Acta Pharmacologica Sinica (2015) 36: 1395–1407
 1671-4083/15
www.nature.com/aps

Review

Toll-like receptors: potential targets for lupus treatment

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by the loss of tolerance to self-nuclear antigens. Accumulating evidence shows that Toll-like receptors (TLRs), previously proven to be critical for host defense, are implicated in the pathogenesis of autoimmune diseases by recognition of self-molecules. Genome-wide association studies, experimental mouse models and clinical sample studies have provided evidence for the involvement of TLRs, including TLR2/4, TLR5, TLR3 and TLR7/8/9, in SLE pathogenesis. A number of downstream proteins in the TLR signaling cascade (such as MyD88, IRAKs and IFN-α) are identified as potential therapeutic targets for SLE treatment. Numerous antagonists targeting TLR signaling, including oligonucleotides, small molecular inhibitors and antibodies, are currently under preclinical studies or clinical trials for SLE treatment. Moreover, the emerging new manipulation of TLR signaling by microRNA (miRNA) regulation shows promise for the future treatment of SLE.

Keywords: systemic lupus erythematosus; autoimmune diseases; Toll-like receptors; TLR antagonists; immune modulatory oligonucleotides; small molecular inhibitors; antibodies; miRNAs

Acta Pharmacologica Sinica (2015) 36: 1395–1407; doi: 10.1038/aps.2015.91; published online 23 Nov 2015

Introduction

Toll-like receptors (TLRs) are a family of evolutionarily conserved, innate immune receptors that play a crucial role in the first-line defense against foreign molecules called pathogen-associated molecular patterns (PAMP)[1, 2]. These receptors are expressed on/in various immunocompetent cells (including macrophages, dendritic cells and B lymphocytes) and some cells with non-immune functions (such as epithelial and mesangial cells of the kidney)[3]. The identified TLRs include cell surface receptors that recognize a variety of bacterial or fungal molecules, including lipopeptide (TLR2), lipopolysaccharide (TLR4) and flagellin (TLR5)[4]. There are also intracellular receptors, which are expressed in the endosomal compartments of cells. The receptors sense nucleic acids, such as double-stranded RNA (TLR3), single-stranded RNA (TLR7 and TLR8) or unmethylated single-stranded DNA containing cytosine–phosphate–guanine (CpG) motifs (TLR9), which are common in viral and bacterial genomes[4].

However, recent findings have revealed that TLRs also recognize and respond to endogenous ligands produced during inflammation or tissue damage[5-12]. Systemic autoimmune diseases are commonly associated with the production of autoantibodies specific for self-determinants[13]. Many of the autoantigens are derived from structures such as chromatin and small nuclear ribonucleoproteins (snRNPs), which are normally sequestered from the immune system by virtue of their intracellular location[14]. However, these nuclear antigens become more accessible for TLRs as a result of cell death or apoptosis. Once engaged, these TLRs act rapidly via adaptor proteins to induce transcription factors for type I IFNs and other pro-inflammatory mediators, which further contribute to the development and progression of autoimmune diseases[15]. Therefore, the identification and characterization of endogenous ligands of TLRs provide a novel perspective for exploring the etiology of autoimmune diseases.

Systemic lupus erythematosus (SLE) is a typical autoimmune disease characterized by the loss of tolerance to self-nuclear antigens[13]. Approximately 40% of SLE patients exhibit defects in the clearance of apoptotic cells, which are removed rapidly by phagocytes in healthy individuals[16]. A lupus murine model showed a defect in the clearance of cellular debris[17]. The inefficient clearance of cellular debris leads to increased release of host DNA and RNA, which can be detected by

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Received 2015-06-23 Accepted 2015-09-08

TLRs and induces the production of autoantibodies[16]. Therefore, targeting TLRs and modulating TLR signaling have emerged as important strategies for the treatment of SLE.

TLRs in SLE

TLRs fulfil many of the criteria required for potential therapeutic targets in SLE. For specific TLRs, evidence includes gene polymorphisms of TLRs contributing to disease susceptibility, overexpression of TLRs in patients and mouse models, TLR knockout mice being resistant to disease and TLR ligands exacerbating and TLR inhibitors alleviating disease. However, different TLRs provide distinct or synergistic contributions to the pathogenesis of SLE.

