Review

Molecular mechanisms of autoimmunity triggered by microbial infection

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Abstract

Autoimmunity can be triggered by microbial infection. In this context, the discovery of Toll-like receptors (TLRs) provides new insights and research perspectives. TLRs induce innate and adaptive antimicrobial immune responses upon exposure to common pathogen-associated molecules, including lipopeptides, lipopolysaccharides, and nucleic acids. They also have the potential, however, to trigger autoimmune disease, as has been revealed by an increasing number of experimental reports. This review summarizes important facts about TLR biology, available data on their role in autoimmunity, and potential consequences for the management of patients with autoimmune disease.

Introduction

Autoimmunity is believed to develop from genetic predispositions while the onset of autoimmune tissue injury or disease flare is often triggered by microbial infection. Diseases such as type I diabetes mellitus, lupus erythematosus, myocarditis, rheumatoid arthritis, and multiple sclerosis often manifest themselves in association with microbial infection. Patients with chronic forms of autoimmunity may experience symptomatic disease flares following infections. These clinical observations raise a set of questions: what classes of receptors recognize microbes or vaccine adjuvants in the host? What molecular mechanisms induce immune activation upon recognition of the pathogen? And how does antimicrobial immunity modulate tolerance? Answers to these questions are expected from the recent discovery of Toll-like receptors (TLRs). TLRs have been identified as a new family of innate receptors that recognize a set of microbial molecules known as pathogen-associated molecular patterns (PAMPs). The role of TLRs in antimicrobial immunity continues to be extensively studied. However, an increasing number of reports provide evidence that TLR ligation can trigger autoimmune tissue injury. In this review, we summarize important facts on TLR biology and available data on their role in autoimmunity. Furthermore, we provide future research perspectives that could influence the management of patients with autoimmune disease.

TLRs recognize pathogen-associated molecular patterns

To date, 11 proteins related to the *Drosophila* gene *Toll* have been characterized in vertebrates [1] (Figure 1; Table 1).

TLR2, TLR1 and TLR6

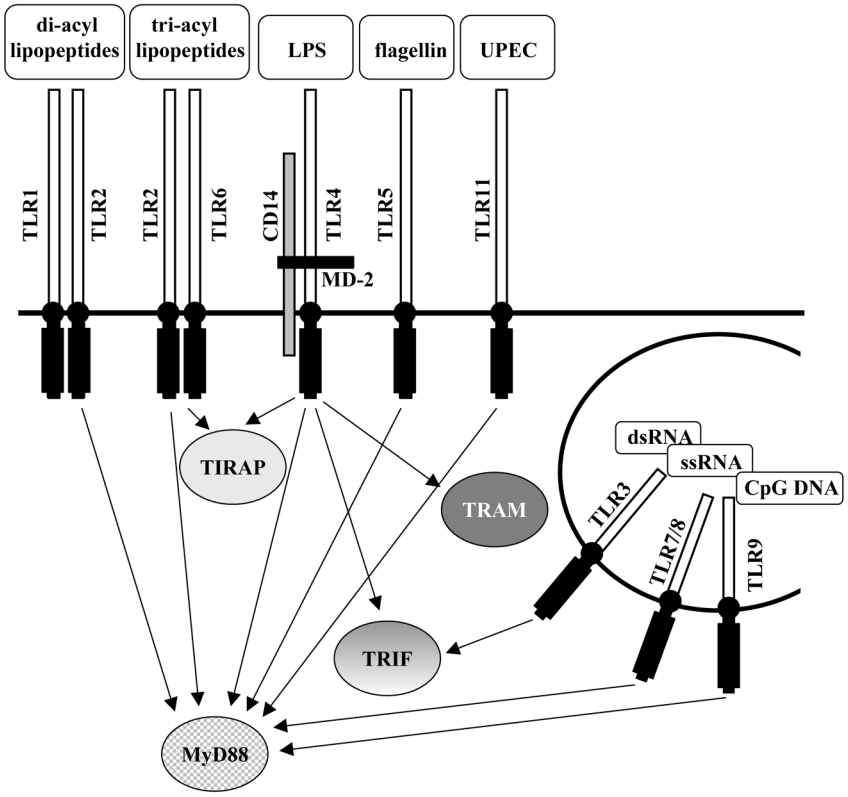
TLR2 recognizes several different ligands (Table 1). It responds to lipoproteins, which are the main cell wall components of Gram-positive bacteria [2,3], and heterodimerizes with TLR1 and TLR6, which enables discrimination between diacetylated and triacetylated lipopeptides in human monocytes [4]. TLR1-TLR2 heterodimerization activates dendritic cells, B cells, NK cells, mast cells, and keratinocytes [1]. TLR2 and TLR6 collaborate in detecting yeast zymosan [1]. In addition, components of necrotic, but not apoptotic, cells activate fibroblasts and macrophages via TLR2 [5]. Such endogenous ligands may play a role in both bacterial as well as aseptic arthritis [6-8]. Studies that suggest TLR recognition of self proteins such as heat shock protein-70 have been questioned, however, as such preparations may have been contaminated by other TLR ligands [9].

