

Toll-like receptors in autoimmunity with special reference to systemic lupus erythematosus

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The Toll-like receptor (TLR) family plays a fundamental role in host innate immunity by mounting a rapid and potent inflammatory response to pathogen infection. TLRs recognize distinct microbial components and activate intracellular signaling pathways that induce expression of host inflammatory genes. Several studies have indicated that TLRs are implicated in many inflammatory and immune disorders. Extensive research in the past decade to understand TLR-mediated mechanisms of innate immunity has enabled pharmaceutical companies to begin to develop novel therapeutics for the purpose of controlling an inflammatory disease. The roles of TLRs in the development of autoimmune diseases have been studied. TLR7 and TLR9 have key roles in production of autoantibodies and/or in development of systemic autoimmune disease. It remains to be determined their role in apoptosis, in the pathogenesis of RNA containing immune complexes, differential expression of TLRs by T regulatory cells.

Key words: Toll-like receptors, autoimmunity, systemic lupus erythematosus

Introduction

TLRs are transmembrane proteins which contain extra-cellular domains composed of leucine - rich regions (LRR) that interact with specific Pathogen Associated Molecular Patterns (PAMP) ligands. The cytoplasmic domains of TLRs, which are homologues to

the interleukin-1 Receptor (IL-1R) signaling domain, are called Toll/Interleukin-1 receptor (TIR) domains. All TLRs recruit certain adaptor proteins that contain a TIR domain and a death domain. 5 adaptor proteins are linked to the TIR domain-MyD88, Mal (MyD88 adaptor-like)/TIRAP (TIR domain-containing adaptor protein), TRIF (TIR domain-containing adaptor inducing interferon-beta), TRAM (Trif related adaptor molecule), and SARM (SAM and ARM containing protein).^[1,2]

Mammalian TLRs comprise of at least 11 members, which are categorically divided into 2 membrane receptor groups.^[3] The TLRs associated with the cell surface are TLR1, TLR2, TLR4, TLR5, TLR6, TLR10, TLR11, TLR12, and TLR13, whereas the TLRs associated primarily with endosomal membranes are TLR3, TLR7, TLR8, and TLR9. Phylogenetically, TLRs are generally divided into 6 families-TLR1, TLR3, TLR4, TLR5, TLR7, and TLR11.^[4] Different TLRs recognize different microbial products i.e. TLRs are differentially activated by various types of PAMPs such as bacterial DNA, LPS, peptidoglycan, teichoic acids, flagellin, pillin, viral dsRNA, and fungi zymosan. Activated TLRs differentially trigger the expression of cytokines like the interferons, the interleukins-IL2, IL6, IL8, IL12, IL16, and TNF- α . Binding of adaptor proteins to the TIR domain of TLRs trigger the downstream signaling cascade, leading to the activation of different transcription factors.^[5,6] PAMP-specific activation of TLRs converges at the level of TIR domain signaling to induce activation of NF- κ B and inflammatory gene expression to clear infectious agents. TLR2 is essential in recognition of microbial lipopeptides. TLR1 and TLR6

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cooperate with TLR2 to discriminate certain differences between triacyl and diacyl lipopeptides, respectively. TLR4 is the receptor for LPS. Cellular TLR4 is present in 2 different sizes, the larger is a 130 KDa heavily glycosylated, MD-2-dependent, surface translocated form, and a smaller 110 KDa partially glycosylated form is found in the Golgi. TLR9 is also essential for CpG. DNA recognition and TLR3 is associated with the recognition of viral dsRNA, whereas TLR7 and TLR8 are implicated in viral-derived ssRNA recognition. Flagellin is recognized by TLR5. The TLR family members thus recognize specific patterns of microbial components.^[3]

Role of Toll-like receptors in Innate and Acquired Immunity

TLRs are the key initiators of innate and adaptive immune response due to high production of proinflammatory cytokines and chemokines, upregulation of co-stimulatory molecules, and activation of antigen presentation. TLR recognition of pathogens leads to expression of genes such as inflammatory cytokines and co-stimulatory molecules. Antigen-specific acquired immunity developed due to phagocytosis-mediated antigen presentation together with TLR-dependent gene expression of inflammatory cytokines and co-stimulatory molecules.^[5,6]

In human naïve B cells, most of the TLRs are expressed at low to undetectable levels, but TLR9 and TLR10 expression is highly increased following B-cell receptors (BCR) triggering where as memory B cells express several TLRs at constitutively high levels. This difference in TLR9 expression correlates with responsiveness to its agonist, CpG DNA. In response to CpG, the human memory B cells proliferate and differentiate to immunoglobulin secreting cells. While naïve B cells do the same only if simultaneously triggered through BCR. The expression of TLRs induced by BCR in human naïve B cells prevents polyclonal activation in a primary response as it restricts stimulation of antigen-specific B cells. Thus, in human B cells, TLRs are downstream of BCR and plays a role both in primary response and in the memory phase.^[7]

