Toll-like receptor signalling in B cells during systemic lupus erythematosus

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Abstract | B lymphocytes have a central role in autoimmune diseases, which are often defined by specific autoantibody patterns and feature a loss of B cell tolerance. A prototypic disease associated with B cell hyperactivity is systemic lupus erythematosus (SLE). In patients with SLE, the loss of B cell tolerance to autoantigens is controlled in a cell-intrinsic manner by Toll-like receptors (TLRs), which sense nucleic acids in endosomes. TLR7 drives the extrafollicular B cell response and the germinal centre reaction that are involved in autoantibody production and disease pathogenesis. Surprisingly, TLR9 seems to protect against SLE, even though it is required for the production of autoantibodies recognizing double-stranded DNA-associated antigens, which are abundant in SLE and are a hallmark of this disease. The protective function of TLR9 is at least partly mediated by its capacity to limit the stimulatory activity of TLR7. The roles of TLR7 and TLR9 in the effector function of B cells in lupus-like disease and in patients with SLE, and the unique features of TLR signalling in B cells, suggest that targeting TLR signalling in SLE might be therapeutically beneficial.

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The diagnosis of autoimmune diseases often relies on the identification of characteristic autoantibody profiles, emphasizing their association with the activation of autoreactive B cells. Furthermore, B cell depletion therapy can have beneficial effects in patients with these disorders[1], highlighting the importance of B cells in the pathogenesis of autoimmune diseases. In autoimmune diseases B cells have been regarded almost exclusively for their role in autoantibody production, although we now know that they also mediate deleterious functions through antibody-independent activities, including: the presentation of antigen to T cells, co-stimulatory functions via the expression of accessory molecules engaging stimulatory receptors on T cells and the production of cytokines[2]. These findings highlight the need to extend the repertoire of effector B cell subsets studied with regard to autoimmune diseases beyond antibody-secreting plasmablasts and plasma cells.

Identifying the signalling pathways controlling the differentiation of effector B cell subsets might shed light on the pathophysiological mechanisms at play during autoimmune diseases. B cell activation is controlled by four classes of receptors, namely B cell receptors (BCRs) that bind autoantigens, cytokine receptors, receptors implicated in cognate T cell–B cell interactions (including checkpoint molecules), and innate immune receptors including Toll-like receptors (TLRs). The implication of TLRs in some autoimmune diseases is underlined by their association with polymorphisms in *TLR* genes (for example, *TLR4* and *TLR7*)[3,4]. TLR signalling promotes three key activities through which B cells can contribute to autoimmune diseases: the production of antibodies, the presentation of antigens to T cells and the production of cytokines[5–7]. The importance of both B cells and TLRs in autoimmune diseases suggests a role for intrinsic TLR signalling in B cells in these disorders.

In this Review we discuss how intrinsic TLR signalling controls the differentiation of effector B cells during autoimmune diseases, with a particular focus on systemic lupus erythematosus (SLE). First, we document the importance of intrinsic TLR signalling in B cells in the development of SLE. Second, we consider the contribution of intrinsic TLR signalling to the generation of pathogenic effector B cell subsets. Third, we highlight features of TLR signalling that are specific to B cells, some of which regulate their anti-inflammatory functions. Fourth, we discuss current therapeutic opportunities and perspectives for targeting TLR signalling in autoimmune diseases.

TLR signalling in B cells drives SLE

TLR7 predisposes humans to SLE. Genetic association studies implicate TLR signalling in SLE[8–10]. In particular, polymorphisms resulting in increased expression of TLR7 (the ligand for which is single-stranded RNA) are associated with an increased risk of developing SLE[11–13]. TLR7 expression is higher in women than in men owing to the localization of *TLR7* on the X chromosome[14]. One X chromosome is normally inactivated in women; yet, some genes on the X chromosome, including *TLR7*,

Key points

- Intrinsic TLR7 and TLR9 signalling in B cells plays an important role in the development and pathogenesis of systemic lupus erythematosus (SLE).
- In patients with SLE, effector plasma cells are generated via the extrafollicular response and via the formation of spontaneous germinal centres.
- TLR7 plays key roles in the extrafollicular response and the response mediated by germinal centres.
- Some plasma cells produce IL-10 and can have protective roles in lupus-like disease.

