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TOLL-LIKE RECEPTORS: LINKING INNATE AND ADAPTIVE IMMUNITY

Chandrashekhar Pasare and Ruslan Medzhitov*

1. ABSTRACT

Work in recent years has shown an essential role for Toll-like receptors (TLRs) in the activation of innate and adaptive immunity in vertebrate animals. These germ-line encoded receptors, expressed on a diverse variety of cells and tissues, recognize conserved molecular products derived from various classes of pathogens, including Gram-positive and -negative bacteria, DNA and RNA viruses, fungi and protozoa. Ligand recognition induces a conserved host defense program, which includes production of inflammatory cytokines, upregulation of costimulatory molecules, and induction of antimicrobial defenses. Importantly, activation of dendritic cells by TLR ligands is necessary for their maturation and consequent ability to initiate adaptive immune responses. How responses are tailored by individual TLRs to contain specific classes of pathogens is not yet clear.

2. INTRODUCTION

In all animals, the innate immune system provides essential protection against invading pathogens. A key component of this system is a collection of germ-line encoded receptors called pathogen recognition receptors (PRRs), which recognize a highly conserved set of molecular structures specific to microbes (Pathogen associated molecular patterns, or PAMPs) [1]. In addition to this system, vertebrates have a second line of defense called the adaptive immune system, which employs a diverse set of somatically rearranged receptors (T- cell receptors [TCRs] and B-cell receptors [BCRs]) with the ability to recognize a large spectrum of antigens.

*Howard Hughes Medical Institute, Section of Immunobiology, 300 Cedar Street, TAC S660, Yale University School of Medicine, New Haven, CT 06510.

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The best understood and perhaps the most important subgroup of PRRs is the Toll-like receptor family. These receptors have the ability to recognize pathogens or pathogen derived products and initiate signaling events leading to activation of innate host defenses. Signaling by TLRs initiates acute inflammatory responses by induction of anti-microbial genes and inflammatory cytokines and chemokines [2,3]. In addition, TLRs have an important role in activation of adaptive immune responses [4,5]. Although T and B cells of the adaptive immune system express receptors of enormous diversity, activation of these cells depends on induction of co-stimulatory molecules and secretion of cytokines and chemokines by the cells of the innate immune system. Effective response to microbial infection requires several levels of interactions between innate and adaptive immune systems. A variety of cell surface receptors, secreted cytokines and chemokines participate in the induction of protective immunity. We will discuss here current paradigms of the importance of innate immune recognition by TLRs and the significance of that recognition for the outcome of adaptive immune responses. We will also discuss how inappropriate activation of TLRs under certain circumstances can lead to autoimmune diseases.

3. TLRs AND THEIR LIGANDS

The mammalian TLR family consists of 10 members with distinct ligand specificities and gene targets [2,3]. TLR4 recognizes lipopolysaccharide (LPS) [6,7] from gram-negative bacteria, TLR2 recognizes peptidoglycan from gram-positive bacteria [8], TLR3 recognizes double-stranded RNA from double stranded and negative strand viruses [9], TLR7 and 8 recognize RNA from single stranded viruses [10,11], and TLR9 recognizes unmethylated CpG DNA found abundantly in prokaryotic genomes and DNA viruses [12,13]. A comprehensive list of ligands and signaling events downstream of various TLRs is described elsewhere [3,14]. In addition, several reports have suggested that some TLRs can also recognize host-derived ligand. One example is the recognition of heat shock proteins by TLR2 and TLR4 [15-17]; however, it remains possible that the recombinant heat shock proteins used in these studies were contaminated with endotoxin (or other TLR ligands), consistent with more recent reports that more stringent purification of the hsps resulted in a loss of stimulatory activity [18-20]. Another example of recognition of a host ligand by a TLR (chromatin associated DNA by TLR9) is discussed in more detail below.

4. ADAPTIVE IMMUNE SYSTEM: MECHANISMS OF TOLERANCE AND IMPORTANCE OF INNATE IMMUNE RECOGNITION

Although most auto-reactive T and B lymphocytes undergo clonal deletion during their development in primary lymphoid organs (central tolerance), a few nevertheless escape into the periphery and must be held in check by mechanisms of peripheral tolerance [21].