Toll-like receptors and Autoimmunity

The roles of TLRs in development of autoimmune diseases have been studied recently. Autoreactive B cells are present in the lymphoid tissues of healthy individuals, but since they are subject to self-tolerance mechanisms, they remain silent. However, when tolerance to self-antigens fails, a complex of self-reactive antibodies against self- or cross-reactive DNA co-engages the antigen receptor and the TLRs, leading to a continuous activation of these auto-reactive B cells and the development of autoimmune diseases. The maturation of antigen-presenting cells (APCs), in response to signals received by innate immune system, may lead to the breakdown of tolerance. This process is mainly activated by TLRs that have been triggered by self-antigens.^[8]

High proliferation of autoreactive immune cells leads to development of autoimmunity, which is mainly due to defects in the maintenance of tolerance. Mechanisms of clonal deletion along with the regulation of expression of co-stimulatory molecules on APCs and T regulatory cells (T regs) are known to maintain the tolerance. Clonal deletion maintains the central tolerance, whereas a co-stimulatory molecule expression maintains the peripheral tolerance. Several factors play a role in the breakdown of tolerance such as genetic predisposition, self- antigenic load, immature self T-cells, and co-expression of co-stimulatory molecules during APC maturation and absence of treg functions.^[2] In autoimmunity, self antigens cannot be easily eliminated because of its excess number or is ubiquitous.^[9] Many microbial infections have been found to be associated with development of autoimmune diseases. TLR ligand binds to their specific microbial products and to the Dendritic Cells (DCs) which further activate them and thus induce signals for maturation towards tolerogenic type. Autoreactive T cells develop when these DCs are exposed to microbial ligands. These developments of autoreactive T cells and the suppression of Tregs cells result in the breakdown of tolerance. Thus, a set of events that follow activation through TLR results in the production of autoantibodies and cytokine response leads to autoimmune diseases.

Immunoglobulin G containing immune complexes (ICs) bound to mammalian chromatin effectively through the process which involves BCR recognition of the ICs, and the DNA is delivered to TLR9 in an endosomal/lysosomal compartment. TLR9 breaks the tolerance of autoreactive B cells by hypomethylated CpG DNA present in DNA. However, low affinity B cells do not proliferate in response to protein containing ICs. Plasmacytoid DCs (pDCs) and Myeloid DCs (mDCs) get stimulated and secrete cytokines through a link of Fc gamma receptors (Fc γ Rs) and TLR9. Mammalian chromatin can also directly stimulate DNA-reactive cells through TLR9-dependent process but under appropriate conditions. RNA and RNA-associated autoantigens stimulate the BCR/TLR7 and thus leads to the activation of autoreactive B cells.^[10] TLR 3, 9 and 7 induces the production of IFN- α , Viral dsRNA binds to TLR 3 and activates NF- κ B which helps in the induction for formation of apoptotic bodies that further promote pDCs to produce IFN- α .^[11]

Despite of many potential mechanisms for triggering of autoantibody production by microorganisms, it is also possible that certain autoantigens, independent of infection, are endogenous ligands for Pattern Recognition Receptors (PRRs) and have a proactive role in loss of immune tolerance. Similar to pDCs, B cells express both TLR7 and TLR9. Importantly, IFN- α markedly upregulates expression of TLR7 and TLR adaptor protein myeloid differentiation primary-response gene 88 (MyD88) and can also increase the response of B cells to TLR9 ligands. Aberrant expression or regulation of relevant TLRs might render B cells hyper-responsive to endogenous ligands, and therefore, predispose an individual to the development of systemic autoimmune disease. Responses to both RNA- and DNA-containing ICs are blocked by inhibitors of TLR7 or TLR9, and B cells that are deficient in TLR7 or TLR9 respond poorly to RNA and DNA-containing immune complexes, respectively.^[11]

TLRs in Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is a prototype autoimmune disease of multifactorial origin. The age of onset is between 16 and 55 years, and there is a higher

frequency of SLE in women typically during their child bearing years. Increased serum levels of IFN- α and chronically activated pDCs are characteristic of SLE patients. It has been reported that pDCs selectively express TLR7 and TLR9. The cellular activation by ICs by DCs, neutrophils, and monocytes play an important role in pathogenesis of SLE. TLR9 is thought to play a role in the production of autoantibodies in SLE.^[12-14]