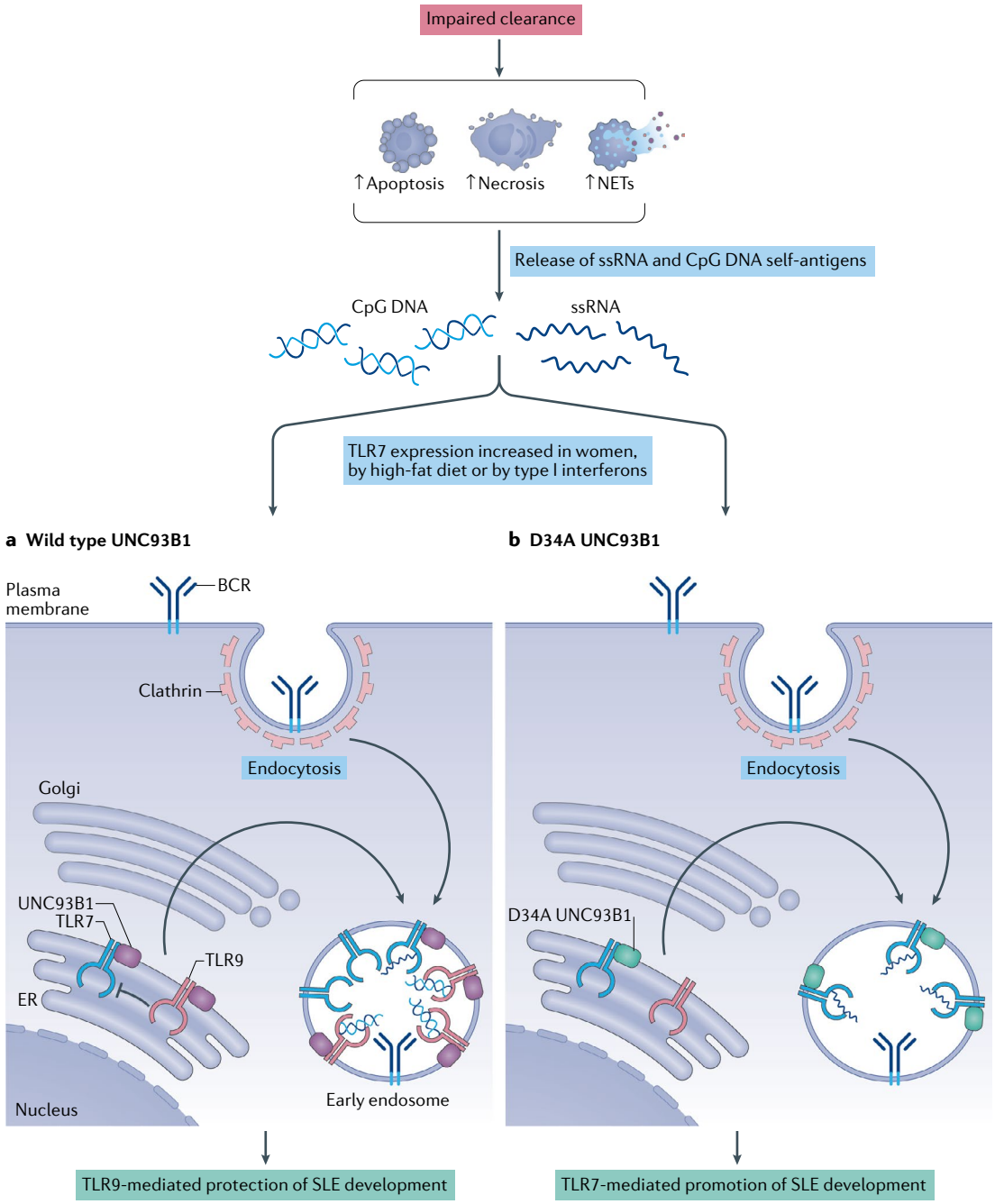
always seem to escape inactivation[14]. As a result, *TLR7* is biallelically expressed in plasmacytoid dendritic cells (pDCs), monocytes and B cells, and TLR7 is thus present at a higher level in these cells in women than in men[14]. In line with this finding, B cells from women exposed to TLR7 agonist in vitro differentiate more efficiently into $CD27^{hi}$ plasmablasts than B cells from men; this gender difference is not observed upon the addition of agonists of TLR9, the gene encoding which is on chromosome 3 (REF.[14]). This observation is consistent with a higher prevalence of SLE in women than in men[15]. Further documenting how X chromosome number affects susceptibility to SLE, the presence of two X chromosomes in men with Klinefelter's syndrome is associated with a higher predisposition to SLE than in men with a single X chromosome[16], and women with a single X chromosome (for example, those with Turner syndrome) are less prone to SLE than women with two X chromosomes[15]. A reduction in TLR7 activity might thus reduce the development of SLE. TLR7 expression is also modulated by metabolic parameters (for example, it is increased by a high-fat diet, which exacerbates SLE)[17], and by cytokines such as type I interferons, which augment the expression of TLR7 but not TLR9 in pDCs[18]. It might thus also be possible to reduce the symptoms of SLE by modulating TLR7 function.

TLR7 predisposes mice to lupus-like disease. TLR7 expression similarly modulates predisposition to lupus-like disease in mice. Overexpression of TLR7 induces systemic autoimmunity in mouse strains not prone to lupus[10,19,20], and the deletion of *Tlr7* reduces lupus development in strains that spontaneously develop such diseases[21,22]. Genetic analysis of the cell types implicated in this reduction underlined the importance of intrinsic TLR7 signalling in B cells in the pathogenesis of lupus-like disease in mice[23]. Specifically, mice that are genetically predisposed to lupus-like disease but have a B cell-specific *Tlr7* deletion displayed reduced disease, lower levels of autoantibodies against RNA-associated and apoptosis-related autoantigens and diminished immune activity, as indicated by a lower number of germinal centre B cells, T follicular helper (T_{FH}) cells, macrophages and neutrophils, including in kidneys; kidneys in these mice had no sign of glomerulonephritis, in contrast to control mice, which were genetically predisposed to lupus-like disease without deletion of *Tlr7* (REF.[23]).

TLR8 and TLR9 protect mice from lupus-like disease. In addition to TLR7, intracellular nucleic acids are detected by TLR8, which also senses single-stranded RNA, and by TLR9, which is a receptor for DNA sequences containing unmethylated cytosine-phosphate-guanosine motifs[24]. Different roles have been identified for these TLRs in distinct models of lupus-like disease. In some models both TLR8 and TLR9 exerted protective effects[25,26]. Specifically, *Tlr8*-null mice and *Tlr9*-null mice displayed more severe lupus than controls, with increased deposition of immunoglobulins and more severe lupus nephritis. These mice also displayed enhanced immune activity and had more germinal centres and antibody-secreting cells, as well as increased autoantibody titres, than controls[26]. Disease exacerbation was abrogated when *Tlr7* was also deleted from *Tlr8*- or *Tlr9*-null mice, indicating that TLR8 and TLR9 might limit the pathogenesis of lupus by limiting the deleterious effects of TLR7 (REF.[26]). Indeed, TLR8 and TLR9 restricted TLR7 activity in dendritic cells and B cells respectively[26,27]. As expected, disease in $Tlr8^{-/-}$ $Tlr9^{-/-}$ double-knockout mice was worse than disease in mice with a single gene defect, reflecting the additive effect of these two abnormalities[26]. Of note, TLR8 does not always act protectively in lupus-like disease in mice because it facilitated the production of anti-RNA antibodies in the absence of *Tlr7* in a model of lupus-like disease in which mice carry a transgenic autoreactive BCR[28]. The cell type responsible for this TLR8-mediated effect was not formally identified in this model, in which TLR7 was the main TLR driving anti-RNA autoantibody production by B cells and TLR9 acted protectively. There is thus no direct evidence that TLR8 signalling can inhibit or increase TLR7 activity in B cells; it might act in other cell types, for instance in neutrophils to increase their secretion of type I interferons[28].