TLR2 and TLR4

Environmental factors play a role in the onset of SLE. The recognition of these factors is mediated by TLRs, in particular, TLR2 and TLR4, which bind PAMP of $Gram^+$ and $Gram^-$ bacteria, respectively. Although the gene polymorphism of TLR2 (Arg677Trp and Arg753Gln) and TLR4 (Asp299Gly and Thr399 Ile) has no effects on the predisposition and clinical characteristics of SLE[18], the expression levels of *TLR2* and *TLR4* mRNAs in SLE patients' PBMCs are much higher than those in healthy subjects'[19]. Deficiency in TLR4 and, to a lesser extent, in TLR2 down-regulates the production of autoantibodies and attenuates the development of renal injuries in *lpr* mutation-induced mouse lupus[20]. $TLR4^{-/-}$ mice also have decreased pathogenic cytokines, anti-dsDNA and anti-RNP antibodies and attenuated renal injury in pristane-induced experimental lupus[21]. In addition, TLR4 up-regulation at the protein or gene level is a potent trigger to induce lupus-like autoimmune disease[22]. Furthermore, TLR2 and TLR4 may be involved in anti-DNA autoantibody-induced kidney damage in lupus nephritis by recognizing HMGB1 (high mobility group box-1 protein), which binds with DNA and pathogenic anti-DNA autoantibodies and is implicated in the pathogenesis of SLE[9].

TLR5

The chromosomal region lq41-42 contains major susceptibility genes of SLE[23, 24]. Intriguingly, the TLR5 gene maps to chromosome lq41 and contains a common stop codon polymorphism (allele C1174T). Allele 1174C, but not 1174T (with the stop codon), was preferentially transmitted to SLE-affected offspring[25]. Additionally, populations with this stop codon produce reduced levels of pro-inflammatory cytokines, suggesting that the TLR5 stop codon polymorphism is associated with protection from the development of SLE[25]. Other evidence revealed that the expression level of TLR5 mRNA correlates significantly with IFN-α mRNA in the PBMCs of SLE patients[19]. Furthermore, because of its central role in regulating inflammatory pathways, the biological plausibility of TLR5's association with SLE is compelling.

TLR3

TLR3 recognizes double-stranded RNA from viruses and the synthetic mimic Poly I:C[1]. Although it is also intracellularly localized, TLR3 signaling is distinct from the TLR7/8/9 signaling pathways. Rather than utilizing Myeloid differentiation factor 88 (MyD88) as an adaptor protein, TLR3 uses TIR-domain-containing adaptor-inducing interferon-β (TRIF)[26]. In MRL/*lpr* mice, TLR3, TLR7 and TLR9 are expressed by intrarenal macrophages, whereas only TLR3 is expressed by mesangial cells[27]. Additionally, the expression of TLR3 mRNA increases with the progression of glomerulonephritis, and Poly I:C aggravates lupus nephritis through TLR3 on glomerular mesangial cells and APC in MRL/*lpr* mice[27, 28]. Nevertheless, Poly I:C injection does not increase the titer of anti-dsDNA antibodies, and ablation of TLR3 does not inhibit the formation of autoantibodies, suggesting that TLR3 participates in the pathogenesis of SLE in a B cell-independent way[28, 29].

TLR7, TLR9 and TLR8

Production of autoantibodies is the main feature of SLE[13]. Disease-related autoantibodies in SLE focus on particular targets, including DNA-containing antigens, such as dsDNA, and RNA-containing antigens, such as Sm/RNP[14, 30]. A variety of *in vitro* studies in mouse cells show that RNA- and DNA-containing immune complexes, respectively, activate TLR7 and TLR9 through BCR-mediated internalization in B cells or through FcγR-mediated internalization in dendritic cells (DCs)[31–33]. TLR engagement in B cells increases BCR signaling and antibody production, whereas in pDCs, TLR induces IFN-α production, which causes mDCs to release B-cell activating factor (BAFF) and further activates autoreactive B cells[34]. Additionally, upregulated TLR7 and TLR9 mRNA expression has been reported in PBMCs from SLE patients, and the levels correlate with the expression of IFN-α[19, 35].

The link between TLR7 and RNA-associated antibody production is supported by studies of lupus-prone mice harboring the Y-linked autoimmune acceleration (*Yaa*) cluster, which includes a duplication of the *TLR7* gene. The overexpression of *TLR7* is the cause of the autoimmune phenotypes associated with *yaa*[36] and autoreactive B cell responses to RNA-related antigens due to *TLR7* gene replication in male BXSB mice[37]. Ablated TLR7 signaling in the BXSB mouse models results in decreased autoantibody production[38]. IFN-α production in pristane-induced mice is also dependent on TLR7[39]. Murine $TLR7^{-/-}$ pDCs stimulated with U1snRNP/anti-Sm ICs produce markedly reduced levels of IFN-α and IL-6[40]. The overall impact of TLR7 on SLE has also been investigated. MRL/*lpr* mice lacking TLR7 display ameliorated disease manifestation, vanished anti-Sm antibodies, decreased serum IgG and decreased lymphocyte activation[41].

