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Toll-like receptor driven B cell activation in the induction of systemic autoimmunity

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Abstract

Studies over the past decade have demonstrated a key role for pattern recognition receptors in the activation of autoreactive B cells. Self reactive B cells that manage to escape negative selection often express relatively low affinity receptors for self antigens (ignorant B cells), and can only be activated by integrating a relatively weak BCR signal with signals from additional receptors. Members of the toll-like receptor (TLR) gene family, and especially the nucleic acid binding receptors TLR 7, 8 and 9, appear to play a key role in this regard and promote the production of autoantibodies reactive with DNA- or RNA-associated autoantigens. These autoantibodies are able to form immune complexes with soluble or cell-bound ligands, and these immune complexes can in turn activate a second round of proinflammatory cells that further contribute to the autoimmune disease process. Recent data have emerged showing a pathogenic role for TLR7, with an opposing, protective role for TLR9. Targeting these disregulated pathways offers a therapeutic opportunity to treat autoimmune diseases without crippling the entire immune system. Further understanding of the role of specific receptors, cell subsets, and inhibitory signals that govern these TLR-associated pathways will enable future therapeutics to be tailored to specific categories of autoimmune disease.

Keywords

TLR; SLE; B cell; autoimmune disease

1.1 Introduction

Systemic autoimmune diseases are frequently associated with the production of autoantibodies reactive with nuclear or cytosolic cellular components. These autoantibodies play an important role in the pathogenesis of various autoimmune diseases, as they can form immune complexes (ICs) that can deposit in the kidneys, skin, or vessel endothelium driving local pathology. Antibodies can contribute to disease pathogenesis through a variety of mechanisms, including complement fixation, engagement of activating Fc receptors, and direct cell activation. Each autoimmune disease is associated with a particular spectrum of autoantibody specificities. For example, Systemic Lupus Erythematosis (SLE) is frequently

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associated with anti-chromatin or anti-SmRNP antibodies [1,2], while antibodies reactive with centromeric or nucleolar components are found in patients with Systemic Sclerosis (SSc), a systemic connective tissue disease characterized by vascular damage and fibrosis [3]. Sjögren's syndrome (SjS), an autoimmune disease associated with salivary pathology, is characterized by antibodies against the antigens SSA (Ro), and SSB (La) [4]. The availability of autoantigens may drive the autoantibody repertoire, as described in the autoimmune myopathy polymyositis (PM): striated muscle has above normal levels of histidine [5], and the autoantibody frequently seen in PM, anti-Jo-1, is directed against histidine-tRNA synthase, an enzyme preferentially expressed by regenerating muscle cells [6]. Even though these autoimmune diseases are quite diverse in their targets and pathology, many of the key autoantigens are part of larger macromolecular complexes associated with either DNA or RNA. It is now known that the nucleic acid components are of central importance to immune activation in systemic autoimmune disease through the activation of Toll-like receptors (TLRs) [7]. The activation of autoreactive B cells with low affinity receptors for DNA- or RNA-associated autoantigens is promoted by a second signal emanating from the nucleic acid engagement of TLR9 or TLR7 (or perhaps TLR3 or TLR8).

Accumulation of cellular debris derived from dying cells is likely to be the source material for the activation of autoreactive B cells and subsequent production of autoantibodies. Patients with SLE have an increased rate of lymphocyte apoptosis [8], and patient macrophages are less efficient at phagocytosing apoptotic cells [9]. Nucleosomes, a frequent antigen target for autoantibodies, become exposed on the surface of apoptotic blebs [10], and along with other autoantigenic moieties, are released from the cells as apoptotic bodies [11]. A range of intracellular autoantigens that are normally sequestered inside the cell become differentially exposed on dying cells as apoptosis progresses [12,13].

To better understand the capacity of mammalian DNA and RNA to engage TLR9 and TLR7 effectively and how such responses can be regulated, our lab has relied on mouse strains expressing the AM14 BCR, a low affinity receptor for autologous IgG2a [14,15]. The AM14 receptor was derived from a lupus-prone Fas-deficient mouse, in which the IgG2a reactivity is a common specificity of the autoantibody repertoire. These cells have provided a very useful experimental tool, as they have enabled us to evaluate efficiently the stimulatory capacity of immune complexes that incorporate either (undefined) cell debris or defined DNA or RNA fragments.