TLR7 and TLR9 functionally interact in B cells. Understanding the functional interaction between TLR7 and TLR9 in B cells relies on understanding how these TLRs are engaged. These TLRs are intracellular and as, unlike dendritic cells, B cells do not internalize extracellular material through micropinocytosis or endocytosis, in B cells they are not directly accessible to natural extracellular nucleic acids[29]. Instead, in B cells, the main portal of antigen entry into cells is through the BCR, which, after engagement, is internalized with the bound antigen and delivered to intracellular compartments, including late endosomes in which TLR7 and TLR9 are present[29–31]. The arrival of BCR–antigen complexes in late endosomes activates these TLRs and triggers the co-stimulation of B cells[29]. This co-stimulation is crucial for autoreactive B cell activation in mouse models of lupus-like disease because deletion of *Tlr7* or *Tlr9* results in the loss of autoantibodies against RNA- or DNA-containing antigens respectively[21,32]. Thus, no pathway appears to be able to compensate for the absence of *Tlr7* or *Tlr9* in the development of lupus-like disease in mice.

Mechanisms of antagonism between TLR7 and TLR9 in B cells. Antagonism between TLR7 and TLR9 can occur within a single B cell if this B cell expresses a BCR that recognizes autoantigens comprising both TLR7 and TLR9 agonists. In this scenario, it was found that TLR9 engagement restrained the differentiation of B cells instructed by TLR7 in vitro[33]. In fact, B cells did



not differentiate into CD138^{hi} antibody-secreting cells unless *Tlr9* was deleted or TLR9 was pharmacologically inhibited upon antigen stimulation[33]. In agreement with this observation, antigens engaging both BCR and TLR7 (but not TLR9) could induce antigen-specific B cell differentiation into CD138^{hi} antibody-secreting cells; this differentiation was not observed for antigens co-engaging the BCR and TLR9, or with synthetic agonists of TLR7 or TLR9 that did not trigger the BCR[33]. Although these in vitro findings do not perfectly recapitulate what is happening in vivo (where the engagement of TLR9 in B cells contributes positively to the production of anti-DNA autoantibodies[27]), they underline functional differences between TLR7 and TLR9 during B cell activation, and the unique response induced upon co-engagement of BCR and TLR7 that is likely relevant to the development of lupus in mice. In keeping with a role for TLR7 in the development of lupus, TLR7 signalling (but not TLR9, TLR2, TLR3 or TLR4 signalling) is strictly required for the formation of spontaneous germinal centres in vivo in mice[34].

The antagonistic interaction between TLR7 and TLR9 within one B cell is underscored by the competition of these TLRs for the intracellular protein UNC93B1, which promotes their trafficking to endosomal compartments[35,36] (FIG. 1). Different amino acids within UNC93B1 bind to TLR7 and TLR9, and UNC93B1 harbouring a N-terminal D34A mutation interacts normally with TLR9 but more strongly with TLR7 than wild type UNC93B1; enhanced UNC93B1–TLR7 binding

Fig. 1 | **Opposing roles of TLR7 and TLR9 in SLE.** Systemic lupus erythematosus (SLE) is characterized by the presence of autoreactive B cells that recognize DNA-associated antigens (such as unmethylated cytosine-phosphate-guanosine (CpG) motifs) and RNA-associated antigens (such as the single-stranded RNA (ssRNA) small nuclear RNA, U11). These self-antigens are thought to be released owing to dysregulated processes that increase the abundance of neutrophil extracellular traps (NETs), necrotic cells or apoptotic cells. These autoantigens can be recognized at the surface of B cells by the B cell receptor (BCR), which initiates their cellular internalization. Once in endosomes, self-antigens can trigger Toll-like receptor 7 (TLR7) and/or TLR9 in B cells. Genetic or environmental signals leading to the overexpression of TLR7 (such as gender, diet and the cytokine environment, including the level of type I interferons) increase the susceptibility of individuals to SLE. Under physiological conditions, TLR7 signalling is restrained by TLR9, which protects individuals from the development of SLE. By contrast, the disruption of TLR9 function can favour TLR7 signalling and facilitate the development of SLE. Such disruption can involve the intracellular protein UNC93B1, which drives TLR7 and TLR9 trafficking to endosomal compartments (**a**). D34A mutation in transmembrane UNC93B1 favours its interaction with TLR7 (**b**), which can raise the abundance of TLR7 in endosomes, intensify TLR7 signalling and initiate a fatal systemic inflammatory syndrome in an experimental model. ER, endoplasmic reticulum.

increases the export of TLR7 from the endoplasmic reticulum to the endosomal compartment, favouring TLR7 signalling over TLR9 signalling. Mice carrying this mutation in *Unc93b1* develop a fatal systemic inflammatory syndrome[35]. In the endolysosomal compartment, the duration of TLR7 signalling is controlled by the interaction of UNC93B1 with Syntenin-1 (also known as syndecan-binding protein (SDCBP)), which can terminate transmembrane receptor signalling by promoting their transport to intralumenal vesicles of multivesicular bodies[37]. Mice with a mutation in *Unc93b1* that negatively affects the interaction of UNC93B1 with Syntenin-1 develop a systemic inflammatory disease similar to TLR7-overexpressing mice[37]. These findings underscore the importance of intracellular TLR7 trafficking in regulating TLR7 signalling. These processes can be altered via naturally occurring mutations; for instance, dogs with a mutation in the C-terminal domain of *UNC93B1* that reduces the interaction of UNC93B1 with Syntenin-1 spontaneously develop cutaneous lupus[38].