Whereas the pathological role of TLR7 in human SLE and lupus nephritis in mouse models is relatively accepted, the role of TLR9 remains controversial. Multiple mouse studies have shown the indispensable role of TLR9 in B cells for the production of anti-dsDNA, anti-chromatin and anti-nucleosome autoantibodies[29, 41, 42]. However, the deletion of TLR9 in these lupus-prone models does not lead to disease amelioration as predicted but to disease exacerbation, suggesting a protective

role of TLR9 in lupus in mice[41, 42]. In addition, although TLR7 and TLR9 act in parallel on different subsets of autoantibodies, TLR9 suppresses the production of TLR7-dependent, RNA-associated autoantibodies[42, 43]. The proportions of TLR9-expressing B cells, plasma cells and monocytes increase in SLE patients, and the increase in TLR9-expressing B cells is correlated with the production of anti-dsDNA antibodies[44, 45]. Additionally, B cells and monocytes from patients with active disease express higher TLR9 levels compared to patients with inactive disease[44, 46]. Paradoxically, the increased expression of TLR9 does not give rise to stronger responsiveness to TLR9 ligands. Despite increased TLR9 expression in B cells and DCs from patients with severe disease, cells are less activated and are hyporesponsive to ODN-CpG stimulation[47]. Overall, further studies are required to establish the role of TLR9 in mediating the progression of SLE.

TLR8, phylogenetically similar to TLR7, also recognizes single-stranded RNA (ssRNA) and synthetic ligands, such as R848. To date, few studies on the function of TLR8 in SLE have been published. TLR8 may participate in the pathogenesis of SLE by recognition of RNA autoantigens[11]. However, recent studies reveal that TLR8 in dendritic cells restrains TLR7-mediated lupus manifestations in C57BL/6 mice[43]. TLR8 deletion accelerates autoimmunity in lupus-prone mice through a TLR7-dependent mechanism[48]. Moreover, TLR8 contributes to the gender differences in the development of SLE[49].

Other potential targets involved in the TLR signaling cascade

MyD88 is a common adaptor protein present in most TLR signaling[50]. Because both TLR7 and TLR9 utilize this protein, MyD88 is an excellent target to intervene in the abnormal signaling in SLE. MyD88-knockout MRL/*lpr* mice exhibit a prolonged lifespan with no apparent development of autoimmune nephritis in comparison with wild-type mice[51]. Shlomchik's group further investigated the separate pathogenetic mechanism of MyD88 in different cell types, revealing that MyD88 in B cells controls lupus nephritis, whereas MyD88 in DCs is critical for dermatitis in MRL/*lpr* mice[52]. The intracellular tyrosine kinase Lyn mediates the inhibitory receptor function in B cells and myeloid cells, and $Lyn^{-/-}$ mice spontaneously develop an autoimmune and inflammatory disease that closely resembles human SLE. Production of germinal center, antinuclear antibody in Lyn-deficient mice also requires MyD88 signaling in B cells and DCs[53]. Deletion of MyD88 in DCs alone completely reversed the lupus manifestation shown in Lyn-mutant mice[54].

Another potential target for abolishing aberrant TLR signaling in SLE is IL-1R–associated kinases (IRAKs). IRAK1 and IRAK4 function as active kinases and as scaffolds for interacting with MyD88 and TRAF6 in TLR signaling. All TLRs, except TLR3, require IRAKs and MyD88 for signaling[55]. IRAK4-deficient patients and MyD88-deficient patients do not display autoreactive antibodies in their serum and do not develop autoimmune diseases, suggesting that IRAK4 and MyD88 pathway blockades may thwart autoimmunity in humans[56]. An IRAK1/4 kinase inhibitor abrogated the TLR7/9-induced IFN-α responses in both mouse and human pDCs[57].

IFN-α, one of the end-product effectors of TLR signaling, has been an intense research focus in SLE pathogenesis. Genome-wide association studies provide strong genetic evidence that type I IFNs (with IFN-α as the dominant mediator) are important for SLE risk. Of 47 genetic variants associated with SLE, over half (27/47, 57%) can be linked to type I IFN production or signaling[58]. The accumulated data indicate that the levels of IFN-α in circulation are significantly elevated among lupus patients compared with control subjects, and the high levels of IFN-α are associated with worsened measures of disease activity[59–61]. Recombinant IFN-α, when administered as a therapy to patients with malignancy or hepatitis infection, can induce SLE[60]. In addition, deficiency of the type I IFN receptor protects mice from experimental lupus[62, 63].

Potential therapeutic treatment by blocking TLR signaling transduction

Currently, there are diverse agents under development for lupus treatment by targeting TLRs or TLR accessory proteins (including MyD88, IRAK-4 and IFN-α) at different stages of TLR signaling pathways (Figure 1). Here, we focus on a series of agents in the discovery phase or in clinical trials, including oligonucleotides (Table 1), small molecular inhibitors (SMIs) (Table 2), antibodies (Table 3) and new emerging modulators, such as microRNAs, which might offer further possibilities for therapeutic manipulation.