TLR3

TLR3 is a detector of double-stranded (ds)RNA that may originate from single-stranded (ss)RNA or dsRNA viruses [10,11]. It is believed that TLR3 recognizes secondary RNA structures as synthetic RNAs, mRNA, and siRNA similarly induce the production of type I IFNs and pro-inflammatory

CpG = unmethylated cytosine-guanosine; ds = double stranded; IFN = interferon; IL = interleukin; LFA = lymphocyte function associated antigen; LPS = lipopolysaccharide; MHC = major histocompatibility complex; MyD88 = myeloid differentiation (primary-response) factor 88; NF = nuclear factor; ODN = oligodeoxyribonucleotide; PAMP = pathogen-associated molecular pattern; ss = single stranded; Th = helper T cell; TIR = Toll-IL-1 receptor; TLR = Toll-like receptor.

Figure 1



Toll-like receptor (TLR) signalling. TLRs recognize pathogen-associated molecules through homophilic or heterophilic interactions. Nucleic-acid-specific TLRs localize in intracellular endosomes. Specificity of TLR signalling is provided by a group of cytosolic adaptor molecules redistributing to the intracellular Toll-IL-1 receptor (TIR) domain upon activation. CD14, cluster of differentation (Cd) 14 antigen, a surface protein preferentially expressed on monocytes/macrophages that binds to LPS-binding protein; CpG, unmethylated cytosine-guanosine; ds, double stranded; LPS, lipopolysaccharide; MD-2, myeloid differentiation protein-2; MyD88, myeloid differentiation (primary-response) factor 88; ss, single stranded; TIRAP, TIR-domain-containing adaptor protein; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain-containing adaptor protein-inducing-interferon beta; UPEC, uropathogenic *Escherichia coli*.

cytokines. Viral dsRNA induces dendritic cell maturation through TLR3 [10]. Apparently, viral RNA can act as a natural adjuvant promoting loss of tolerance against presented endogenous or exogenous antigens and that modulates the T helper cell (Th)1/Th2 balance of the subsequent T cell response. Among the monocytic immune cell subsets, TLR3 is expressed on murine macrophages; whereas in humans, TLR3 is exclusively expressed on myeloid dendritic cells [10,12,13]. In addition, TLR3 is reported to be expressed on an increasing number of non-immune cells, including glomerular mesangial cells [14], astrocytes [15], uterine epithelial cells [16], and fibroblasts [17]. These cells express TLR3 constitutively at low levels and upregulate TLR3 upon exposure to dsRNA or other TLR ligands. Most cell types express TLR3 in an endosomal compartment, which supports the idea that viruses need to be processed before their RNA can be exposed to TLR3. Fibroblasts have been reported, however, to express TLR3 also on their outer surface membrane [17].

TLR4

TLR4 is a critical component of the lipopolysaccharide (LPS) receptor complex that activates cells upon exposure to Gram-negative bacteria. However, it also responds to other ligands (Table 1). Endotoxic shock is mediated by TLR4, which induces the release of pro-inflammatory cytokines and chemokines from immune and non-immune cells. For example, lack of appropriate TLR4 signalling can predispose to septicemia in patients with rheumatoid arthritis treated with an anti-tumour necrosis factor regimen [18], but functional TLR4 does not predispose to rheumatoid arthritis *per se* [19]. TLR4 is required for neutrophil sequestration during endotoxin-induced lung injury [20]. In 1998, a point mutation in the gene encoding TLR4 was found to be the molecular basis of LPS hyporesponsiveness in C3H/HeJ and C57BL/10ScCr mice, the latter having a chromosomal defect that leads to a loss of the *Tlr4* gene [21]. Mice belonging to these strains show a similar phenotype to mice with target deletions of the *Tlr4* gene. In humans, TLR4 mutations are