TLR7 and TLR9 signalling in B cells might also exert opposite effects on SLE via cell-extrinsic mechanisms that condition the immune environment. Little is known about such possible cell-extrinsic effects of B cells in SLE, which might involve B cell-mediated production of cytokines (such as IL-10) that can increase protection against autoimmune diseases including SLE (see below).

Understanding TLR autoantigens in SLE. A complete understanding of the role of these TLRs (TLR7, TLR8 and TLR9) in SLE pathogenesis requires the source and biochemical properties of the relevant autoantigens to be elucidated. Extracellular nucleic acids are involved in SLE pathogenesis, and mutations in *DNASE1* (encoding deoxyribonuclease-1) have been associated with the development of SLE in humans and lupus-like disease in mice[39,40]. These self-antigens are thought to be released owing to dysregulated cellular processes resulting in an increase in abundance of neutrophil extracellular traps, necrotic cells or apoptotic cells (FIG. 1). Many intracellular autoantigens are redistributed from intracellular sites to the plasma membrane during apoptosis[41], and the impaired clearance of apoptotic cells has been associated with SLE pathogenesis[42]. The small nuclear RNA U11 is also an endogenous agonist of TLR7 that drives immune pathogenesis[43]. Thus, abundantly available nucleic acids in patients with SLE contribute to active and chronic disease, likely by triggering persistent T cell-dependent and T cell-independent B cell activation. Autoreactive lymphocytes might also be activated through 'epitope mimicry' with the microbiota, as documented for autoreactive T cells targeting the SLE autoantigen Ro60 (a protein element of small cytoplasmic ribonucleoprotein hY-RNA complexes) from the host and microbiota[44]. Furthermore, rearrangements of the heavy-chain variable (VH) gene *VH4-34*, which are preferentially employed in autoimmunity, especially the idiotype 9G4 (a particular group of VH4-34-containing antibodies[45]) in SLE[46], which contributes to the autoantibody repertoire against RNA and dsDNA[47], cross-react with the gut microbiota[48]. Notably, impaired B cell selection of $9G4^+$ B cells has been observed in various autoimmune conditions including SLE[46]. Identifying where, when and how these TLR antigens drive effector B cell differentiation will provide important insights into the pathophysiology of SLE.

Effector B cell subsets driven by TLR7

SLE is associated with alterations in B cell homeostasis. Disease flares are marked by expansions in plasmablasts that are discernible in blood and correlate in magnitude with disease exacerbation[49]. These antibody-secreting cells have a more diverse repertoire of BCRs than antibody-secreting cells generated after vaccination with tetanus or influenza[50] and contain cells of irrelevant antigen specificity, with up to 1% of IgG-antibody-secreting cells producing antibodies against influenza virus or tetanus toxin[50,51]. Antibody-secreting cells in these waves also contain few expanded clones that are autoreactive, indicating the presence of an autoantigen-specific response and of cells activated in a bystander manner[50,51].

Differentiation of antibody-secreting B cells. The trajectory of effector B cell subsets leading to these antibody-secreting cells was reconstructed by combining the phenotyping of the peripheral blood B cell compartment by flow cytometry with analysis of the immunoglobulin gene repertoire. This reconstruction identified activated naive B cells ($CD11c^+IgD^+CD27^-CD21^-MTG^+CD23^-$) and the DN2 subset of IgD^-CD27^-double negative B cells (DN2 B cells; $IgD^-CD27^-CD11c^+$T-$Bet^+CD69^+CD21^-CD24^-CD38^-CXCR5^-FCRL4^-FCRL5^+$)[52], both of which are abundant in patients with SLE but rare in healthy individuals, as having a role in the production of antibody-secreting cells. Activated naive B cells have been considered to be the precursors of DN2 B cells, which are prone to differentiating into antibody-secreting cells[52]. Interestingly, activated naive B cells and DN2 B cells have a similar transcriptional profile, which differs by the expression of only 42 genes[52,53]. The transcriptome of DN2 B cells is consistent with their status as precursors to antibody-secreting cells: they express higher levels of BLIMP-1 and IRF4, master transcription factors of plasmablast and plasma cell differentiation[54], as well as of SLAMF7, which is encoded by an IRF4 target gene and also found at higher levels in plasma

cells than in other B cell subsets[52]. Furthermore, they express less ETS1, a transcription factor that inhibits antibody-secreting cell formation, than other B cell subsets[52]. As expected based on these observations, DN2 B cells rapidly secrete antibodies after polyclonal stimulation, including anti-Smith and anti-ribonucleoprotein (RNP), indicating that they express autoreactive BCRs that recognize RNA-associated autoantigens. Supporting the notion that these cells play a notable role in the production of such antibodies, the frequency of DN2 B cells correlates with the levels of anti-Smith and anti-RNP autoantibodies, the two RNA-associated antigens investigated in this study, in patients with SLE[52]. Furthermore, accumulation of $CD11c^+T\text{-}Bet^+CD21^-CD38^-$ B cells resembling DN2 B cells, which were autoreactive and correlated with clinical manifestations, was similarly reported in a different cohort of patients with SLE[55].

