiation. As indicated in the figure, Type 1 pathogens will be recognized as lacking self, but they will also engage TLRs, which will predominate and lead to a Type 1 response. It is not known whether the two types of receptors that regulate these responses are expressed on the same DC or on different DC subsets.

choice between Th1 and Th2 effector responses is not well understood and is an area of intense interest. In general, this decision is believed to be determined by the receptors of the innate immune system through their effect on DC maturation and the involvement of different subsets of DCs in the initiation of Th1 and Th2 responses [7,9–11].

Several subsets of DCs exist in mammalian organisms; the DC subsets differ from each other by expression of distinct sets of pattern-recognition receptors (PRRs) and by cytokines they can produce upon maturation. Accordingly, a number of studies have suggested that different DC subsets induce T cells to differentiate preferentially into Th1 or Th2; however, in many cases conflicting data exist (for a review, see [12]). In general, lymphoid DCs appear to induce Th1 differentiation whereas myeloid and in some cases plasmacytoid DCs have been shown to induce Th2 differentiation [13]. These experiments are complicated by the difficulty in isolating large numbers of different subsets. In addition, the process of isolating DCs may alter their phenotype and affect their function. Nevertheless, these results have led to a model in which T cell differentiation is determined by which DC subset is involved in priming [9,11].

Whether different DC subsets control T cell differentiation, the question remains: how are pathogens that induce distinct responses recognized? Since Type 1 and 2 adaptive immune responses are associated with such different pathogens, the immune system must have evolved a means to distinguish between them. A recent focus within this field has been whether TLR signaling in DCs is involved in the induction of Th1 versus Th2 responses by discriminating between different types of infections [7,11].

Linking the specificity of TLRs for microbial patterns to T cell differentiation would seem to make sense. A number of known TLR ligands such as LPS, peptidoglycan, and CpG DNA lead to potent production of IL-12 by some DC subsets. Consequently, DCs treated with these stimuli induce the differentiation of Th1 cells (Figure 1). Whether there are TLR ligands that can induce a DC maturation program that will lead to Th2 differentiation remains unclear, although there is increasing evidence that this many not be the case. Nevertheless, control of Type 2 responses by TLRs could be achieved in two ways, as discussed below.

The first alternative is that different TLRs may lead to different signaling events in the same cell that result in DC maturation without the production of IL-12. Thus far, there is no evidence for this possibility. All TLRs appear to induce the same conserved signaling pathway. Although there is evidence for differential gene expression downstream of different TLRs, the same core set of NF-κB dependent genes is induced by all tested TLR ligands [14••,15•].

The second alternative is that certain subsets of DCs may be preprogrammed to respond to TLR signals differently than others. These subsets would necessarily express largely non-overlapping TLRs so that a particular stimulus would only activate the relevant DCs. There is some evidence supporting this possibility. An analysis comparing TLR expression in human plasmacytoid and myeloid DCs found that plasmacytoid DCs express TLR7 and TLR9, whereas myeloid DCs express many of the remaining TLRs, but not TLR7 or TRL9 [16•,17•]. Interestingly, plasmacytoid DCs produce type I interferons when stimulated with ligands for TLR9 but do not produce IL-12 [16•–18•]. The production of these anti-viral cytokines seems to fit with the recent finding that TLR7 may be involved in viral recognition and also with the possibility that TLR9 may recognize CpG motifs in viral DNA [19•]. This lack of IL-12 production may also explain why plasmacytoid DCs induce Th2 differentiation in some *in vitro* systems [13]. However, it seems unlikely that these cells have evolved specifically to prime Th2 cells but rather that they are in some way important for anti-viral responses.

The strongest evidence against the possibility that certain TLRs induce DCs to prime Th2 cells comes from the recent analysis of adaptive immune responses in MyD88-deficient mice [20•]. As mentioned above, MyD88 is the adaptor required for signaling by all TLRs, so MyD88-deficient mice can be used as a model for mice with impaired TLR function. MyD88-deficient mice immunized with complete Freund's adjuvant, which was mixed with the antigen (ovalbumin), were unable to generate Th1 immune responses. The mice failed to produce antigen-specific IgG_{2a} antibodies and T cells from these mice did not produce IFNγ when restimulated with ovalbumin. Surprisingly, though, the production of antigen-specific IgE and IgG_1 antibodies was unaffected by MyD88 deficiency. These results suggest that TLR signaling may be necessary for the induction of Th1 responses but not for the induction of Th2 responses.

