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Linking complement and anti-dsDNA antibodies in the pathogenesis of systemic lupus erythematosus

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Abstract

Systemic lupus erythematosus is a severe autoimmune disease that affects multiple organ systems resulting in diverse symptoms and outcomes. It is characterized by antibody production to a variety of self-antigens, but it is specifically associated with those against anti-dsDNA. Anti-dsDNA antibodies are present before the onset of clinical disease and are associated with severe manifestations of lupus such as glomerulonephritis. Their levels fluctuate with changes in disease activity and, in combination with the levels of complement proteins C3 and C4, are strong indicators of disease flare and treatment response in patients with lupus. The decreased complement levels that are noted during flares of lupus activity are believed to be secondary to increased autoantibody production and immune complex formation that results in tissue damage; however, recent data suggest that complement activation can also drive development of these pathogenic autoantibodies. This review will explore the various roles of complement in the development and pathogenesis of anti-dsDNA antibodies.

Keywords

SLE; Autoantibodies; Anti-dsDNA antibodies; Complement; Clearance

Introduction

Systemic lupus erythematosus (SLE) is a potentially fatal and severe chronic autoimmune disease that affects multiple organ systems including the skin, heart, brain, and kidneys [1]. It is remarkably heterogeneous, with diverse and dynamic symptoms manifested by flares of disease activity. The disease burden of SLE in the United States is greater than 250,000 patients with ~90 % of the cases being female [1]. It is a prototypical autoimmune disease in that it involves multiple components of the immune system and results in the production of autoantibodies against a variety of targets including, but not limited to, double-stranded DNA (dsDNA), RNA-binding proteins (RBPs), and phospholipids [2].

Many autoimmune diseases result in autoantibody production, but anti-dsDNA antibodies are highly specific to SLE: less than 0.5 % of healthy people or patients with other autoimmune diseases have anti-dsDNA antibodies, whereas 70 % of SLE patients are positive [3]. Anti-dsDNA antibodies in SLE were first described in 1957 in the blood [4] and were later found in the kidneys of nephritic patients [5]. Their presence in the blood of lupus patients for several years prior to their first clinical manifestations suggests that they may be involved in the progression to clinical disease [6]. Furthermore, increased levels of anti-dsDNA antibodies are associated with disease flares [7–9], usually in combination with decreased levels of the complement proteins C3 and C4 [10]. Although disease activity is not always correlated with altered levels of anti-dsDNA antibodies and complement proteins, renal involvement is the most strongly associated clinical manifestation [11], and both anti-dsDNA and complement levels normalize after treatment with immunosuppressive therapy [12]. The long-standing observation that complement depletion and anti-dsDNA antibodies are associated with increased activity and severe manifestations of SLE is intriguing, and recent data suggest new mechanisms for these associations. Here, we review the current concepts of how the complement system contributes to anti-dsDNA antibody development and pathogenic mechanisms in SLE.

Development of anti-dsDNA antibodies

The vast diversity of the immune system enables receptor-mediated recognition of virtually any substance that it encounters [13]. This diversity is essential for protecting the host from invasive organisms, but also requires the ability to discriminate self and not initiate a response to one's own tissue: a mechanism known as tolerance. B and T cells of the adaptive immune system are subjected to receptor editing and deletion during development to ensure that self-reactive cells are not released into the periphery. Despite these mechanisms, some autoreactive cells escape tolerance mechanisms and enter the circulation. The presence of autoreactive B cells in healthy individuals is demonstrated by the transient appearance of autoantibodies, including those with anti-dsDNA specificity, after infection [14]. Importantly, not all anti-dsDNA autoantibodies are pathogenic as evidenced by lupus patients who have elevated anti-dsDNA titers without active disease and mice that do not develop disease after passive transfer of some anti-dsDNA autoantibodies [15]. One factor that influences the pathogenic potential of anti-dsDNA auto-antibodies is the antibody isotype: active disease in humans is associated with IgG and not IgM or IgA [16], and in murine models, the subclass of IgG2a is more pathogenic than IgG1 due to more efficient complement and Fc receptor activation [17].