Roles of TLR7 in B cell differentiation. TLR7 can instruct successive steps in the differentiation of resting naive B cells to activated naive B cells, DN2 B cells and, subsequently, to antibody-secreting cells, which defines a pathway of extrafollicular B cell differentiation[52]. TLR7 has a stimulatory activity in all of these effector B cell subsets and can promote the differentiation of DN2 B cells, which are hyperresponsive to TLR7 agonists, into antibody-secreting cells in the presence of IL-21 and IFNγ[52]. By contrast, DN2 B cells are less responsive to CD40 engagement than activated naive B cells[52], indicating a change in the responsiveness of B cells to external stimuli during differentiation, perhaps owing to a progressive reduction in the expression of TRAF5, a factor that is required for CD40 signalling and inhibitory for TLR signalling[56,57].

The role of TLR7 in the differentiation of effector B cells in patients with SLE has been further documented through molecular studies in an in vitro culture system that generates DN2-like B cells with a transcriptome similar to that of DN2 B cells from patients with SLE[58]. This culture system relies on the stimulation of naive human B cells with agonists of TLR7 and BCR as well as IFNγ, IL-2, IL-21 and B cell activating factor, and generates $IgD^-CD27^-CD11c^+T\text{-}Bet^{hi}CD21^-CXCR5^-IRF4^{int}FcRL5^+$ B cells resembling DN2 B cells found in patients with SLE[58]. TLR7 signalling was crucial for the subsequent differentiation of these cells into antibody-secreting cells[58]. The sensitivity of B cells to TLR7 agonists is augmented by IFNγ, which confers them with the capacity to respond productively to amounts of TLR7 agonist that are otherwise insufficient[58]. Of note, the concentration of IFNγ is increased in the serum of some patients with SLE[59,60]. By contrast, B cells from patients with SLE are hyporesponsive to TLR9 agonists[61,62], suggesting that the balance between these two opposing TLR signalling pathways is distorted in patients with SLE.

TLR7 also plays a key role in the differentiation of naive B cells into $CD11c^+T\text{-}Bet^+CD21^-$ B cells (which would comprise activated naive and DN2 B cells in humans) in animal models of lupus, because these cells are absent in mice when *Tlr7* is deleted[63]. Furthermore, the repeated administration of TLR7 agonists (but not of TLR3, TLR4 or TLR9 agonists) promotes the accumulation of these cells in mice via a mechanism involving intrinsic TLR7 signalling in B cells[63]. Remarkably, mice with B cell-specific deletion of *Tlr9* display a higher number of $CD11c^+CD11b^+$ activated B cells than their counterpart with functional *Tlr9*, underlining the correlation between the abundance of these cells and the development of lupus-like disease and providing another example of the opposing roles of TLR7 and TLR9 (REF.[23]). Of note, TLR7 also facilitates the formation of spontaneous germinal centres in mice which, in addition to the extrafollicular plasma cell response involving DN2 B cells, can lead to autoantibody production in patients with SLE[64] (FIG. 2). As in patients with SLE, IFNγ is also important for the pathogenic functions of B cells in animal models of lupus, underlining the conserved role of these pathways in this disease across species[65–68].

In conclusion, and as previously discussed[69] suggests that two pathways of B cell activation lead to the formation of autoreactive antibody-secreting cells in patients with SLE: the extrafollicular response (supported by activated naive B cells and DN2 B cells) and germinal centre reactions[47]. Remarkably, intrinsic TLR7 signalling in B cells has emerged as a key player in both responses (FIG. 2). These findings provide a framework in which to carry out the biochemical analyses of the molecular mechanisms implicated in these cellular processes. Interestingly, these pathways are not uniquely confined to autoimmunity as they have been reported to be active in patients with COVID-19 (REF.[70]).

Mechanisms of TLR signalling in B cells

B cells uniquely respond to TLR agonists. B cells are defined by the expression of a cell surface BCR and, as they also express multiple TLRs, these cells are thus at the intersection of adaptive and innate immunity. Intrinsic innate signalling in B cells is essential for the development of lupus-like disease in mice, as lupus nephritis was absent in mice with a B cell-type specific ablation of *Myd88* (*Myd88* encodes the signalling adaptor protein MyD88, which acts downstream of TLRs and IL-1 receptors)[71–73]. The response of B cells to TLR agonists is unique compared with other cell types of the immune system. B cells proliferate intensively and produce large amounts of IL-10 upon TLR stimulation, a combination not observed in myeloid cells[7]. Underlining the complex role of intrinsic TLR signalling in B cells during disease, B cell-derived IL-10 can be protective in autoimmune diseases[74], including in mouse models of lupus-like disease[75]. Thus, in mice deficient in *Lyn*, which encodes a non-receptor tyrosine-protein kinase that regulates innate and adaptive immune responses, IL-10 production by B cells inhibits the progression of lupus-like disease even when no other cell types can produce this cytokine[75].