1.2 TLR detection of mammalian nucleic acids

TLRs all share certain structural features including a recognition domain made up of leucine rich repeats, a transmembrane domain and a cytoplasmic TIR domain [16]. TLRs 3, 7, 8 and 9 are all located intracellularly and are of particular interest because of their recognition of nucleic acids, specifically double-stranded RNA (TLR3), single-stranded RNA (TLRs 7 and 8) and DNA (TLR9) [17]. They are directed to an endolysosomal compartment by the chaperone protein UNC93B [18,19]. Murine B cells with a loss of function mutation in UNC93B fail to respond to TLR3, TLR7, and TLR9 ligands, and B cells from patients with active SLE have increased levels of UNC93B [20,21]. Disregulation of TLR responses directly in B cells is likely to be an important factor in the pathogenesis of autoantibody driven autoimmune diseases.

TLR9 was originally shown to distinguish bacterial DNA from mammalian DNA, based on the presence of hypomethylated CpG motifs; cytosine methylation rendered bacterial DNA significantly less stimulatory [17]. By contrast, mammalian DNA was considered a poor TLR9 ligand, because mammalian DNA contains relatively few CG dinucleotides; most of the CpGs are methylated, and mammalian DNA also contains inhibitory motifs [22].

However, our lab and others have shown that both autoreactive B cells and other antigen presenting cells can be activated by DNA-associated complexes through a mechanism dependent on TLR9, and therefore, TLR9 under certain circumstances can detect immunostimulatory endogenous DNA [15]. Our initial studies involved IgG2a-reactive B cells from the AM14 line, stimulated with prototypic autoantibodies that bound cell debris of an undefined nature, always present in cultures of primary B cells. To address directly the importance of DNA sequence in activation, we used IgG2a antibodies specific for either TNP or biotin to form complexes with defined dsDNA fragments that incorporated either haptenated or biotinylated bases. These studies demonstrated that hypomethyated CG content of the DNA was a major factor for the capacity of mammalian DNA to activate AM14 B cells through TLR9 [23]. A more detailed analysis demonstrated that CpG islands, stretches of DNA in mammalian genomes that are GC rich and not depleted for CG dinucleotides, are endogenous TLR9 ligands able to activate autoreactive B cells when delivered by the BCR to the TLR9 containing compartment. By contrast, comparably sized ICs incorporating dsDNA fragments more representative of the CG content of total genomic DNA exhibited little if any activity [24].

There is a similar, but less clearly defined case for the specificity of TLR7. TLR7 has been shown preferentially to detect uridine-rich RNA [25]. The presence of modifications, such as methylation and pseudouridines can silence the TLR7 response, and RNAs with fewer modifications, such as bacterial or mitochondrial RNA, are more stimulatory [26]. Other potential physiologically relevant sources of uridine-rich RNA include viral leader RNA [27], small nuclear RNAs associated with small nuclear (sn)RNPs [28,29], or transcripts from small, highly repetitive, retrotransposed sequences (SINEs) [30]. Our group showed that both spontaneous RNA ICs and sm/RNP ICs can activate RF B cells in a TLR7 and RNA dependent manner [31]. Moreover, ICs formed from SLE patient sera and U1 snRNA complexes induce human PBMCs to produce type I IFN [32]. Our lab has also tested ICs incorporating a panel of RNA fragments, known to be associated with common autoantigens, for their capacity to activate AM14 B cells; many of these RNAs stimulate through a TLR7 dependent pathway, while a subset stimulate through a TLR7 independent pathway (Green NM, unpublished observation). Another potential source of endogenous TLR7 ligands are microRNAs. In fact, ago 2, a protein known to bind the single-stranded RNA component of the Dicer complex, is the autoantigen Su, commonly targeted in the TLR7-dependent pristane-induced model of SLE [33].