Table 1

Important microbial ligands of Toll-like receptors

Receptor	Ligand	Pathogen
TLR1	Triacyl lipopeptides	Bacteria, mycobacteria
TLR2	Peptidoglycan	Gram-positive bacteria
	Lipoteichoic acid	Gram-positive bacteria
	Lipoprotein, lipopetides	Different pathogens
	Atypical lipopolysaccharide	*Leptospira interrogans*
	Glycoinositolphospholipids	*Trypanozoma cruzii*
	Zymosan	Fungi
TLR3	Double-stranded RNA	Viruses
TLR4	Lipopolysaccharide	Gram-negative bacteria
	Fusion protein	Respiratory syncytial virus
	Taxol	Plants
TLR5	Flagellin	Bacteria
TLR6	Diacyl lipopeptides	*Mycoplasma*
TLR7	Single-stranded RNA	Viruses
	Imidazoquinoline, Loxoribine, Bropirimine	Synthetic compounds
TLR8	Single-stranded RNA	Viruses
	Imidazoquinoline	Synthetic compounds
TLR9	CpG-DNA	Bacteria, viruses
	Hemozoin	*Plasmodium falciparum*
TLR10	Nd	Nd
TLR11	Nd	Uropathogenic *E. coli*
	Profilin-like molecule	*Toxoplasma gondii*

CpG, unmethylated cytosine-guanosine; nd, not determined; TLR, Toll-like receptor.

also associated with impaired responsiveness to LPS, but lack of TLR4 in humans does not affect the outcome of bacterial sepsis [22,23]. Reports showing that endogenous molecules act through TLR4 have been questioned as studies using low-endotoxin preparations of such molecules failed to confirm these data (reviewed in [9]).

TLR5

The only known ligand for TLR5 is bacterial flagellin present in both Gram-positive and Gram-negative bacteria [24]. TLR5 induces maturation of human but not murine dendritic cells [25]. In addition to immune cells, TLR5 is also expressed on the basolateral, but not on the apical, membrane of human intestinal epithelial cells. This finding supports the hypothesis that TLR5 recognizes flagellin on the surface of enteropathogenic bacteria, such as *Salmonella*, that can translocate across epithelia in contrast to commensual bacteria such as *Escherichia coli* [26].

TLR7 and TLR8

Similar to TLR3, TLR7, TLR8, and TLR9 are located intracellularly in endosomes and recognize phagocytosed ligands [27,28]. TLR7 and TLR8 both recognize viral ssRNA as well as distinct synthetic guanosine analogs [1,29]. Both can activate dendritic cells to mature and produce pro-inflammatory cytokines [29].

TLR9

Unmethylated cytosine-guanosine (CpG)-DNA is an important ligand for TLR9 [30]. The CpG dinucleotide is the stimulatory motif of bacterial and viral DNA [31]. CpG-DNA is a B cell mitogen and a strong activator of plasmacytoid dendritic cells in humans [28]. In complex with other proteins it induces an enhanced antigen-specific humoral and cellular immune response of the Th1 type [32]. TLR9 resides in the endoplasmic reticulum but redistributes to late endosomes for the interaction with ingested CpG-DNA [27]. Various synthetically manufactured CpG-oligodeoxynucleotides represent powerful tools for research in this field. A recent study described plasmodia-derived hemozoin as another natural ligand for TLR9, which raises doubt about the concept that TLR9 recognizes specific nucleic acid sequences [33,34]. TLR9 might recognize particle-related secondary structures rather than specific DNA sequences. This is supported by the observation that the formation of DNA nanoparticles can modulate TLR9 signalling towards production of high levels of type I IFNs [35].