Recognition of Type 2 pathogens

As discussed above, the ability of the TLR family to discriminate between different classes of pathogens does not appear to control Type 2 immune responses directly. Instead, the multicellular parasites and allergens that induce Type 2 responses may be recognized through a different system. Understanding the molecular basis of this recognition is essential if we hope to understand how innate recognition controls adaptive immune responses.

In general, the innate immune system uses two strategies of recognition: recognition of 'microbial non-self' and recognition of 'missing self'. Microbes can be identified as non-self through recognition of unique microbial products by PRRs such as TLRs. Alternatively, a pathogen may be identified and targeted because of its lack of host self markers. Whether Type 2 pathogens have unique molecular features that can be recognized by an as-yet-unidentified family of PRRs remains unclear. Multicellular eukaryotic pathogens lack the unique metabolic products of prokaryotic pathogens that are recognized by TLRs. However, certain carbohydrates present on *Schistosoma mansoni* eggs have been implicated in the potent Type 2 response induced by egg extracts [21,22]. Parasite-associated glycans may be targeted by the large number of C-type-lectin and lectin-like receptors expressed on macrophages and DCs [23]. Additional products capable of inducing Type 2 responses have been identified from other helminth infection models [24,25]. Such potent inducers of Type 2 responses may represent Type 2 PAMPs.

The other possibility is that Type 2 pathogens lack any unique features that identify them as non-self. In this case, these pathogens may be recognized by their lack of self markers. This latter possibility could be mediated by cell-bound receptors or a soluble system such as complement. In either case, recognition would be based on the absence of self. For example, activation of complement by the alternative and/or lectin-dependent pathways, which is prevented on host cells by a variety of mechanisms, may serve as a very general way to identify Type 2 pathogens.

Although these two possibilities may appear to be largely overlapping, the one important distinction is that the former will be unique to Type 2 pathogens whereas the latter will not (Figure 1). Bacteria and other Type 1 pathogens are recognized through their lack of self markers. In this case, though, TLRs are also engaged by PAMPs and signal for a Type 1 response. Such a system requires that Type 1 responses be dominant over Type 2 responses, which appears to be the case, at least in several model systems. If Type 2 responses are induced through a missing-self-recognition system, this recognition, combined with the lack of TLR-activating PAMPs on Type 2 pathogens, may lead to the generation of Type 2 responses. In fact, these criteria fit with the features associated with multicellular parasites and allergens.

Undoubtedly, multiple mechanisms have evolved to recognize Type 2 pathogens. However, we believe that these mechanisms will share many of the features that we have described above. Recognition will result in activation of DCs; however, this activation will be distinct from that induced by TLR signaling. Although MHC and costimulatory molecules will most probably be upregulated, there may be differences in the costimulatory molecules that are induced. In addition, the cytokines expressed will certainly differ significantly from those induced by TLRs. It remains to be seen whether a cytokine analogous to IL-12 exists for Th2 differentiation. Most importantly, the molecular recognition of Type 2 pathogens will be independent of TLRs.

Conclusions

There is accumulating evidence that molecular recognition of pathogens by cells of the innate immune system is

responsible for determining the nature of the adaptive immune response. Bacteria, viruses and other microbes are identified as non-self through recognition of PAMPs by the TLR family. The ensuing response involves activation of a signaling pathway in DCs that leads to upregulation of MHC and costimulatory molecules and the expression of NF-κB dependent genes such as IL-1, IL-6, TNFα and IL-12. This maturation profile primes CD4 T cells to differentiate into Th1 cells and produce IFNγ, which in turn induces B cells to produce IgG_2 antibodies. Within the TLR family, there may be differences in the response that are based on which TLR is activated. However, these differences are unlikely to determine the choice between Th1 and Th2 responses.

Infection by multicellular parasites or exposure to allergens leads to the induction of Type 2 responses, characterized by differentiation of Th2 cells and production of IgE. This class of immune response appears to be regulated through a different system of innate recognition that is independent of the TLR family. Whether recognition of Type 2 pathogens occurs by unidentified PRRs or by recognition of 'missing self' remains unclear. Identifying the basis for this recognition and the associated signals that lead to Type 2 immune responses will be an important focus for future research.

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