Natural antibodies

One prominent source of autoantibodies is the natural antibody repertoire. Natural antibodies are usually IgM and utilize germline-encoded genes largely devoid of somatic mutations [18]. Unlike antigen-induced antibodies, production of natural antibodies does not require B cell contact with an external antigen, and therefore, they are considered to be part of the innate immune system. Another feature of natural antibodies is that they recognize a large number of diverse antigens, including pathogens and self, with moderate to low affinity [19–21]. The self-reactive nature of these antibodies suggests that they play a role in

maintaining homeostasis of the immune system [22]. Additionally, natural antibodies may participate in removal of apoptotic debris and maintenance of immunological tolerance [23].

Although natural antibodies can be reactive to self-antigens, pathogenic anti-dsDNA antibodies from SLE patients are typically high-affinity IgG antibodies that show extensive affinity maturation [24, 25]. These features are distinct from those of the majority of natural antibodies and suggest that these antibodies originate from germinal center reactions in response to antigen-driven selection [26]. Different anti-dsDNA antibodies share structural features, especially in the heavy chain complementarity-determining regions (CDR) [25, 27]. Arginine residue position and frequency in CDR3 correlate with DNA binding, and when the somatically mutated residues are returned to germline, DNA binding is abrogated [28–30]. Furthermore, sequence analysis of anti-dsDNA antibodies in mice has indicated that IgM and IgG autoantibodies are clonally related and are likely derived from an antigen-driven response [31].

Self-derived antigens

DNA itself is a poor immunogen, but association of DNA with an immunogenic protein can result in the production of anti-dsDNA antibodies [32, 33]. A potential source for DNA–protein antigens is apoptotic debris, and several autoantigens are found on the surface of apoptotic bodies [34]. Under normal conditions, apoptotic cells are rapidly cleared without inflammation or the initiation of an immune response. Some SLE patients demonstrate a clearance defect that can lead to secondary necrosis and inflammation [35, 36]. Consistent with this, injection of apoptotic cells into mice induces transient production of anti-dsDNA antibodies that results in IgG deposition in the kidneys [37].

Studies of milk fat globule epidermal growth factor 8 (MFG-E8) further implicate apoptotic cell debris in anti-dsDNA antibody development. MFG-E8 was initially discovered in mouse mammary glands [38], but since has been shown to function more ubiquitously by enhancing phagocytosis of apoptotic cells [39]. In mice, MFG-E8 deficiency results in lupus-like disease including anti-dsDNA antibody production and proteinuria [40]. Efficient clearance of apoptotic debris by the complement system and other mechanisms is essential to control inflammatory exposure of autoantigens. Many naturally occurring biological processes such as germinal center formation, pregnancy, and lactation are associated with large amounts of apoptosis and could therefore provide a source of immunogenic autoantigens if the debris is not rapidly cleared.

Although late-stage apoptotic bodies are a potential source for immunogenic dsDNA–protein complexes, other cellular processes can also produce these antigens. Microparticles are particulate structures released by most cells that can promote thrombosis and stimulate multiple cell types with effects on cytokine production and inflammation [41–43]. Although microparticles are released during apoptosis, they are distinct from the apoptotic bodies discussed above in both size (smaller) and when they are generated (earlier) [44]. Furthermore, microparticle production is not always part of a cell death pathway [45], and as such, microparticles could be a source of dsDNA and protein that is independent of dead or dying cells.

Another potential source for DNA-protein antigens is neutrophil extracellular traps (NETs). NETs are composed of DNA and proteins and are generated through a unique cell death program [46, 47]. Generally, NETs are found at sites of infection and function in an antimicrobial capacity [46, 48], but more recently, NETs were described in the marginal zone of the spleen where they exhibited B cell helper function in generating T-independent immune responses [49]. NETs are degraded by DNase-I, and a cohort of SLE patients had impaired degradation that was attributed to anti-NET antibodies shielding the DNA [50]. Furthermore, SLE disease flares are often associated with infection [51], and NET release in response to invading pathogens is a potential source of autoantigens that, like apoptotic debris, may not be efficiently cleared in patients with lupus.