Chronic stimulation of TLR9 by SLE DNA-ICs leads to overproduction of IFN- α that exacerbates SLE pathogenesis. Both chloroquine and CpG oligonucleotides, known inhibitors of TLR9 signaling, blocked SLE DNA-IC- induced IFN- α production in pDCs. The interaction of TLR9 with SLE DNA-ICs in lysosomes was found to be rapid, but transient. It is also possible that an exacerbated response to pathogens initiated by TLR2 and TLR4 may potentiate SLE attacks.^[12] TLR9 was originally identified as a receptor that could distinguish between bacterial (or viral) DNA and mammalian (host) DNA, on the basis of high frequency of hypomethylated CpG motifs in non-mammalian DNA. TLR7 was identified as a receptor for viral single-stranded RNA (ssRNA). The link between DNA-ICs, Fc γ Rs, and TLR9 has been formally established by using TLR9-deficient primary cell populations and TLR9-transfected cell lines. DNA-ICs from SLE patients were shown to stimulate production of cytokine mRNA by HEK293 (human embryonic kidney 293) cells that had been transfected with TLR9 and Fc γ RIIA, this complex co-localize with TLR9 and Fc γ RIIa in acidic lysosomes. SLE or Sjögren's Syndrome (SS) patients often have high titers of antibodies specific for small nuclear ribonucleoproteins (snRNPs), which are macromolecular complexes consisting small nuclear RNA (snRNA) and associated proteins. It has been found that IgG purified from the sera of SLE patients induce IFN- α production by pDCs when IgG is mixed with necrotic-cell debris or purified snRNPs. This IFN- α -inducing activity is inhibited by chloroquine or bafilomycin, agents that interfere with the acidification of endosomes and block activation of TLR7 and TLR9. Cytokine production is also blocked by oligodeoxynucleotide (ODN) sequences that are known to inhibit the activation of TLR7 and TLR9.^[11]

Toll-like receptors Studies on Experimental Autoimmune Animal Models

Data originating predominantly from experimental animal models of autoimmune disease suggest that inappropriate activation of TLR pathways by endogenous or exogenous ligands may lead to the initiation and/or perpetuation of autoimmune responses and tissue injury.^[7] It is reasonable to assume that the association between infection and autoimmunity is often caused by TLR-mediated induction of proinflammatory cytokine and chemokine expression and upregulation of co-stimulatory molecule expression by APCs. The ability of microbial TLR ligands to trigger disease onset has been documented in experimental models of arthritis, multiple sclerosis (MS), experimental allergic encephalomyelitis (EAE), myocarditis, diabetes, and atherosclerosis. Adoptive-transfer studies in mouse models of arthritis and in mice with EAE indicate that endogenous TLR ligands might contribute to the pathogenesis of related autoimmune diseases. Transfer of serum to TLR4-deficient mice induces joint swelling that resolves more quickly than in TLR4-sufficient (control) mice, indicating that endogenous TLR4 ligands have a role in the perpetuation of disease. The transfer of T cells to MyD88-deficient recipients lead to only minimal disease, and TLR9-deficient recipients had a much attenuated clinical score compared with TLR-sufficient (control) mice. TLR2-deficient mice subjected to occlusion of renal arteries produce significantly less pro-inflammatory cytokines and chemokines, showed less leukocyte infiltration, and developed less severe renal injury than TLR-sufficient (control) groups of mice.^[11]

To evaluate the overall impact of TLR activity on production of autoantibodies and on development of systemic autoimmune disease, *in vivo* verification of the *in vitro* studies is needed. TLR7 and TLR9 have key roles in production of pathogenic autoantibodies and/or in development of clinical features of autoimmunity in experimental animals. Lpr/Lpr mice deficient in the TLR adaptor protein, MyD88 do not produce anti-nuclear antibodies (ANAs), and these mice develop marked lymphoproliferative disease. TLR9-deficient lpr/lpr mice show strong cytoplasmic reactivity, but this reactivity

is relatively rare among lpr/lpr mice that are sufficient in TLR9. Remarkably, renal disease in TLR9-deficient autoimmune-prone mice was significantly worse than in TLR9-sufficient mice. It remains to be determined whether this reflects a role for TLR9 in the clearance of cell debris, an increase in the pathogenicity of RNA-containing immune complexes, differential expression of TLR7 or TLR9 by a treg population or any another mechanism. B-cell expression of TLR9 has an important role in promoting antibody response to DNA and DNA-binding proteins, such as histones. The absence of functional TLR9 has a marked effect on disease outcome, and TLR7 deficiency can also influence autoantibody production.^[11]