Interplay between TLR signalling and BCR signalling. These unique features of TLR-driven cellular activation are related to the distinctive expression of BCR on B cells; B cells in which BCR has been genetically ablated fail to proliferate upon TLR stimulation[76,77]. At the intracellular level, TLR-stimulated B cell

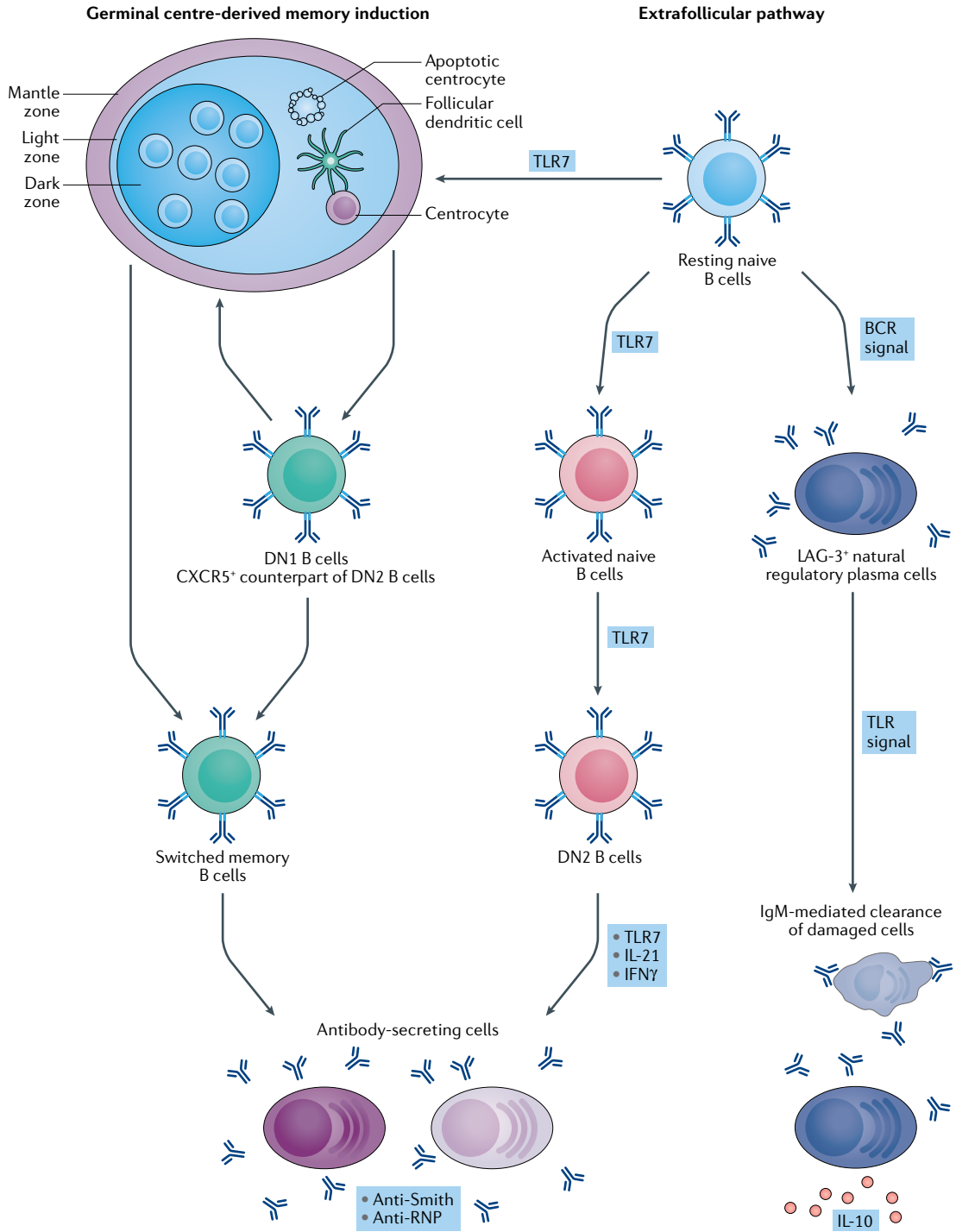


Fig. 2 | **TLRs drive plasma cell differentiation in SLE via different pathways.** Two distinct pathways generate pathogenic antibody-secreting cells in patients with systemic lupus erythematosus (SLE): germinal centre reactions and the extrafollicular pathway, both of which engage resting naive B cells. The germinal centre pathway generates the DN1 subset of double negative B cells (DN1 B cells; that is, IgD^- $CD27^-$ cells that are $CXCR5^+$) and the memory B cells produced in germinal centres can re-enter the germinal centre reaction or differentiate into antibody-secreting cells that produce isotype switched anti-Smith and anti-RNP. The spontaneous generation of the germinal centre is dependent on TLR7. TLR7 also drives the extrafollicular pathway, in which resting naive B cells become activated naive B cells ($CD11c^+IgD^+CD27^-CD21^-MTG^+CD23^-$) and, subsequently, the DN2 subset of IgD^- $CD27^-$ double-negative B cells (DN2 B cells; IgD^- $CD27^-CD11c^+$ $Tbet^+$ $CD69^+CD21^-CD24^-CD38^-CXCR5^-FCRL4^-FCRL5^+$). DN2 B cells are precursors of pathogenic antibody-secreting cells in patients with SLE, the differentiation into which is promoted by TLR7, IL-21 and IFNγ. Of note, resting naive B cells can also generate, in a manner dependent on the B cell receptor (BCR), regulatory plasma cells that are characterized by the cell surface expression of lymphocyte activation gene 3 protein (LAG-3). These regulatory plasma cells produce a uniquely high level of IL-10 in response to TLR signalling. At steady state, they also secrete IgM with reactivity against antigens expressed by damaged cells, suggesting that they might be involved in the clearance of damaged cells.

proliferation involves the adaptor protein Src tyrosine kinase SYK, which promotes BCR signalling by phosphorylating immunoreceptor tyrosine-based activation motifs in the cytoplasmic domains of the Igα and Igβ substructures of the BCR[78,79]. Thus, *Syk*-deficient B cells show impaired proliferation and IL-10 secretion upon activation with TLR agonists[76]. This fact is related to the defective activation of AKT and ERK in the absence of SYK. Remarkably, this TLR–SYK–AKT–ERK pathway is independent of MyD88 as it is still active in *Myd88*-deficient mice[76]. Finally, PI3K also has a key role in this pathway as over-expression of this kinase restored a proliferative response in BCR-deficient B cells stimulated with TLR agonists[77].