In addition to the recognition of endogenous single-stranded RNA by TLR7, it has been suggested that the secondary structure of certain endogenous RNAs with extensive double-stranded regions could be recognized by TLR3 [34]. The significance of the role of TLR3 for B cell activation is unclear, since TLR3 expression is low on most murine B cells [35]. However, TLR3 expression can be induced by cytokines such as type I IFN, or it may be expressed by particular B cell subsets. Naïve and memory human tonsillar B cells do not express TLR3, but CD138 positive (plasmablast/plasma) cells do, and the CD138 positive B cells secrete antibody in response to the TLR3 ligand polyinosinic-polycytidylic acid (poly(I:C)) [36]. Murine splenic B cells respond to the TLR3 ligands poly(I:C) and polyinosinic acid in a manner dependent on the adaptor molecule TRIF [37]. Limited expression of particular RNA receptors could be a mechanism to prevent the inappropriate activation of B cells in the absence of T cell help or an inflammatory environment. B cells reactive to endogenous proteins associated with RNA that has extensive secondary structure, such as the Jo-1 antigen associated with histidine-tRNA [6], may depend on a signal from TLR3 to become activated.

1.3 The role of type I IFN in the activation of autoreactive B cells

SLE patients have been found to have elevated serum levels of IFN-α [38]. Studies of patients receiving IFN-α therapy for chronic hepatitis C virus infections or for malignant midgut carcinoid tumors, show that between 4% and 19% of patients develop some sort of autoimmune disease, including 13 cases of an ANA-positive SLE-like syndrome [39]. SLE patients also express elevated levels of many genes known to be regulated by type I IFN, referred to as a type I IFN signature [40,41]. This IFN signature in SLE patients correlates with class switched autoantibody titers to nucleic acid containing autoantigens, such as U1-RNP, Ro, and La, whereas low IFN signature SLE patients had elevated IgM autoantibodies [42]. IFN-α can act indirectly by making cells more susceptible to apoptosis by causing the upregulation of death receptors (e.g. Fas, TRAIL) or directly through increasing expression of direct mediators of apoptosis, such as XAF-1 (an inhibitor of an inhibitor of apoptosis (IAP)) or caspase-8 and -4 [43], which could increase the availability of dying cells to the immune system. It has also been shown that TLR7 and IFN-α act together to promote class switch to the IgG2a isotype [44]. IgG2a and IgG2b are pathogenic isotypes in autoimmune disease, probably because of their interaction with the activating Fc receptor FcγRIV [45].

Our lab has shown that the response of autoreactive B cells to RNA-associated autoantigens and modestly stimulatory DNA ICs are enhanced by IFN-α. Part of this effect is due to an IFN- α dependent increase in BCR signaling [24,31]. Type I IFN priming also upregulates TLR7 in murine B cells [46] and strongly promotes B cell responses to TLR7 ligands. Naïve B cells respond well to experimental TLR7 ligands, however, naïve B cells that lack a type I IFN receptor fail to proliferate or produce cytokine under the same conditions. This is because TLR7 ligands normally induce B cells to produce their own type I IFN. It follows that IFN-β deficient B cells are also unable to mount normal responses to TLR7 ligands without the addition of an exogenous source of type I IFN [46]. The dependence on type I IFN for robust TLR7 responses is seen in human B cells as well, where naïve human B cells have a very weak response to TLR7 ligands, unless they are provided with an exogenous source of type I IFN [47].

Plasmacytoid dendritic cells (pDCs) play an important role in SLE because they produce type I IFN in response to ICs incorporating endogenous TLR9 and TLR7 ligands. IgG from SLE patients with high SmD titers stimulates DCs to make IFN-α in a manner that is dependent on TLR7 and RNA [48]. pDCs can further amplify B cell responses, because IFN-α can induce other DCs subsets as well as monocytes to produce the B cell survival and differentiation factors BLyS and April [49]. These factors can act in concert with TLR9-mediated upregulation of the BLyS receptor transmembrane activator calcium modulator and cyclophilin ligand interactor (TACI) to support the activation and survival of autoreactive B cells [50]. Overexpression of BLyS leads to increased numbers of B cells and an autoimmune phenotype [51]. Therefore, type I IFNs promote autoantibody production through a variety of mechanisms.