Oligonucleotides

Several TLR ligand mimics that are oligonucleotides that bind to endosomal TLR7 and/or TLR8 and/or TLR9 have been designed and synthesized to treat lupus[64, 65]. Immunoregulatory sequence-954 (IRS-954) is an oligonucleotide-based bifunctional inhibitor of TLR7 and TLR9 and is currently under preclinical research[66]. *In vitro* experiments showed that IRS-954 inhibits the induction of IFN-α by human pDCs in response to DNA/RNA viruses and isolated immune complexes from lupus patients[66]. Preclinical data from animal model studies showed a significant reduction of the serum levels of nucleic acid-specific autoantibodies, as well as decreased proteinuria, reduced glomerulonephritis, end-organ damage and increased survival[67]. The glucocorticoid resistance characteristics of SLE mediated via TLR7 and TLR9 was also reversed by IRS-954[68].

DV-1179, another TLR7/9 dual antagonist developed by the same company that produced IRS-954, is being tested for the treatment of SLE. A phase I study of DV-1179 was conducted to assess its safety and tolerability in healthy volunteers followed by a phase Ib/IIa study of the safety and pharmacodynamics in patients with active SLE. In the SLE study, doses up to 60 mg/week for 8 weeks were well-tolerated, and the most common adverse events were injection site reaction. However, DV-1179 did not achieve the pharmacodynamic endpoints related to reduction in IFN-α-regulated genes[69].

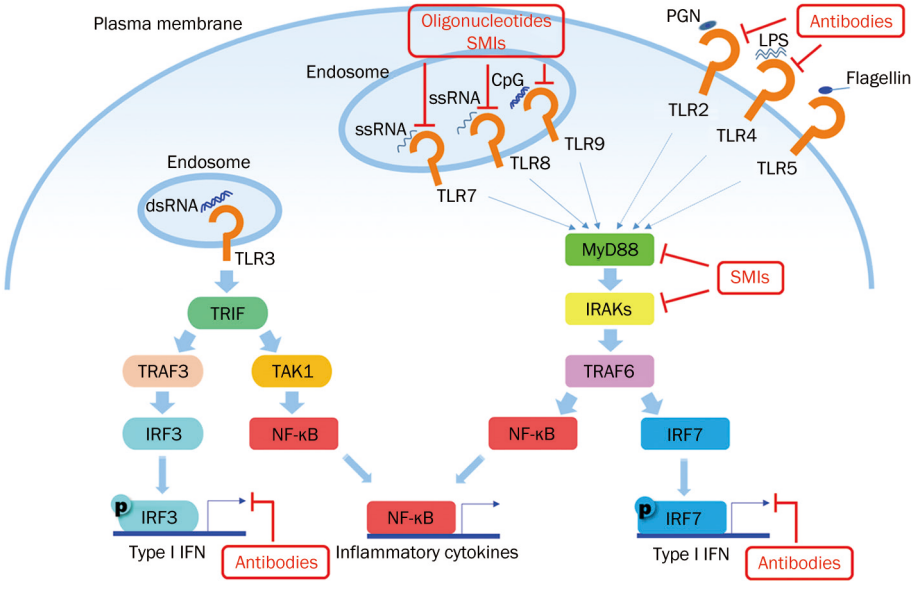


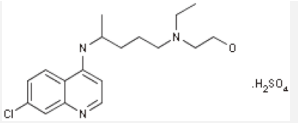
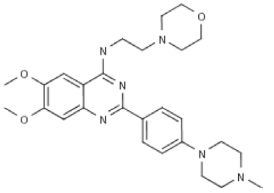
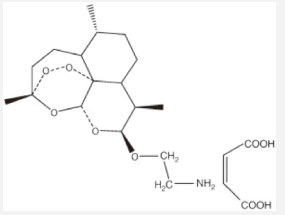
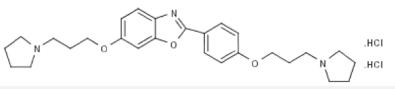
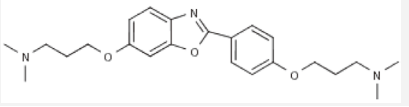
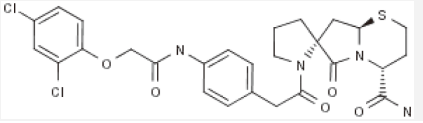
Figure 1. Potential targets involved in TLR signaling pathways for SLE treatment. TLR-mediated responses are controlled mainly in a MyD88-dependent way, which is used by all TLRs except TLR3. In the pathogenesis of SLE, both exogenous ligands from infection and endogenous ligands from apoptotic debris contribute to the activation of TLRs and initiate the downstream signaling cascade, resulting in the production of type I IFN and inflammatory cytokines. A series of potential therapeutics, including oligonucleotides, SMIs and antibodies, modulate TLR signaling pathways at different stages. Some oligonucleotides, SMIs and TLR-neutralized antibodies directly block TLRs; some SMIs target TLR accessory proteins, such as MyD88 and IRAKs. Anti-IFN-α antibodies are also under intensive development for the treatment of SLE. TAK1, transforming growth factor-β-activated kinase; IRF, interferon regulatory factor.