TLR10 and TLR11

TLR10 is expressed on human B cells, but the ligands for human TLR10 as well as the effects of its activation remain to be identified [36]. TLR11 was only recently described [37]. TLR11 recognizes a yet undefined molecule of uropathogenic

Table 2

Toll-like receptor expression on human and mouse immune cell subsets

Receptor	Monocyte/ macrophages	Plasmacytoid dendritic cells	Myeloid dendritic cells	B cells	T cells
TLR1	m, h	–	m, h	m, h	?
TLR2	m, h	–	m, h	m, h	–
TLR3	m	–	m, h	–	–
TLR4	m, h	–	m, h	m, h	–
TLR5	m, h	–	m, h	–	?
TLR6	m, h	–	m, h	m, h	–
TLR7	m, h	m, h	m, h	m, h	–
TLR8	–	–	h		–
TLR9	–	m, h	m	m, h	–

m, mouse; h, human; TLR, Toll-like receptor.

E. coli and a profilin-like molecule of *Toxoplasma gondii* [37,38]. Whether humans express TLR11 protein is uncertain, as published TLR11 sequences include a stop codon within the coding sequence [37].

Species-specific expression of TLRs on immune cell subsets

Species-specific differences in TLR expression are important when data derived from rodents are to be interpreted in the human context. Differences between mice and humans include: the expression of TLR3 by murine but not human macrophages (Table 2); the expression of TLR8 by human but not murine myeloid dendritic cells; and the expression of TLR9 by murine but not human myeloid dendritic cells.

Specificity of TLR signalling

The specificity of TLR signalling depends on their cell type-specific expression, the potential for heterodimerization of certain TLRs, and on a group of cytoplasmic adaptor molecules [1] (Figure 1). Myeloid differentiation (primary-response) factor 88 (MyD88) was the first adaptor identified [39]. Studies with MyD88-deficient mice revealed other MyD88-independent signalling pathways involving the Toll-IL-1 receptor (TIR) domain-containing adaptor molecules TRIF (TIR domain-containing adaptor protein-inducing-interferon beta) and TRAM (TRIF-related adaptor molecule). MyD88 is the only adaptor molecule for TLR9, but TLR2, TLR4, and TLR6 can use TIR domain-containing adaptor proteins or MyD88, and TLR3 and TLR4 activation can involve MyD88 or TRAM [39] (Figure 1). Downstream of the adaptor molecules, TLR signalling involves members of the IL-1 receptor-associated kinase family and IFN regulatory factors that ultimately activate the transcription factor nuclear factor (NF)-κB or members of the IFN-regulatory factor family [39]. A detailed description of TLR signalling is beyond the scope of this article, so the reader is referred to one of the excellent in depth reviews about this issue [39]. Genetic defects in TLR signalling cause immunodeficiency rather than autoimmune syndromes [40], although a recent linkage analysis study of 44 single nucleotide polymorphisms in 13 genes from the type I IFN pathway identified two polymorphisms in the tyrosine kinase 2 and IFN regulatory factor 5 genes that are associated with systemic lupus erythematosus [41].

Ligating TLRs induces innate and adaptive antimicrobial immunity

PAMPs that ligate TLRs induce potent mechanisms of innate antimicrobial immunity. TLRs that signal through MyD88 induce NF-κB-dependent expression of pro-inflammatory cytokines and chemokines, which trigger local and systemic inflammation including arthritis [42]. For example, both CpG DNA and LPS can cause a massive release of tumour necrosis factor-α and other pro-inflammatory mediators in mice, while TLR4-deficient and TLR9-deficient mice fail to respond to the respective ligands [21,30]. In macrophages and antigen-presenting cells, TLR ligation induces the phagocytic capacity as an important component of pathogen control and antigen-processing [43]. Viral nucleic acids ligate a selected set of TLRs. For example, TLR3 signals through the adaptor TRIF, which induces expression of type I IFNs, a major component of antiviral immunity [44]. TLR9 signalling through MyD88 by plasmacytoid dendritic cells can also produce high amounts of type I IFNs upon recognition of bound viral CpG-DNA [35]. Antimicrobial immunity involves adaptive immunity, however, which also is readily activated by TLRs [45,46]. Upon TLR ligation, antigen-presenting cells upregulate expression of co-stimulatory molecules and secrete modulatory cytokines. TLR ligation mostly induces secretion of Th1 cytokines, which drives subsequent T cell functions towards Th1-type immunity [47]. Thus, TLR ligands are being increasingly explored as vaccine adjuvants [48,49].

Many other aspects of TLR biology in antimicrobial immunity have been described in great detail in several excellent reviews [1,45,46,50,51]. In the following part of this review we focus on those mechanisms that link TLR-induced immunity to mechanisms clearly involved in autoimmunity.