Nevertheless, additional factors can promote autoantibody production independently of type I IFNs. For example, B cells can also be modulated by the inhibitory Fc receptor, FcyRIIb: FcyRIIb-deficient mice on the C57BL/6 background develop autoantibodies [52]; B cells from humans with a SLE-associated mutation in FcyRIIb are hyperresponsive to intact anti-IgM [53]; and memory B cells from SLE patient fail to upregulate FcgRII expression [54]. We found that FcyRIIb deficiency did not affect the AM14 B cell response to ICs incorporating strong TLR9 ligands such as CG-rich dsDNA fragments, but did result in enhanced responses to ICs containing weakly stimulatory CG-poor DNA [55]. An even more dramatic effect of FcgRII deficiency was observed when AM4 B cells were stimulated with RNA ICs. The absence of FcyRIIb essentially bypassed the requirement for exogenous

type I IFN priming for the RNA ICs. Perhaps the increased sensitivity to inhibition points to tighter regulation of a potentially more dangerous response.

1.4 Role of TLRs for *in vivo* autoantibody production

The *in vivo* relevance of TLRs for the production of autoantibodies in autoimmune prone mouse strains has now been shown in multiple models (summarized in Table 1). Our group initially showed that MyD88, an adaptor molecule downstream of most of the TLR family members, was necessary for the *in vivo* production of autoantibodies in autoimmune prone Fas-deficient mice [31]. In contrast to the MyD88-sufficient littermates, the MyD88 deficient mice were almost completely ANA negative and had significantly reduced titers of anti-SmD antibodies [56]. This result has been confirmed in extensively backcrossed cohorts of multiple autoimmune-prone phenotypes, including strains that develop SLE-like disease as a result of dysregulated receptor signaling components ($Lyn^{-/-}$)[57] or elevated B cell survival factor (BLyS Tg) [58]. In general, these mice exhibited dramatically reduced autoantibody and circulating IgG titers, decreased isotype switching to IgG2a, less severe renal disease, and improved survival. These studies were all consistent with the premise that TLR signaling is involved in the production of autoantibodies, but MyD88 is also downstream of the IL-1R family as well as BAFF, and therefore the data could not rule out a role for a non-TLR component [59,60]. More recent studies involving both Unc93b-deficient and double TLR7/TLR9 KO mice have therefore provided important confirmation of a key role for the nucleic acid binding receptors. Not only do these mice also develop minimal autoantibody titers, but also they are dramatically improved by numerous criteria including much less severe renal disease and improved survival [56,61].

The relative importance of TLR7 versus TLR9 in the overall disease process has been addressed by a number of labs. As predicted from the *in vitro* data, deletion of TLR7 in MRL/lpr mice did not reduce anti-dsDNA titers as detected by immunofluorescent staining of HEp2 cells or crithidia. However, the titers of autoantibodies reactive with a variety of RNA-associated autoantigens were dramatically reduced [62]. Similar results were obtained in mice injected with the hydrocarbon oil 2,6,10,14-tetramethylpentadecane (TMPD; also known as pristane); pristane induces a sterile injury in the mice that leads to the production of IgG autoantibodies, including anti-RNPs and anti-Su [33]. Both TLR7-deficient MRL/lpr and TLR7-deficient pristane treated normal mice had significant reductions in serum IgG2a and IgG3 and in composite renal disease scores. 564 Tg mice express a BCR reactive with an RNA associated autoantigen and these B cells become spontaneously activated through a TLR7-dependent process [63]. The importance of TLR7 in SLE was further revealed by the discovery that the genetic element known as the Y-linked autoimmune accelerator (Yaa), initially described in the BXSB strain, was due to the duplication of a genetic interval that included TLR7 [64,65].

By contrast, TLR9-deficiency has been shown to have the opposite effect, again in multiple animal models of SLE. In patient populations, anti-dsDNA antibodies are considered the hallmark of SLE and an important parameter of disease severity. As predicted by the *in vitro* studies, TLR9-deficient autoimmune prone lines, including MRL/lpr, B6/lpr, Ali5 (driven by hyperactivation due to a gain of function mutation in Plcγ2), B6.Nba2 (containing the major lupus susceptibility locus from the NZB model), and B6.Nba2.Yaa, generally have dramatically reduced dsDNA/chromatin titers, as determined by homogeneous nuclear staining and/or nucleosome-specific ELISA readouts [66-69]. By contrast, several labs reported elevated DNA titers as measured by DNA ELISA; the discrepancy between these ELISA data and ANA staining patterns suggests that the “DNA” ELISAs are likely to be detecting either ssDNA or other autoantibody specificities. Moreover, as also predicted from the *in vitro* data, the TLR9-deficient autoimmune-prone mice continued to produce and