Table 1. Development status of oligonucleotide agents that target TLRs for SLE treatment.

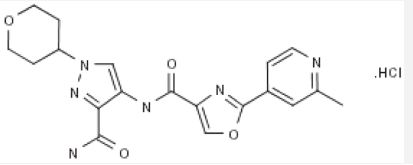
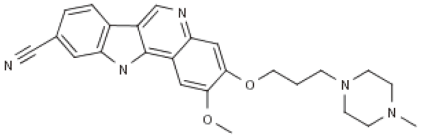
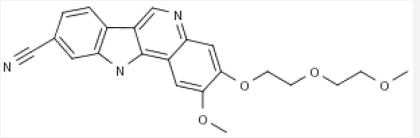
Compound	Target	Sequence	Indication	Clinical phase	Dosage regimen	Ref
IRS-954	TLR7 and TLR9 antagonist	5'-TGCTCCTGG AGGGGTTGT-3'	SLE	Preclinical	15 and 45 μg, twice/week, sc (NZB/W F1 mice)	[66-68]
DV-1179	TLR7 and TLR9 antagonist	_	SLE	Phase I/II	≤60 mg/week, sc (patients, tolerability study)	[69]
IMO-3100	TLR7 and TLR9 antagonist	_	Multiple sclerosis; Plaque psoriasis; Rheumatoid arthritis; SLE	Phase II	0.16 and 0.32 mg/kg, once/week, sc (patients)	[70, 71]
IMO-8400	TLR7, TLR8 and TLR9 antagonist	_	Graft-versus-host Disease; Arthritis; Sjogren's syndrome; Plaque psoriasis; SLE	Phase II	0.3 and 0.6 mg/kg, once/week, sc (patients)	[72]
IRO-5	TLR9 antagonist	5'-CTATCTGAC	SLE	Biological	100 μg, once/week, sc (MRL/*lpr* mice)	[73]
		GTTCTCTGT-3'		Testing	300 μg, once/2 week, sc (NZB/W F1 mice)	
INH-ODN-24888	TLR7 and TLR9 antagonist	5'-TCCTGGCG GGGAAGT-3'	SLE	Preclinical	_	[74, 75]

A number of oligonucleotides-based immune modulatory oligonucleotides (IMO) are also in the pipeline for use in autoimmune disease treatment. IMO-3100, a TLR7 and TLR9 antagonist, is in phase II clinical trials for the treatment of psoriasis. The company evaluated the compound pre-clinically for SLE treatment. IMO-3100 inhibits TLR-induced increases in the gene expression of TNF-α, IFN-α and IL-17 in human PBMCs and inhibits disease development in lupus-prone NZBW/F1 mice[70, 71]. However, no recent development has been reported for this research. IMO-8400, which antagonizes TLR7, TLR8 and TLR9, has shown efficacy in mouse models of lupus[72, 73]. Early clinical trials are now underway for the

Table 2. Development status of SMIs that target TLR signaling pathway for SLE treatment.

Compound	Target	Chemical structure	Indication	Clinical phase	Dosage regimen	Ref
HCQ	TLR7, TLR8 and TLR9 antagonist		Malaria; Rheumatoid arthritis; SLE	Launched-1956	200–400 mg, once daily, *po* (patients)	[76-81]
CpG-52364	TLR7, TLR8 and TLR9 antagonist		Rheumatoid arthritis; SLE	Phase I	1–100 mg, *po* (healthy objects, safety and tolerability study) 2.5 and 5 mg/kg, ig (MRL/*lpr* mice);	[82, 83]
SM934	TLR7/9 signal transduction modulator		Autoimmune diseases; SLE	IND Filed[1]	10 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$, ig (NZB/W F1 mice) 20 and 60 mg/kg, ig (MRL/*lpr* mice)	[84-87]
E-6446	TLR7, TLR9, and IL-6 antagonist		Antimalarials; Sepsis; Multiple sclerosis; Rheumatoid arthritis; SLE	Preclinical	20 mg/kg, ig (BALB/c mice)	[88]
AT-791	TLR7, TLR9, and IL-6 antagonist		Multiple sclerosis; Sepsis; Rheumatoid arthritis; SLE	Preclinical	–	[88]
VTX-763	TLR8 antagonist	–	Inflammation; Autoimmune diseases	Preclinical	–	–
TMX-302	TLR7 antagonist	–	Antipsoriatics; Autoimmune diseases	Preclinical	5 and 10 mg/kg, once/week, sc (MRL/*lpr* mice);	–
IMO-9200	TLR7, TLR8 and TLR9 antagonist	–	Autoimmune diseases	Phase I	1.5 and 9 mg/kg, once/2 week, sc (monkeys)	[89, 90]
ST-2825	MyD88 dimerization inhibitor		Cancer; Viral infection; Transplant rejection; Arthritis; Autoimmune disease	Biological testing	100 and 200 mg/kg, ig (C57BL/6 mice) 0.3–3 mg/kg, bid, ig (5/6 Nx	[91, 92]