The recruitment of the BCR signalling cascade downstream of TLR engagement in B cells underscores the interconnection between the innate and cognate functions of these cells. Along these lines, the engagement of the BCR upregulates TLR expression in human B cells, endowing these cells with the capacity to respond to TLR agonists[5,80]. In this context, genome-wide association studies identified the BCR signalling pathway as the biological process most affected by genetic polymorphisms facilitating the development of SLE[81]. These polymorphisms might not only facilitate BCR signalling but also increase TLR signalling in B cells, which might enable the growth of autoreactive B cell clones and the release of cytokines by them. Interestingly, the two B cell subsets with the highest capacity to produce IL-10 upon TLR stimulation in the mouse, namely LAG-3^{+}CD138^{hi} natural regulatory plasma cells and CD1d^{hi} B cells, develop in a BCR-dependent manner[82,83] (FIG. 2).

TLR signalling also comprises a SYK-independent pathway because the activation of NF-κB and the secretion of IL-6 (events downstream of TLR signalling) occurred normally in *Syk*-deficient B cells stimulated via TLR[76]. IL-6 production by B cells is directly relevant to the pathogenesis of SLE, as IL-6 expression is increased in patients with active SLE[84] and correlates with disease activity in patients with lupus nephritis[85]. IL-6 expression can be induced in B cells by TLR7 agonists[86,87], and this expression is further enhanced by IFNγ[88]. Remarkably, the ablation of IL-6 production specifically from B cells abrogated the spontaneous formation of germinal centres in lupus-prone mice and inhibited disease development[88]. Thus, elimination of the arm of intrinsic TLR signalling in B cells that results in IL-6 production, which seems to be independent of SYK, might be more beneficial in patients with SLE.

From a therapeutic standpoint, the fact that BCR signalling adaptors such as SYK have been implicated in TLR-mediated cell function suggests that inhibitors of BCR signalling might block the TLR-driven functions of B cells. Accordingly, the SYK inhibitor entospletinib reduced human B cell responses to TLR9 agonist[89], and SYK expression was increased in activated CD21^{low} B cells[90].

Targeting TLR signalling to treat SLE

Inhibiting signalling adaptors. There is much interest in inhibiting signalling adaptors implicated in the pro-inflammatory functions of TLRs to treat inflammatory diseases. Although TLRs are prototypic pathogen recognition receptors, humans with loss of function mutations in *MYD88*, which transduces signals via all TLRs except TLR3, display a narrow susceptibility to pyogenic bacterial infections by *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but are resistant to other common microbial pathogens[91]. Remarkably, their susceptibility to these pathogens decreases with age. Similarly, children with a deficiency in *IRAK4* (which encodes IL-1 receptor-associated kinase 4, a component of the TLR signalling pathways important for TLR7 and TLR9, as well as other TLRs) display an increased susceptibility to pyogenic bacterial infections during their first 10 years of life, which improves with age[92]. The improvement of pathogen control in both of these groups of individuals is likely related to the emergence of compensatory adaptive mechanisms involving T lymphocytes or B lymphocytes[93]. The inhibition of key molecules of the TLR signalling pathway, such as MYD88 or IRAK4, might thus allow inflammation to be tapered without compromising host defence against pathogens.

Inhibiting TLR activation in endosomes. The inhibition of endosomal TLR activation appears to be the most pertinent for treating patients with SLE. Existing treatments already target this pathway; notably, hydroxychloroquine and bafilomycin inhibit endosome acidification and/or maturation, thereby inhibiting both TLR7 and TLR9 signalling[94]. Hydroxychloroquine only moderately inhibits TLR signalling, and does not result in a surge of infection, consistent with the concept that TLR inhibition modulates, rather than suppresses, the immune system[95]. It inhibits the inflammatory response of human memory B cells, including their TLR-stimulated production of IL-6 (REF.[96]). Several other inhibitors of endosomal TLRs are under clinical evaluation for use in rheumatic diseases, including SLE and psoriasis, that also involve TLR7 signalling. TLR7, TLR8 and TLR9 signal through IRAK4, the inhibition of which has been studied in various assays and showed superior effects compared with hydroxychloroquine on the inhibition of cytokine production and inflammatory gene expression in peripheral blood mononuclear cells[97]. An early trial using the IRAK4 inhibitor PF06650833 provided promising phase I data in healthy individuals, showing a favourable safety and pharmacokinetic profile as well as evidence of pharmacological effect[98]. Additional inhibitors of IRAK1 and IRAK4 or of TAK1, a signalling molecule involved in TLR signalling, are under development[99]. Of note, these approaches do not differentiate between different endosomal TLRs[95] and they affect multiple cell types in addition to B cells. The safety of these inhibitors thus needs to be considered carefully.