made exceedingly high titers of antibodies against a wide range of RNA-associated autoantigens, including SmD, RNP, and nucleolar components. Sera from TLR9$^{-/-}$ mice showed a much stronger cytoplasmic staining pattern, as detected by immunfluorescence, than their TLR9^{+} littermates. Most importantly, by many criteria the TLR9-deficient mice developed more severe clinical disease, including more extensive renal pathology and decreased survival, compared to the control group.

Intriguingly, the TLR9-deficient autoimmune prone mice had routinely detectable levels of circulating IFNa in their serum [67]. Exactly why TLR9-deficiency leads to exacerbated disease outcome is not clear, especially considering the historical association in patient populations between anti-DNA titers and SLE. It has been shown that TLR7 expression is increased in B cells and pDCs of TLR9 deficient, autoimmune mice, and that B cell proliferation is higher in the absence of TLR9 [69]. This is consistent with overexpression systems, which suggested that TLR9 could inhibit TLR7, but not vice-versa [70], or that TLR9 outcompetes TLR7 for the endoplasmic reticulum membrane protein Unc93b1 [71], which is required for signaling [19]. The relative importance for type I IFN signaling for TLR7 but not TLR9 is supported by the requirement for IRF9 for TLR7 expression in B cells and anti-RNP IgG responses in the Pristane model of SLE [72]. Other possible explanations include: a dominant role for TLR9 in the elimination of self-reactive clones during the establishment of the B cell repertoire, preferential expression of TLR9 in a key regulatory population, or positive feedback loops preferentially driven by RNA-associated immune complexes. Whatever mechanism is involved, it must regulate TLR7-driven responses, as the double-deficient TLR7/TLR9 MRL/lpr mice develop less severe disease than their TLR7-deficient counterparts. The surprising pattern of these studies suggested that while anti-DNA antibodies are a hallmark of SLE, TLR9 independent, anti-RNP autoantibodies might be just as, if not more important in disease.

The role of TLR8 in animal models of SLE has been somewhat unclear. A recent study examined the effect of TLR8-deficiency in normal B6 mice and found an unexpected spontaneous development of SLE-like disease. Further analysis revealed a four-fold increase in the level of TLR7-expression in these mice, associated with increased severity of TLR7 mediated autoantibody production [73]. Whether TLR8 deficiency affects TLR7 because of the absence of an inhibitory signal or because of a change in regulation due to the physical proximity of TLR8 to TLR7 on the X chromosome remains to be determined.

The final nucleic acid binding TLR, TLR3, has also been examined in the MRL/lpr mice, where it did not appear to impact significantly either autoantibody production or other disease parameters [67]. However, TLR3 expression in B cells is highly dependent on type I IFN, and it is possible that TLR3 plays a role in a more IFN-α-driven model, such as TLR9-deficient mice. It is also relevant that TLR3 appears to play a role in a poly-IC driven model of fibrosis that in some ways reflects the pathology of SSc patients [74].

1.5 Genetic evidence of TLR association in systemic autoimmune disease

There is mounting human genetic evidence for an association between several polymorphisms associated with genes affecting the TLR or IFN signaling pathways and SLE. These genes include interleukin-1 receptor-associated kinase 1(IRAK-1), and TLR8 [75]. Of particular interest is an association between multiple haplotypes of interferon regulatory factor 5 (IRF5), a key transcription factor downstream of the type I IFN receptor and TLRs, including one haplotype that confers higher expression with an increased risk of SLE [76]. In support of the careful balance between IRF5 expression and SLE, humans with at least one copy of an SLE risk haplotype of IRF5 had increased type I IFN activity [53]. There is also a TLR7 polymorphism associated with increased expression levels which

confers increased risk for SLE, especially in males [77]. There is a somewhat paradoxical relationship between TLRs and autoreactive B cells, in that patients deficient in MyD88 or the downstream adaptor IRAK-4 have an accumulation of autoreactive mature naïve B cells, presumably due to a role for TLRs in tolerance induction. However, the autoreactive B cells are apparently not activated *in vivo*, as these patients do not develop serum autoantibodies or develop autoimmune disease [78]. Another group of polymorphisms associated with cell death genes BCL6 and caspase 10 could lead to a defect in eliminating autoreactive B cells [75]. These data suggest that disregulation in genes important for tuning the responses to TLRs and B cell activation and survival can tweak the balance towards inflammation and disease.