(To be continued)

Compound	Target	Chemical structure	Indication	Clinical phase	Dosage regimen	Ref
AS-2444697	IRAK4 inhibitor		Arthritis; Gout; Sepsis; SLE	Preclinical	rats)	[93]
PF-05387252	IRAK4 inhibitor		Rheumatoid arthritis; SLE	Preclinical	–	–
PF-05388169	IRAK4 inhibitor		Rheumatoid arthritis; SLE	Preclinical	–	–
PF-06650833	IRAK4 inhibitor	–	SLE	Phase I	1–1000 mg of immediate release 10–500 mg of modified release (healthy objects, safety and tolerability study)	[94]
DZ2002	TLR signal transduction modulator		SLE	Preclinical	50 mg·kg^{-1}·d^{-1}, ip (BXSB mice); 50 mg·kg^{-1}·d^{-1}, ip (MRL/*lpr* mice); 0.5 mg·kg^{-1}·d^{-1}, ig (NZB/W F1 mice)	[95, 96]

[1]IND, Investigational New Drug Application

Table 3. Development status of antibody agents targeting TLR signaling pathway for SLE treatment.

Compound	Target	Description	Indication	Clinical phase	Dosage regimen	Ref
OPN-305	TLR2 antagonist	Humanized monoclonal IgG4κ antibody targeting TLR2	Myelodysplastic syndrome; Transplant rejection; Autoimmune diseases	Phase II	1.5 mg/kg, iv (patients, efficacy and safety study)	[97, 98]
NI-0101	TLR4 antagonist	Humanized monoclonal antibody binding to human TLR4	Inflammation; Autoimmune disorders	Phase I	≤15 mg/kg, iv (healthy objects, tolerability study)	[99]
Mab 9F3	Anti-IFN-α	Murine monoclonal antibody that neutralizes human IFN-α	Type 1 diabetes; SLE	Biological testing	–	–
13H5	Anti-IFN-α	Human monoclonal IgG1 antibody against human IFN-α	Transplant rejection; SLE	Biological testing	–	–
ACO-1	Anti-IFN-α	IgG2a murine monoclonal antibody against IFN-α	Psoriasis; SLE	Biological testing	–	–
AGS-009 (NNC-0152-0000-0001)	Anti-IFN-α	Humanized monoclonal IgG4 antibody targeting human IFN-α	SLE	Phase I	0.01–30 mg/kg, iv (patients, tolerability study)	[105]
Rontalizumab (PRO-155767)	Anti-IFN-α	Recombinant humanized monoclonal antibody targeting human IFN-α	SLE	Phase II (discontinued)	750 mg, once/4 week, iv or 300 mg, once/2 week, sc (patients, efficacy and safety study)	[100]
Sifalimumab (MEDI-545; MDX-1103)	Anti-IFN-α	Full human monoclonal IgG1 antibody targeting human IFN-α	Inflammatory myopathy; Myositis; Polymyositis; Psoriasis; SLE	Phase II	200, 600 and 1200 mg, once/2 week, iv (patients, efficacy and safety study)	[101-104]
Anifrolumab (MEDI-546; MDX-1333)	Anti-IFN α receptor 1	Full human monoclonal IgG1κ antibody targeting the type I IFN receptor 1	Scleroderma; SLE	Phase II/III	100, 300 and 1000 mg, once/4 week, iv (patients, efficacy and safety study)	[106, 107]

treatment of SLE.

At the end of 2013, preclinical research on INH-ODN-24888, a guanine-modified inhibitory oligonucleotide (INH-ODN) derived from INH-ODN-2088, was initiated for the treatment of lupus based on activity as a TLR7 and TLR9 antagonist. The guanine modification of INH-ODN potentiates the suppressive function of its prototypic agent and efficiently impairs the TLR7- and TLR9-mediated immune responses of human immune cells[74, 75]. Thus, INH-ODN-24888 represents a promising therapeutic agent for the treatment of SLE.