TLR ligation and autoimmunity

Autoimmunity is obviously caused by several coincident mechanisms that relate to the presence of autoreactive immune cell subsets and loss of tolerance. Tolerance is maintained by controlling autoreactive T and B cells as well as by tolerogenic stimuli provided by dendritic cells that constantly process autoantigens [52]. It is thought that autoimmunity develops upon uncontrolled proliferation of autoreactive immune cell subsets and non-tolerogenic cytokine signalling by antigen-presenting cells in a host with defects in tolerance control. Consistent with the observation that microbial infections are common triggers of autoimmunity, several lines of evidence show that TLR ligation can cause autoimmune tissue injury.

Exposure to TLR ligands can induce local inflammation

Intra-articular injection of bacterial CpG-DNA causes aseptic arthritis characterized by macrophage infiltrates in healthy mice [53]. Similar findings were reported after intraventricular CpG-DNA injections as a model of meningitis [54]. Macrophages produce large amounts of CC-chemokines and express chemokine receptors on their surface upon exposure to bacterial CpG-DNA to promote further leukocyte recruitment to the site of injection [55]. Thus, aggravation of local inflammatory tissue injury during microbial infection can be a consequence of circulating TLR ligands activating tissue macrophages. We traced CpG-DNA and viral dsRNA by fluorescence labelling and detected their uptake by glomerular macrophages present in nephritic kidneys of MRLlpr mice with lupus nephritis [56]. As predicted, CpG-DNA-treated mice developed a marked aggravation of lupus nephritis characterized by strong CCL2 and CCL5 expression in inflammatory cell infiltrates. Similar results have been obtained in other models of autoimmune tissue injury, including collagen-induced arthritis [57] or experimental encephalomyelitis [58]. Thus, pre-existing autoimmune tissue injury can be exacerbated by exposure to TLR ligands via activation of TLRs on parenchymal or infiltrating immune cells, which leads to increased local production of pro-inflammatory mediators. Studies with human synovial fibroblasts suggest similar mechanisms occur in human arthritis [59].

TLR ligation modulates dendritic cell functions

Dendritic cells play a central role in coordinating both adaptive antimicrobial immunity and control of tolerance [60]. Immature dendritic cells process microbial antigens as well as autoantigens. In the absence of stimulatory signals, dendritic cells remain immature, providing tolerogenic signals to T cells [60]. In the presence of additional signals, dendritic cells mature, upregulate co-stimulatory molecules, and secrete cytokines, which all provide mitogenic signals to T cells with appropriate antigen-specificity. PAMPs that ligate TLRs on dendritic cells provide such stimulatory signals to dendritic cells. As TLR ligands are usually in complex with other microbial antigens, TLRs are potential adjuvant receptors for microbial antigens. Furthermore, exposure to microbial TLR ligands can break tolerance by activating dendritic cells that present autoantigens and thereby induce autoimmunity. For example, Eriksson *et al*. [61] showed that dendritic cells loaded with a heart-specific self peptide induce CD4+ T-cell-mediated myocarditis in non-transgenic mice after transfer of dendritic cells that had been pulsed with LPS or CpG-DNA. Similar mechanisms may contribute to loss of tolerance to self DNA after exposure to bacterial CpG-DNA. For example, bacterial DNA induces production of cross-reactive DNA autoantibodies in autoimmune NZB/NZW mice [62]. The latter argues for a role of TLR9 ligation by exogenous CpG-DNA in lupus induction in genetically predisposed individuals.

TLRs on dendritic cells and control of regulatory T cells

In vertebrates, autoreactive T cells are kept under tight control by a number of mechanisms, including the suppressive effect of regulatory T cells. Autoimmunity is commonly associated with uncontrolled proliferation of autoreactive T cells, which can, in part, be attributed to a modified function of regulatory T cells. Recently, it was shown that dendritic cells play a major role in determining the functional state of regulatory T cells. Pasare *et al*. [63] attempted to identify the critical factor among the many cytokines that were produced by LPS or CpG-DNA-pulsed dendritic cells that determines the functional state of regulatory T cells. They identified IL-6 to be this factor and showed that IL-6 can induce proliferation of autoreactive T cells through functional blockade of CD4+/CD25+ regulatory T cells. Thus, dendritic cells exposed to microbial PAMPs signal for proliferation of T cells specific for microbial antigens as well as for autoreactive T cells, which may link host defence to loss of tolerance or autoimmunity.