1.6 Sources of TLR ligands

The underlying trigger for systemic autoimmune disease has remained elusive. There is likely a significant environmental factor in SLE, as evidenced by the fact that the concordance for SLE among monozygotic twins is as low as 24% [79]. Inflammatory events over a lifetime are likely to be important triggers, and could explain the waxing and waning seen in SLE. Sources of inflammation can include sterile injury, infection, or inflammatory injury secondary to infection. The damage caused by infection or injury increases the availability of cellular debris, and inflammation primes the activation of B cells.

Cells from SLE patients have a decreased ability to phagocytose apoptotic debris [80]. Deficiency in scavenger receptors for apoptotic cells, such as TIM-4, lead to immune hyperactivation and the production of autoantibodies [81]. Another apoptotic debris scavenger receptor, MerTK, is a member of a family of receptors, comprised of Tyro3, Axl, and MerTK (TAM). These receptors are probably important for suppressing autoimmune responses, because, in addition to the clearance of apoptotic debris, TAM receptors can shut down signaling by cytokine receptors, including the receptor for type I IFN. When MerTK is knocked out in combination with another TAM receptor, the mice develop autoimmune disease, which includes the production of autoantibodies, which is more severe in the absence of all three receptors [82,83]. While the specific roles of these receptors on B cells have not yet been determined, the combination of increased circulating apoptotic debris and inflammation is sufficient to drive the activation of autoreactive B cells *in vivo*.

One source of sterile injury is sun exposure. Exposure to UV irradiation in certain autoimmune prone mice aggravates disease [84,85]. While SLE patients do not have a consistently measurable increase in apoptotic debris in response to UV exposure, there is an increase in inflammatory infiltrates and type I IFN production in response to UV exposure to their skin [86,87]. Interestingly, UV triggers the exposure of RNA-associated autoantigens on the surface of keratinocytes [88], which could be an important mechanism by which they become available to autoreactive B cells. Similarly, another model of sterile injury, mineral oil injection [33,89], causes immune infiltrates, and RNA-associated autoantibody production and kidney disease are dependent on TLR7.

Another potential trigger for autoreactive B cells by TLR ligands is endogenous retroviruses. Inflammation caused by TLR ligands can upregulate endogenous retroviral genes in autoimmune mouse strains, which already make detectable levels of endogenous retroviral RNA [90]. Similarly, the expression of retroviral SINE elements are induced by cell stress, such as heat shock [30]. In fact, endogenous retrovirus expression is so dangerous, that in the absence of the 3′ repair exonuclease 1 Trex1, DNA derived from endogenous retroelements builds up and causes autoimmune disease [91]. A broad role for endogenous retroviruses in human SLE has not been clearly demonstrated, but there is a genetic association between two rare TREX1 mutations and SLE [92]. Uncontrolled expression of

endogenous retroviruses seems to be an important risk factor in at least some fraction of SLE cases and animal models.

Another case where the failure to degrade DNA could lead to increased antigen availability is with neutrophil extracellular traps (NETs). NETs are anti-microbial sticky webs, formed by neutrophils extruding granule proteins and chromatin in response to inflammatory stimuli and chemokines. Sera from SLE patients have a defect in degrading NETs, and this corresponds with anti-NET and anti-DNA antibodies [93]. Many SLE patients have elevated circulating numbers of granulocytes, which are mostly neutorphils [41]. Taken together, increased numbers of neutrophils in SLE patients could lead to increased NETs, which would not be cleared efficiently, creating a large source of nucleic acid-associated cellular debris to amplify the activation of the immune system.