Boosting TLR signalling. Although the dominant rationale for targeting TLRs to treat autoimmune diseases is to inhibit TLR signalling, the data outlined above indicate that some TLR signalling is protective in inflammatory diseases, including in lupus-like disease; thus, boosting TLR signalling to strengthen its regulatory function might be an alternative treatment strategy. Indeed, there is already some insight into the use of TLR agonists in

Table 1 | **TLR modulators in clinical development for inflammatory diseases**

TLR target	Compound	Target disease	Mechanism of action	Development phase (NCT number)	Refs
TLR7	Imiquimod	Actinic keratosis	Immune-stimulator	Phase I (NCT01151956); phase IV (NCT00777127, NCT01453179)	108–112
	GSK2245035	Rhinitis	Induces type I IFN; immune-stimulator	Phase II (NCT01788813, NCT02446613, NCT01607372); phase I (NCT01480271)	113,114
		Asthma	Induces type I IFN; immune-stimulator	Phase II (NCT03707678); phase I/II (NCT02833974)	115
TLR9	CYT003-QbG10	Asthma	Induces a T_H1 cell-mediated immune response	Phase II (NCT02087644, NCT00890734)	116,117
	AZD1419	Asthma	Induces T_H1-type IFN response	Phase II (NCT02898662)	118,119
	Hydroxychloroquine	Sjögren's syndrome	Immune modulator	Phase III (NCT00632866, NCT01601028)	120,121
IRAK4	ND-2158	Rodent models of: lipopolysaccharide-induced TNF production; collagen-induced arthritis; gout; activated B cell like-diffuse large B cell lymphoma; chronic lymphocytic leukaemia	Small molecule inhibitors of inflammatory pathways	Preclinical	122,123
	BMS-986126	Systemic lupus erythematosus	Inhibitor	Preclinical	124
	PF-06650833	Rheumatic autoimmune diseases	Inhibitor	Phase I (NCT02224651, NCT02485769); phase II (NCT02996500)	98
	BAY1834845	Psoriasis; pelvic inflammatory disease	Small-molecule inhibitor	Phase I (NCT03493269, NCT03054402)	125
IRAK1, IRAK4 and TAK1	HS-243	Autoimmune diseases	Inhibitor	Preclinical	99

IRAK-, IL-1 receptor-associated kinase; TAK1, transforming growth factor-β-activated kinase 1; T_H1, T helper 1; TLR, Toll-like receptor.

the clinic, which shows the feasibility and safety of such approaches. Cancer therapy has undertaken approaches to target TLRs with FDA-approved agonists, including: the locally administered Bacillus Calmette–Guérin vaccine (comprising live attenuated *Mycobacterium bovis*) for bladder cancer, which can stimulate TLR2 and TLR4 (microbial cell wall) and TLR9 (bacterial DNA); topical imiquimod for pre-malignant actinic keratosis, which targets TLR7 in basal cell carcinoma; and monophosphoryl lipid A, which is a bioactive part of a lipopolysaccharide targeting TLR4 in human papillomavirus-associated cervical cancer[100]. Further TLR agonists and antagonists are in clinical development for cancer. In this context, TLR agonists are expected to facilitate programmed cell death and enhance immune surveillance[101]. TLR antagonists are used to limit the TLR-driven growth of tumour cells and it should be considered if some of these strategies could be beneficial in autoimmune diseases.

Conclusions

There is increasing evidence that TLR7 has an important role in SLE pathogenesis, with functions conserved in humans and in mice. TLR7 is critical for the extrafollicular and germinal centre responses associated with the activation of autoreactive B cells that is implicated in this disorder. Genetic studies have shown that TLR7 signalling in B cells is particularly important in orchestrating disease. It is remarkable that the different endosomal TLRs that act as nucleic acid sensors, namely TLR7, TLR8 and TLR9, have distinct roles in patients with SLE. In fact, TLR8 and TLR9 might even have beneficial functions in patients with SLE. Uncovering the biochemistry of these molecular processes is thus important and might lead to the identification of novel targets for drug development.

The ability of TLR signalling to activate and inhibit immune signalling, which is not completely understood, suggests that several strategies could target this pathway to treat disease. Although current clinical developments for targeting TLR signalling are still limited compared with treatments for other biological targets[102,103], a number of molecules are currently in development for targeting TLR signalling in inflammatory diseases[104] (TABLE 1). It will be of great interest to follow their clinical development and possible application. Beyond the currently developed approaches, it will also be of interest to identify strategies to rebalance the TLR7 and TLR9 pathways and thus readjust immune homeostasis. It is possible that B cell depletion therapy could reset these pathways by replacing B cells in which this dysregulation might be epigenetically imprinted by novel naive B cells. By contrast, this defect might persist in B cells, including memory B cells that have resisted depletion, thus favouring the restart of disease. Finally, it is important to consider that the inhibition of BCR signalling might interrupt some functions of TLR signalling in B cells[76]. This interruption might be pertinent for the use of the SYK inhibitor fostamatinib, which is approved for treating the autoimmune disease chronic

immune thrombocytopenia, a disease in which the role of intrinsic TLR signalling in B cells is not defined[105].

It remains incompletely understood why TLR7 might be deleterious and TLR9 might be protective in SLE, although it is possible that this phenomenon is related to the distinct types of autoantigens that these TLRs recognize. Some immune complexes containing RNP induced TLR7-mediated production of TNF by macrophages and type I IFN by pDCs[106,107]. However, it is unclear if immune complexes containing TLR9 agonists have similar immunological properties to those containing TLR7 agonists as there has been no systematic comparison of the myeloid cell response to immune complexes associated with RNA (and thus TLR7) versus DNA (and thus TLR9) moieties. There is also some evidence that TLR7 and TLR9 signalling might have opposing roles in SLE given that they distinctively impact B cell activation and differentiation, as mentioned above[33]. However, the relevance of this difference in patients with SLE has not been tested experimentally. Of note, we still have a limited knowledge of the differences in signalling and cellular responses driven by distinct TLRs; these differences might be a fruitful area for future drug development, especially considering that TLR signalling in B cells can have potent anti-inflammatory functions by eliciting the production of IL-10. The distinct molecular mechanisms associated with the control of immunity, including B cell responses, by TLRs thus seems directly relevant for the development of novel therapeutic strategies for autoimmune diseases.

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