Non-self-antigens

Immunogenic dsDNA-protein antigen complexes can clearly be generated by natural processes gone awry, but non-self-sources of antigen have also been implicated. Epstein-Barr virus (EBV) infects B cells and is the cause of infectious mononucleosis. Although the vast majority (95 %) of healthy individuals are serologically positive for EBV, SLE patients have an even higher seropositive rate (99.5 %) [52]. The difference is more evident in juvenile patients, suggesting that EBV could be an environmental factor influencing the development of SLE [53]. Because EBV infects B cells, it is possible that the process of viral DNA integration into the host and latent gene expression could alter B cell activation pathways and induce autoimmunity [54]. Indeed, expression of latent membrane protein (LMP) 1 can enhance autoimmune phenotypes in susceptible mice [55], and LMP2A directly alters B cell activation via disruption of signaling microdomains [56, 57]. Although the infectious process of EBV (e.g., DNA integration and latency) may be involved in the overall immune dysregulation in SLE, EBV antigens can also directly contribute to the development of anti-dsDNA antibodies through molecular mimicry. Antibodies generated against the EBV nuclear antigen EBNA-1 were shown to cross-react with dsDNA, but did not cause lupus-like disease in mice [58]. In addition to EBNA-1 of EBV, other microbial products can induce antibodies that cross-react with dsDNA, including cell wall components of *Mycobacterium tuberculosis* and *Streptococcus pneumoniae* as well as proteins from *Burkholderia fungorum* [59–61]. It is apparent that an assortment of self- and non-self-antigens can result in the production of anti-dsDNA antibodies, and whether the source of the antigen influences the pathogenicity of the autoantibodies is not known.

Pathogenic mechanisms of anti-dsDNA antibodies

Direct effects

The cellular localization of the target antigens is an important factor in determining the pathogenic potential of autoantibodies [62]. Generally, autoantibodies directed against extracellular antigens are considered to be pathogenic, whereas the pathogenic potential of autoantibodies against intracellular antigens remains unclear [14]. Although anti-dsDNA antibodies have an intracellular target, there is a well-defined association with kidney disease in SLE patients [63–65]. One potential mechanism of action is the ability of anti-dsDNA antibodies to enter cells, traffic to the nucleus, and directly exert cytopathic effects via induction of apoptosis after engaging cellular DNA (Fig. 1) [66–69]. Intracellular

transport mechanisms remain unclear, but initial antibody penetration can occur via several mechanisms including Fc receptor-mediated uptake, non-Fc receptor-mediated endocytosis, and electrostatic interactions [67, 70, 71]. This process could lead to increased cell death and buildup of apoptotic debris resulting in additional substrate for amplification of autoreactive B cell responses.

The pathogenic potential of anti-dsDNA antibodies may also depend on polyreactivity (Fig. 1) [72]. A potential target for polyreactive anti-dsDNA antibodies is the actin-interacting protein α -actinin. In support of this theory, high-avidity anti-dsDNA antibodies that cross-react with α -actinin are associated with renal disease [73]. Furthermore, α -actinin cross-reactivity is most commonly observed in SLE patients with renal disease, and anti-dsDNA antibody affinity for α -actinin is associated with glomerular binding [74, 75]. Polyreactivity is not a phenomenon limited to kidney antigens, as demonstrated by anti-dsDNA antibody binding to the NMDA (*N*-methyl-D-aspartate) receptor. NMDA receptors bind the neurotransmitter glutamate and are found in the hippocampus and cortex of the brain [76]. SLE patients can display central nervous system manifestations, including cognitive impairment and mood disorder [77]. Interestingly, cognitive function and mood stability both require the NMDA receptor, and it is hypothesized that anti-dsDNA antibody polyreactivity with this receptor contributes to some of the neurological manifestations of SLE [78–80]. Indeed, administration of these polyreactive anti-dsDNA antibodies to mice results in Fc receptor-independent neuronal death [81]. The polyreactive nature of anti-dsDNA antibodies could, at least in part, explain why some anti-dsDNA antibodies are pathogenic and others are not.