TLR ligands can come from exogenous sources. Leader RNA from vesicular stomatitis virus (VSV) binds to the autoantigen La [94], and SLE patients with anti-Ro antibodies have VSV N protein reactive antibodies [95]. Another study also showed that immunizing autoimmune prone NZB.NZW mice with VSV N yielded anti-Ro antibodies [96]. Further analysis of anti-Ro antibodies found that a large fraction of anti-Ro antibodies was not against the common octapeptide between Ro and VSV, ruling out molecular mimicry of the epitope as the etiologic source of the autoantibody [97]. An alternative role that viral infection could play in SLE would be an inflammatory trigger that is accompanied by extensive cell death. VSV is a potential candidate in this context, because the infection elicits significant amounts of type I IFN, the virus is cytolytic in mammals, and infection is relatively asymptomatic. Another candidate, Epstein-Barr virus (EBV), makes two RNAs, EBER1 and EBER2, which are found in association with the La autoantigen [98]. Again, exposure rates to EBV are far too high for EBV exposure alone to be the driving etiological force in SLE, but it might act as a trigger in a genetically prone individual.

1.7 Outlook

The production of autoantibodies by autoreactive B cells is the central pathogenic event in SLE. Autoreactive B cells are activated to make autoantibodies by the co-engagement of their B cell receptor, which is specific for the autoantigen, and a TLR, which is specific for the nucleic acid associated with the autoantigen. While antibodies specific for both DNA- and RNA-associated autoantigens are common features of SLE, there seems to be a particularly important role for the recognition of RNA-associated autoantigens by TLR7 in SLE. Further work is needed to fully understand how TLR7 can drive a more pathogenic autoimmune response than TLR9.

Understanding the specific pathways involved in the pathogenesis of SLE will allow highly specific therapeutics which could block the autoimmune response without crippling the adaptive immune system as a whole. We have shown that inhibition of TLR7 and TLR9 can significantly prolong the survival and decrease the kidney pathology of autoimmune MRL.lpr mice [99]. The major caveat of this study and many other inhibitor studies is that it is a disease prevention study, not a treatment study. Blocking disease becomes more difficult once disease is established. DCs and B cells in autoimmune mice are resistant to glucocorticoid (GC) mediated killing due to activation of endogenous TLR7 and TLR9 ligands. Combining GC treatment with a TLR inhibitor that is capable of blocking IC complex mediated pDC type I IFN production allows for the killing of B cells and DCs in autoimmune mice [100]. The blocking of TLR7 specifically, or blocking both TLR7 and TLR9, potentially in combination with therapies targeting the deletion of B cells, is an exciting avenue that needs to be explored clinically.

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Table 1

Effect of genetic removal of TLRs and related genes on murine models of SLE

Genetic Deficiency	Model	DNA autoantibodies		RNA autoantibodies	Disease		Reference
		HEp-2 ANA nuclesome ELISA	dsDNA ELISA		Renal	Mortality	
MyD88	MRL/lpr × B6	↓↓		↓			[31]
	MRL/lpr	↓↓		↓↓	↓		[56]
	B6 FcgR2b$^{-/-}$		↓↓		↓↓	↓↓	[45]
	Lyn$^{-/-}$	↓↓			↓		[57]
	BAFF-Tg		↓↓		↓		[58]
Unc93B1	B6/lpr	↓↓			↓↓		[61]
	BXSB	↓↓	↓	↓	↓↓	↓↓	[61]
TLR7 & TLR9	MRL/lpr	↓↓		↓	↓		[56]
	B6.Nba2	↓↓	↓	↔	↓	↓↓	[69]
IRF5	FcgR2b.yaa	↓↓		↓↓	↓↓	↓↓	[52]
TLR7	MRL/lpr	↑		↓	↓		[56]
	564			↓	↓		[63]
	B6 Pristane	↔	↔	↓↓	↓↓		[89]
	BALB/c Pristane			↓↓			[33]
TLR9	MRL/lpr	↓↓		↑	↑	↑	[62,67]
	B6/lpr	↓↓	↑	↑	↑		[66]
	Ali5	↓	↑	↑	↑↑		[68]
	B6.Nba2.Yaa	↓	↑	↑		↑	[69]
	B6.Nba2	↓	↑	↑	↑	↑↑	[69]
TLR8	B6	↑	↑	↑	↑		[73]
TLR3	MRL/lpr	↔		↔	↔		[67]

↑ modest increase, ↑↑ major increase, ↔ no change, ↓ modest decrease, ↓↓ major decrease