TLR-induced interferon production

IFN producing plasmacytoid dendritic cells play a dominant role in antimicrobial immunity as well as in various types of autoimmunity, including lupus erythematosus [64-66]. Among the type I IFNs, IFN-α is a dominant mediator of autoimmune disease activity, and is known to drive tolerance towards autoimmunity. Nuclear particles released from ultraviolet light-treated cells induce IFN-α production by plasmacytoid dendritic cells [67]. Most interestingly, this response was abolished by DNAse or RNA digestion, which indicates that endogenous nucleic acids are major modulators of type I IFN production [67]. Using a genetic model of type I diabetes, Lang *et al*. [68] showed that upregulation of major histocompatibility complex (MHC) I on beta islet cells but not on exocrine pancreatic cells was dependent on interaction of circulating IFN-α with beta cell IFN type I receptors. Ligands

for TLR3, TLR7, and TLR8 were found to induce high levels of IFN-α and beta cell MHC I expression in the host, leading to diabetes, which could be neutralization of IFN-α [68]. Ligation of TLR4 or TLR9 that failed to induce IFN-α did not cause diabetes. It was concluded that organ-specific effects secondary to the immunostimulatory effect of systemic exposure to microbial TLR ligands can convert T cell autoreactivity into overt autoimmune disease. Furthermore, IFN-α released by plasmacytoid dendritic cells modulates the sensitivity of TLR7 on B cells to respond to TLR7 ligands, which remains unresponsive in the absence of IFN-α [69].

TLR expression by non-immune cells can modulate autoimmune tissue injury

From the findings discussed above, it becomes clear that autoimmune tissue injury also relates to tissue specific responses during exposure to microbial molecules. We have recently identified that from all known TLRs, glomerular mesangial cells express TLR3 at high levels *in vitro* and *in vivo* in an endosomal compartment [14]. In cultured glomerular mesangial cells, TLR3 ligation with viral dsRNA induces the production of pro-inflammatory cytokines and chemokines. Labelled dsRNA injected into $MRL^{lpr/lpr}$ mice with lupus nephritis was found to localize to endosomes of glomerular mesangial cells, consistent with immunostaining for TLR3. A course of repetitive injections with viral dsRNA caused severe aggravation of lupus nephritis in autoimmune $MRL^{lpr/lpr}$ mice associated with increased glomerular production of CCL2 and CCL5 and subsequent leukocytic cell infiltrates. By contrast, no changes in serum DNA autoantibody levels were detected as viral dsRNA does not induce B cell stimulation. These data indicate that TLR3 on glomerular mesangial cells recognizes circulating viral dsRNA, causing local inflammation in pre-existing lupus nephritis, which may correspond to renal disease flares in lupus patients that experience intercurrent viral infections. As TLR3 is expressed by other non-immune cell types, including astrocytes [15], uterine epithelial cells [16], and fibroblasts [17], similar mechanisms may account for autoimmune tissue injury of organs that harbour these cell types.

TLR ligands are B cell mitogens

B cells are professional antigen-presenting cells that express TLR7 and TLR9. B cells isolated from $MRL^{lpr/lpr}$ mice with lupus-like disease produce large amounts of autoantibodies when exposed to immune complexes that contain CpG-DNA [70]. Autoreactive B cells from these mice recognize the IgG part of the immune complex by their surface B cell receptor, then internalize immune complexes, which exposes CpG-DNA to TLR9 in the endosomal compartment [32]. These mechanisms may also apply *in vivo*, because autoimmune $MRL^{lpr/lpr}$ mice injected with bacterial CpG-DNA produce large amounts of DNA autoantibodies [56] in association with increased MHC II expression on B cells isolated from spleens of these mice [14]. The avidity of DNA autoantibodies present in $MRL^{lpr/lpr}$ mice and those induced by unmethylated CpG-DNA is comparable [71]. Systemic exposure to bacterial CpG-DNA can induce the production of DNA autoantibodies in non-immune mice [72]. Furthermore, CpG-DNA has a strong adjuvant effect on DNA autoantibody production in mice that have been exposed to vertebrate DNA [72]. These data support the hypothesis that exposure to bacterial DNA, for example, during bacterial infection, provides a strong signal for enhanced DNA autoantibody production in systemic lupus erythematosus.