Clonal deletion of auto-reactive B cells is achieved in the bone marrow. Immature B cells that interact with membrane bound self-antigens are promptly deleted, while those that interact with soluble self-antigens are subject to a much more prolonged process of anergy followed by apoptosis [22-25]. In the case of T cells, clonal deletion is the fate of thymocytes with high affinity to self-antigens expressed in the thymus [21]. To make this an effective process, it is important that genes that function only in peripheral tissues and organs are expressed in thymic epithelium and DCs resident in thymus. Recent findings identify a transcriptional regulator called autoimmune regulator (AIRE) that permits thymic expression of many genes that function or are otherwise expressed only in peripheral tissues and organs [26,27]. Mice and humans with defective AIRE have an increased number of self-reactive T cells, which underscores the important role of AIRE in central tolerance [26-28]. Because expression of co-stimulatory molecules on thymic APCs is essential for induction of negative selection, it will be interesting to address the role of TLRs in controlling co-stimulatory molecule upregulation in this context [29-32].

Tolerance to self-antigens in the peripheral tissues and secondary lymphoid organs is achieved by at least two distinct and equally important mechanisms. The first one is through controlled expression of co-stimulatory molecules on professional APCs. The second is by the suppressor activity of a specialized group of cells called suppressor or regulatory T cells (Tr cells). Priming of naive T cells can be induced only when TCR-MHC/peptide interaction is coupled with a second (co-stimulatory) signal provided by interaction between CD28 on T cells and CD80/CD86 molecules on APCs [33,34]. This second signal, a result of dendritic cell (DC) maturation [35], is regulated by Toll-like receptors, and in effect, flags the antigen as being pathogenic [36]. Another consequence of TLR-induced DC maturation is the elaboration of a variety of cytokines and chemokines [2,3]. These soluble signals play an important role in the outcome of adaptive immune responses. Some cytokines are particularly important for overcoming suppression mediated by Tr cells; others like IL-12 are responsible for directing the T cell responses towards a Th1 phenotype.

Recently, several studies have suggested that peripherally-resident DCs, in addition to TLR-induced migration to draining lymph nodes during infection, may also traffick to lymph nodes even in the absence of infection. This homeostatic migration enables DCs expressing self-peptides either to tolerize self-reactive T cells [37,38] or to promote these T cells to acquire properties of suppressor T cells [39,40], thereby promoting peripheral tolerance.

The second mechanism by which peripheral tolerance is maintained is through the activity of Tr cells. These cells can be divided into two major classes based on their function. The first class, which express CD4 and CD25, [41] reside in all the secondary lymphoid organs and develop in the thymus under control of a specialized transcription factor Foxp3 [42,43]. This class of suppressor cells may have evolved to prevent activation of auto-reactive T cells that escape thymic deletion; humans and mice with defective Foxp3 expression develop generalized, fatal autoimmune [42,44]. The second class is characterized by the secretion of either of two anti-inflammatory cytokines, IL-10 (Tr1) [45], or TGF-beta (Th3) [46]. These suppressor cells typically reside in mucosal tissues of the body such as the gut and lung, and may have evolved to prevent destruction of host tissues caused by chronic inflammatory processes.

5. ROLE OF TLRs IN PROTECTION FROM INFECTIONS

5.1. Induction of Innate Immune Responses

The primary function of pattern recognition receptors is to provide an immediate protection from invading pathogens [3,47]. This is achieved through activation of a plethora of defense mechanisms, including inflammatory cytokines, complement, phagocytosis, and killing via anti-microbial proteins and peptides [48]. In addition, many members of the TLR family are potent inducers of type I interferons and the consequent suite of interferon-inducible genes in response to viral DNA and RNA [9-11,13,49]. Below we will discuss data indicating an essential role for TLRs in control of adaptive immunity.

5.2. Induction of Adaptive Immune Responses: Role of Co-stimulation and Cytokines

As described earlier, TLR-induced DC maturation during infection is essential for naive T cell activation. This has been demonstrated in mice lacking MyD88, and adapter protein downstream of all TKRs. optimal activation of naive T cells requires signals through both TCR and CD28 molecules [33,34]. These requirements are met only by a fully mature DC exposed to TLR ligands either in the secondary lymphoid organ or the peripheral tissues. Engagement of TLRs on DCs by TLR ligands (presence of infection) leads to up-regulation of both MHC and co-stimulatory molecules [35]. Migration of this professional APC to the draining lymph node and subsequent interaction with naive T cells ensures that immune responses are mounted only to pathogen-derived antigens. As discussed earlier interaction of immature DC and naive T cells leads to tolerance or induction of suppressor T cells [50]. The scenario of exposure to pathogens is mimicked in most experimental conditions by use of TLR ligands as adjuvants. The hypothesis that induction of DC maturation by microbial stimuli is essential for naive T cell activation was experimentally confirmed using MyD-88 deficient mice. MyD88 is an adapter protein that functions downstream of all TLRs and deletion of this adapter abolish signal transduction downstream of most TLRs