Small molecular inhibitors (SMIs)

Small molecule inhibitors (SMIs) can be taken orally and are promising agents to penetrate the cell membrane, effectively targeting endosomal TLRs and downstream signaling proteins. Thus, SMIs have been designed to treat SLE based on multiple targets in the TLR signaling pathway, including TLRs, MyD88 and IRAKs.

In the treatment of SLE, antimalarial drugs, such as hydroxychloroquine sulfate (HCQ), chloroquine and quinacrine, have been used clinically since 1956 and are now known to act as TLR7/8/9 antagonists. Their suppression of endosomal TLR activation was attributed to the inhibition of endosomal acidification, which is a prerequisite for the activation of these receptors[76–78]. Recently, a new mechanistic model of these antimalarial drugs has proposed that they directly interact with nucleic acids and consequently cause structural modifications of the TLR ligand to prevent the ligand from binding to TLR[79–81].

The quinazoline derivative CpG-52364 is a specific SMI of TLR7/8/9. CpG-52364 was designed to interfere at an early stage of the immune cascade by blocking the inappropriate immune activation of all the three TLRs and to treat the underlying cause of the disease without causing general suppression of immune function. CpG-52364 has been proved to be safer and more efficacious than HCQ in preclinical animal studies[82, 83], and it is currently in phase I clinical trials for the oral treatment of SLE.

A novel artemisinin analogue, β-aminoarteether maleate (SM934), has recently been authorized by the Chinese SFDA to enter clinical trials for SLE treatment. In preclinical study, SM934 exhibited significant protective effects on both MRL/*lpr* mice and NZBW/F1 mice[84–86]. The therapeutic mechanism of SM934 involves inhibiting TLR7/9-triggered B cell activation in a MyD88-dependent way[87]. Since 1956, no new chemical drug for SLE treatment has reached the market in the USA, and no original class I chemical drugs have been developed in China. Therefore, the clinical study of SMI SM934 could be a milestone in the development of lupus treatment drugs in China.

In addition, E-6446 and AT-791, targeting TLR7/9 and IL-6, were designed for the treatment of SLE[88]. However, research on the two SMIs was suspended at the preclinical stage. The TLR8-specific antagonist VTX-763 and the TLR7-specific antagonist TMX-302 are both under active preclinical research for autoimmune disease therapy. IMO-9200, is under phase I clinical trials as a small molecule poly-inhibitor of TLR7/8/9 to treat autoimmune diseases. However, greater therapeutic effects are reported in the treatment of rheumatoid arthritis (RA)[89, 90].

A series of peptido-mimetic compounds was synthesized as MyD88 dimerization inhibitors, including ST-2825, ST-3324, ST-2928, ST-2797, ST-2804, ST-2807, ST-2565 and ST-3375, among which ST-2825 was the most effective. ST-2825 interferes with the recruitment of IRAK4 and IRAK1 via MyD88, causing inhibition of pro-inflammatory factor overproduction[91]. In human PBMCs, ST2825 suppressed B cell proliferation and differentiation into plasma cells in response to CpG-induced activation of TLR9[91]. ST-2825 also blocks autoantibody production in B cells from SLE patients[92].

Given the critical role of IRAK-4 in inflammatory processes, modulation of IRAK-4 kinase activity presents an attractive therapeutic approach for the treatment of SLE. Several IRAK-4 inhibitors have been identified, among which PF-05387252, PF-05388169 and AS-2444697 are in preclinical studies and PF-06650833 is in phase I clinical studies[93, 94].

Abnormal post-translational protein modifications involved in TLR signaling also play a significant role in SLE pathogenesis. We and others have reported DZ2002, a reversible SAHH (S-adenosyl-l-homocysteine hydrolase) inhibitor, as a candidate compound for lupus treatment[95, 96]. In our studies, DZ2002 showed significant therapeutic effects on lupus-prone NZB/W F1 mice via interference with the TLR-mediated APC response[95]. Similarly, Lawson's group demonstrated that DZ2002 could prevent lupus-like disease from developing in both BXSB and MRL/*lpr* mouse models by reducing the TLR-induced activation of immune cells[96]. However, the exact target protein of DZ2002 still requires further exploration.

Antibodies

Several antibodies have been designed to block ligands from binding to their respective TLRs. OPN-305, under phase II clinical research, is the first humanized IgG4 monoclonal antibody against TLR2 in development and is intended for the prevention of reperfusion injury following renal transplantation, among other indications[97]. Recent data suggest a potential role of OPN-305 for TLR2 in SLE[98].