Together, the cell type- and tissue-specific expression of TLR contribute to the ligand-specific immune effects of microbes. Viral dsRNA can promote autoimmune tissue injury through TLR3 expressed on intrinsic parenchymal cells. Bacterial CpG-DNA and possibly ssRNA of viral origin induce B cell proliferation, including autoreactive B cell subsets. Furthermore, TLR ligands induce dendritic cell maturation towards a non-tolerogenic phenotype that promotes antimicrobial immunity as well as autoimmunity through secretion of selected cytokines that modulate subsequent immune responses, including the blockade of the suppressive effect of regulatory T cells.

TLR9 and DNA recognition in lupus

As DNA particles are important autoantigens in lupus, recognition of CpG motifs in self DNA through TLR9 might be involved in the pathogenesis of systemic lupus erythematosus. In fact, immune complexes isolated from lupus patients can activate plasmacytoid dendritic cells to produce cytokines, chemokines, and IFN-α through TLR9 [73]. Whether such patient-derived immune complexes contain self-DNA or microbial DNA remains elusive [74]. CpG motifs in self DNA need to be protected from activating TLR9 to prevent autoimmunity. In vertebrates at least three such mechanisms exist.

Methylation of CpG-DNA

Methylation reduces the immunostimulatory effects of unmethylated bacterial or synthetic DNA [31,75], but in vertebrates only 70% to 80% of CpG motifs are methylated [76]. Hypomethylation of human DNA is associated with autoimmunity [77]. Interestingly, ultraviolet light, hydralazine, and procainamide, all known triggers of lupus-like syndromes, inhibit the activity of DNA methyltransferases and induce autoreactive T cell subsets [78]. Experimentally, DNA methylation inhibitors induce lymphocyte function associated antigen (LFA)-1 positive autoreactive T cells that mediate DNA autoantibody production [78]. Individuals with active systemic lupus erythematosus show decreased DNA methyltransferase activity, lower rates of genomic methylated cytosine nucleotides, increased levels of circulating hypomethylated DNA, and increased numbers of autoreactive T-cells that overexpress LFA-1 [78]. Thus, DNA methylation maintains tolerance to self DNA, which can lead to impaired DNA methylation associated with lupus disease activity.

Number of CpG-motifs

Demethylating vertebrate DNA does not result in equivalent stimulatory activity when compared to bacterial DNA [75]. Thus, additional factors exist that block the activation of immunity by self DNA. Comparative genome analysis for the frequency of CpG-motifs in different species revealed that CpG motifs are present in vertebrate genomes at only 20% of random frequency [76], but are over-represented in *E. coli* DNA [75]. Possibly, during evolution, stimulatory CpG motifs were negatively selected in vertebrate genomes and positively selected in bacterial DNA.

Suppressive DNA sequence elements

In search of oligodeoxyribonucleotide (ODN) with optimal stimulatory activity, several groups have detected sequence motifs that can suppress CpG-DNA-induced immunity, for example, in arthritis [79]. Vertebrates and bacteria show considerably different frequencies of suppressive DNA sequences. In mice, such suppressive DNA sequence elements are present at a high frequency whereas these elements are underrepresented in the *E. coli* genome [75]. Thus, the ratio of stimulatory and suppressive sequence elements may determine the immunomodulatory potential of self DNA. This would imply that human DNA has a high number of suppressive sequence elements that neutralize a small number of unmethylated CpG motifs, representing a mechanism to discriminate self DNA from bacterial DNA via TLR9.