As described earlier, TLR-induced DC maturation during infection is essential for naive T cell activation. This has been demonstrated in mice lacking MyD88, and adapter protein downstream of all TLRs. [3]. Unlike wild-type mice, MyD88-deficient mice failed to induce T cell activation and interferon-gamma production when immunized with antigens emulsified in complete Freund's adjuvant (CFA), which is essentially heat killed Mycobacterium and contains several TLR ligands [4]. Further analysis showed that mycobacterial extracts fail to induce maturation of DCs derived from MyD88-deficient mice [4], underscoring the importance of TLR-induced DC maturation for T cell priming.

In addition to controlling the co-stimulatory pathway, DCs seem to contribute to T cell activation by overcoming suppression mediated by Tr cells. This was shown in experiments using LPS-treated MyD88-deficient DCs, which can still undergo maturation, but cannot produce inflammatory cytokines [51]. Surprisingly, when these DCs were used as APCs in T cell priming assays, they failed to induce effective priming. Additional experiments revealed that the cytokine IL-6, produced by DCs upon TLR ligation, is essential to overcome the function of Tr suppressor T cells, by making responder T cells refractory to

suppression [5]. Importantly, TLR-induced cytokines, in the absence of co-stimulatory molecule induction, seem to be insufficient by themselves to induce T cell activation (unpublished observations, CP and RM), thereby ensuring that bystander T cells are not activated non-specifically by DCs during infection.

6. TLRs AND AUTOIMMUNITY

6.1. Control of B Cell Activation

In addition to their clonally-expressed B cell receptors, B lymphocytes express most known TLRs. TLR ligation on B cells induces polyclonal proliferation and expression of co-stimulatory molecules, and also promotes plasma cell differentiation, but the significance of these events (with regard to inducibility by TLR signaling) is not yet clear. However, that TLR activation on B cells can contribute to pathology in at least some contexts has been shown. In this study, linked recognition of ligands by BCR and TLR9 has been shown to initiate and exacerbate at least one autoimmune disease. While TLR9 evolved to recognize unmethylated CpG motifs found in bacterial genomes and DNA viruses, TLR9 can also be activated by self DNA under certain special circumstances. One such case occurs when apoptotic cells are inefficiently removed, leading to secondary necrosis and release of high quantities of chromatin-bound DNA fragments. B cells specific to these fragments efficient take them up via BCR-mediated endocytosis and deliver them to intracellular sites containing TLR9. Signaling through TLR9 in such a scenario can lead to activation of B cells and differentiation into plasma cells that secrete antibodies specific to chromatin or chromatin-associated antigens and lead to immune pathology. A mouse model has been described recently that demonstrates that uptake of such complexes by rheumatoid factor positive (RF+) B cells leads to highly efficient activation of such B cells [52]. This activation was shown to be dependent on TLR9-mediated recognition of DNA present in the mammalian chromatin. Dual signaling through BCR and TLR9 therefore led to enhanced production of RF antibodies and associated immune pathology. This example illustrates the potential danger inherent in expression of both BCRs and TLRs on B cells, and may explain why continuous signaling through the BCR, which may indicate engagement of self antigens, leads to inhibition of TLR9-triggered plasma cell differentiation [53].

6.2. Role of TLR-Induced Cytokines

As discussed earlier, cytokines secreted by DCs and macrophages in response to TLR ligands contribute significantly to induction of T cell responses. However, many of these cytokines, including IL-6, TNF, and interferons, can in some contexts contribute to autoimmunity [54]. IL-6 has been implicated in autoimmune diseases such as pristine induced lupus, collagen induced arthritis and experimental autoimmune encephalomyelitis (EAE) [55-58]. The fact that IL-6-deficient animals are resistant to several auto-immune diseases suggest that this may be, at least in part, due to the inability of its effector T cells to overcome suppression mediated by Tr cells. During chronic infections, persistence of pathogens and their products can lead to enhanced production of several pro-inflammatory

cytokines including IL-6, leading to a situation conducive to activation of self-reactive T cells. Indeed, several studies have shown a link between chronic infections and autoimmune diseases [59].

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