Similarly, TLR4 antibodies are being developed to block the excessive immune responses associated with autoimmune diseases, among which NI-0101 is the most advanced under phase I clinical research. NI-0101 is capable of binding an epitope on TLR4, thereby inhibiting TLR4 dimerization and reducing pro-inflammatory cytokine production[99].

IFN-α signaling may be suppressed by various strategies: direct neutralization by an anti-IFN-α antibody or suppression of the IFN-α signature using an anti-IFN-α receptor antibody. Although a number of projects aiming to develop antibody agents targeting IFN-α have been launched, only a few of the projects are still under active development. With the discontinued development of MAb 9F3, 13H5, ACO-1 and Rontalizumab[100], two anti-IFN-α monoclonal antibodies (mAbs), Sifalimumab (MEDI-545) and AGS-009, have achieved a safe

and dose-dependent reduction of the IFN-α signature in their clinical trials for SLE treatment[101–103]. Sifalimumab is a fully human mAb in phase II clinical trials for the treatment of SLE. Preclinical data indicate that the levels of IFN-α are elevated in many patients with active SLE[61]. Sifalimumab binds to IFN-α and has been shown to neutralize its activity in previous studies[104]. AGS-009 is a humanized IgG4 mAb for the intravenous treatment of mild to moderate SLE, and it has recently completed a phase I clinical trial. The results announced at the 2012 EULAR Congress show that patients receiving the drug trend toward normal IFN-α signatures after only a single dose, whereas none of the patients receiving a placebo show a similar shift[105].

Anifrolumab (MEDI-546), a fully human mAb against huIFNAR, is now in phase II/III clinical trials for the treatment of SLE. The safety, tolerability, pharmacokinetics, and pharmacodynamics of intravenously administered Anifrolumab in patients with active SLE were assessed in a phase II, open-label, dose-escalation study conducted on 17 Japanese patients. This study exhibited promising results, indicating that Anifrolumab causes increased and more sustained suppression of the IFN gene signature (IFNGS) target compared to Sifalimumab[106, 107].

MicroRNAs

MicroRNAs (miRNAs) are small, non-coding regulatory RNAs of 21–25 nucleotides in length. miRNAs work as post-transcriptional regulators by binding to a target sequence located within the 3′ UTR of the mRNA, promoting degradation of the mRNA or inhibiting translation of the mRNA. Accumulating studies provide evidence for the connection between the dysregulated miRNA network and excessively activated TLR signaling in SLE, indicating the potential of miRNA as a novel SLE therapeutic[108–110]. In this review, we briefly summarize pilot miRNAs involved in SLE therapy.

MiR-146a, targeting TRAF6 and IRAK1, is a negative regulator in autoimmunity[111]. Mice deficient in miR-146a manifest severe autoimmune phenotypes with elevated autoantibodies, splenomegaly and lymphadenopathy[112]. Restoring the loss of miR-146a using an MS2 VLP-based delivery system was effective in eliminating the production of autoantibodies and ameliorating SLE progression in lupus-prone mice[113]. miR-146a negatively regulates abnormal production of type I IFN in human lupus by targeting key proteins in the TLR signaling pathway[111, 114]. miR-155 can be induced by a number of immune cell stimuli, including TLR ligands. Further studies found that several TLR ligands up-regulate miR-155 expression by either MyD88 or TRIF signaling, uncovering the therapeutic potential of miR-155 in SLE[115, 116].

Benefiting from miRNA's fine tuning of gene expression, the potential miRNA therapeutics should be more effective and safer than traditional approaches. Several companies are developing approaches to mimic or block the miRNA regulation of its targeting mRNA, resulting in the design of so-called agomirs or antagomirs. These approaches, if used to target TLR-relating miRNAs, are likely to be successful in the treatment of SLE.

Perspectives

SLE is a polygenic disease with a high degree of heterogeneity in clinical manifestations; therefore, drug development within this area has been challenging and has a high rate of attrition. Currently, the existing therapeutics revolve around non-steroidal anti-inflammatory drugs, antimalarials, steroids, immunosuppressives and biologics. However, several critical defects of those traditional drugs, such as modest therapeutic effects, severe side effects and extremely high costs, necessitate the development of novel therapeutic agents. Further exploration of the exact role of TLRs in SLE will aid in the development of improved therapeutics. We anticipate that new modulators targeting TLR signaling could alleviate disease severity with minimal side effects and lighten the financial burden of SLE patients. Although it is unlikely that all of the therapeutic agents currently under development will progress to approval for marketing, many show promise in clinical trials and will hopefully continue to progress along the approval process. The emergence of novel regulatory molecules, such as miRNAs, presents encouraging new opportunities for drug discovery and development in SLE treatment.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (No 81273524, 81273525, and 81322049) and the National Key Basic Research Program (973 Program, 2014CB541906).

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