If recognition of self DNA is involved in the pathogenesis of lupus, additional amounts of suppressive DNA should reduce autoantibody production and autoimmune tissue injury in experimental lupus [80]. Dong *et al*. [81] used synthetic ODN expressing TTAGGG motifs in the spontaneous lupus model of NZB/NZW mice. Treatment with suppressive ODN improved survival of these mice associated with improved lupus nephritis, proteinuria, and lower serum dsDNA autoantibody levels [81]. This finding was confirmed in $MRL^{lpr/lpr}$ mice, another model of progressive lupus nephritis [82]. As in these studies NZB/NZW mice were not exposed to exogenous CpG-DNA, these data suggest that the suppressive ODN blocked endogenous CpG-DNA-induced immunity. Thus, CpG motifs in self DNA appear to be a pathogenic factor in the progression of established tissue injury in autoimmune mouse strains and possibly in human lupus [83,84]. In order to elucidate the role of TLR9 in the development of lupus, TLR9 deficient mice have to be backcrossed into the appropriate lupus mouse strain. This approach was used in a recent study in which, overt lupus nephritis was reported, despite decreased dsDNA autoantibody production, in TLR9-deficient $MRL^{lpr/lpr}$ mice after two backcrosses [85]. The role of TLR9 in the development of lupus remains unclear and its determination could require data from TLR9-deficient lupus mice that have appropriately backcrossed for at least five generations into their specific genetic background.

Pharmacological blockade of TLR9 signalling

Specific small molecule TLR antagonists are not yet available, although drugs currently in use for the treatment of autoimmune diseases interfere with TLR signalling. For example, chloroquine, an antimalarial drug used to treat milder forms of lupus, inhibits CpG-DNA-induced immunity [86-88]. Chloroquine is a strong base that inhibits endosomal acidification, which is required for the interaction of CpG-DNA with TLR9 [89]. In fact, treatment with chloroquine somewhat reduces mRNA expression of IFN-α-related genes in patients with active lupus [65], which argues for a role of the aforementioned pathways for lupus disease activity. Chloroquine is no TLR9 specific antagonist, however, and may interfere with other endosome-dependent disease mechanisms, for example, TLR7-dependent ssRNA recognition [90,91]

Clinical implications and future perspectives

In view of the aforementioned potential of microbial molecules to trigger or modulate autoimmunity, new hypotheses arise that may influence the management of patients with autoimmune disease.

Concerns about therapeutic use of TLR agonists in patients with autoimmune disease

Mycobacterial vaccine adjuvants have now been identified to ligate TLRs. Experimental studies that exposed rodents with lupus to TLR agonists, for example, experimental autoimmune encephalomyelitis, collagen-induced arthritis, immune complex glomerulonephritis and other types of autoimmune tissue injury, raise considerable concern about the safety of TLR agonists in patients with autoimmune disease [55-57,92]. These studies reported disease aggravation after repeated injections with, for example, CpG-ODN, but side effects of TLR ligands may relate to the dose, treatment intervals, and route of administration [29,93]. Topical application or a single vaccination regimen might have less effects on pre-existing autoimmunity. CpG-DNA is currently in clinical trials for the treatment of cancer, atopy and as vaccine adjuvant [48,93,94]. So far, clinical or serological signs of drug-induced lupus or flares of autoimmunity have not been reported in recently reported trials that applied CpG-DNA as a vaccine adjuvant for vaccination against influenza or hepatitis B virus [93,95,96].

Therapeutic use of TLR antagonists in patients with autoimmune disease

As discussed above, chloroquine already has an established role in the treatment of milder forms of lupus. In addition, suppressive DNA elements may modulate the stimulatory effects of CpG DNA. *In vitro* studies suggest that CpG-DNA-induced activation of B cells, macrophages, or dendritic cells can be blocked with ODN containing suppressive DNA motifs [75,80]. Thus, suppressive ODN may represent a functional antagonist for TLR9 signalling induced by CpG-DNA, a hypothesis supported by our studies with $MRL^{lpr/lpr}$

mice. These findings support a role for TLR9 signalling in the pathogenesis of lupus. Thus, developing specific small molecule TLR9 antagonists may represent a new approach as a preventive therapy for systemic lupus erythematosus.

Conclusion

TLRs are critical receptors for innate pathogen recognition. Their specific role in modulating innate and adaptive immunity also interferes with the mechanisms that maintain tolerance in the host. Thus, TLR ligation can contribute to loss of tolerance by multiple mechanisms. The specific roles of individual signalling pathways for several different autoimmune conditions remain a future challenge in this field. A role for TLR9 in the pathogenesis of lupus is suggested for both infection-induced disease flares as well as for the recognition of CpG motifs in self DNA. Preliminary studies with functional antagonists of TLR signalling suggest that TLRs may provide a new set of potential targets for the treatment of autoimmune diseases.

Competing interests

The author(s) declare that they have no competing interests.

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