Ehlers *et al.* reported that TLR9 is a MyD88-dependent inducer of IgG2a and IgG2b autoantibodies, which are implicated in the progression of lupus-prone mice. Wu and Peng reported that genetic ablation of TLR9 has a protective role in the onset of SLE-like syndrome in MRL/lpr mice because TLR9-deficient animals have low suppressive activity of treg and demonstrated that TLR9-deficient mice possess a higher titer of anti-dsDNA antibody than control C57BL/6 mice. This suggests that diminished expression of TLR9 could be involved in enhanced production of autoantibodies. It will be important to evaluate whether aged TLR9-deficient mice lacking other autoimmune-related genes develop autoimmune diseases.^[15]

Viral double-stranded RNA (dsRNA) activates DCs to secrete type I interferons and cytokines, which are associated with disease activity in SLE or with autoimmunity in general. Systemic exposure to unmethylated CpG-DNA (ligand of TLR9) can induce EAE and aggravate the IC glomerulonephritis in MRL Lpr/Lpr mice. TLR3 is the only known TLR that depends on signaling through the adaptor molecule TRIF (Toll-IL-1 receptor domain-containing adaptor inducing IFN- α) and RNA helicase RIG-1, which is followed by a robust induction of IFN responsive genes. These findings may point toward the recognition of viral dsRNA via TLR3 on DCs not only as an important component of virus-induced immunity but also as hypothetical link to viral infection-induced aggravation of preexisting autoimmunity.^[16]

Toll-like receptors directed Therapies in Treatment of Autoimmune Diseases

Immunotherapeutic role of TLRs is emerging in treating autoimmune conditions, suggesting that the selective targeting of TLRs might be useful. Initially, extracellular TLR agonists were designed to compete with natural microbial ligands for binding to TLRs. More recently, basic research to identify new targets for drug development has begun to explore modulation of TLR intracellular signaling pathways, in addition to TLR ligand binding. The common signaling pathways used by all members of TLR superfamily are being targeted, with drugs that block NF- κ B and p38 mitogen-activated protein kinase (MAPK) in clinical development for diseases such as RA and Psoriasis.^[17] Decoy peptides and mimetics, plant polyphenols, and chemically-modified anti-sense oligonucleotides that inhibit different molecular events in TLR signaling pathways to modulate the inflammatory response have been tried. The molecular mechanisms of these inhibitors range from interference with protein-protein interactions between signaling proteins, inhibition of transcription factor activity, to perturbation of the plasma membrane. These inhibitors are derived from host, pathogen, and plant sources and by rational design. Taken together, these studies represent promising avenues for the development of novel-tailored immune therapeutics that can relieve by inflammatory and autoimmune diseases on human health and quality of life.^[18]

At present, the efficacies of potential TLR7 and TLR9 inhibitors have been tried. They are mainly anti-malarial agents and inhibitory oligonucleotides. Anti-malarial agents have been used to treat SLE for more than a century, and hydroxychloroquine (plaquenil) is still considered to be an effective treatment for cutaneous SLE and for SLE-associated polyarthralgia, pleuritis, and pericarditis.^[11] The potential for therapeutic development of TLR antagonists is also considered. Several biotechnology and pharmaceutical companies have programs to develop new drugs like agonists of TLRs to enhance immune responses against tumors and infectious agents or to correct allergic responses; or antagonists designed to reduce inflammation due to infection or autoimmune disease.^[19] The potent immunogenicity of lipopeptides

is due to their ability to activate DCs by targeting and signaling through TLR-2. In addition, the simplicity and flexibility in their design makes TLR antagonists highly attractive vaccine candidates for humans and animals.^[20]

TLRs represent attractive drug targets for modulation of immune response and hold promising applications for the treatment of infection and inflammation. Two main strategies for targeting TLRs seem to be promising for drug development such as targeting TLRs with synthetic agonists or antagonists and targeting the protein-protein interaction involved in the TLR-activated signaling cascade. Specifically, blockade of TIR/TIR interaction between TLRs and adapter proteins by low molecular weight is particularly encouraging for the development of orally-available agents. To address the need for additional and potentially superior vaccine adjuvants, a number of TLR agonists are currently being tested. While LPS is fairly toxic in humans, monophosphoryl lipid (MPL), another TLR4 ligand with a greatly improved safety profile, has recently won approval for use as an adjuvant.^[21] Finally, with the rapid progress in the development of therapeutic agonists for the TLRs, there is accompanying attention to, the theoretical possibility that such therapy may induce autoimmunity or autoimmune diseases.^[22] TLR3 ligands have been identified in the synovial fluid of patients with RA, and TLR9 ligands have been found in the immune complexes of SLE patients. Such observations raise concerns that pharmacological doses of TLR agonists could precipitate autoimmunity. These considerations have also prompted interest in the potential of TLR antagonists, as therapeutic agents for autoimmune diseases in near future.

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