Indirect effects

Anti-dsDNA antibodies can also exert pathogenic effects in an indirect manner via the circulating antigen–antibody complexes that form during the course of disease. The formation of these circulating immune complexes and their presence in the kidneys of lupus patients was first suggested approximately 50 years ago [5], with levels of these circulating immune complexes subsequently being shown to correlate with active disease [82, 83]. Although antibodies in these immune complexes have specificity for DNA, it is likely that they interact with nucleosomes *in vivo*, as nucleosomes rather than free DNA have been identified in the blood of patients with lupus [84, 85]. The positively charged histones in nucleosomes can bind to the negatively charged basement membrane of the glomerulus via electrostatic interactions and thus target these immune complexes to renal tissue (Fig. 1). Indeed, anti-DNA antibodies alone failed to bind kidney tissue, whereas addition of nucleosomes to generate immune complexes resulted in glomerular antibody deposition and complement activation [86]. Additionally, basement membrane-associated nucleosomes were found to be the targets of nephritogenic antibodies to DNA using electron microscopy [87].

Pathogenic effects can also be mediated when DNA in immune complexes functions as a danger-associated molecular pattern that is recognized by Toll-like receptor (TLR) 9. TLR9 is located in endosomes to provide access to pathogen-derived DNA and to restrict access to self-DNA that can be released during apoptosis or NET formation [88]. Despite the

intracellular localization, TLR9 can be activated by DNA-containing immune complexes after uptake by plasmacytoid dendritic cells (pDCs) via Fc γ RIIa receptors (Fig. 1) [89]. pDCs are potent producers of IFN- α , a cytokine that is elevated in the serum of SLE patients and is thought to be important in the disease process [90]. One possible mechanism of IFN- α enhancing anti-dsDNA antibodies is through its interaction with neutrophils. The neutrophil response to IFN- α includes upregulation of the antimicrobial peptide LL37 on the cell surface and eventually in NETs to enhance bacterial killing capacity. Interestingly, LL37 can also be an autoantigen in some patients, and anti-LL37 antibody binding on the neutrophil surface induces cell death and NET formation. The autoantibody-induced NETs then provide antigen for anti-dsDNA antibodies, and the DNA component of the resulting immune complexes triggers more IFN- α production by pDCs, setting up an inflammatory positive feedback loop [91, 92]. Thus, the presence of circulating DNA-containing immune complexes can initiate a process that results in increasing amounts of substrate that can drive development of anti-dsDNA antibodies as well as formation of DNA-containing immune complexes.

The place of complement in lupus pathogenesis

Overview of complement system

The complement system is a humoral component of the innate immune system that contains about 30 proteins, present both in the fluid phase and anchored to cell membranes. Complement becomes activated by three main pathways: the classical, alternative, and lectin pathways. All three pathways converge on the generation of C3 convertases that result in the production of anaphylatoxins and a proinflammatory cascade. The classical pathway is initiated by C1q directly binding to complement-fixing antibodies (IgM > IgG) or other proteins including serum amyloid P protein or C-reactive protein (reviewed in [93]). Similarly, the lectin pathway begins when mannose-binding lectin (MBL) or ficolins bind microbially derived carbohydrate moieties (reviewed in [94]). The classical and lectin pathways converge on the activation of C4 which, together with C2, leads to the formation of the classical C3 convertase and further complement activation. While the classical and lectin pathways begin with specific interactions, the alternative pathway can autoactivate and requires constant regulation to control complement activation and inflammation (reviewed in [95]). C3 will spontaneously change conformation and bind factor B, which is then cleaved by factor D to Bb, and Bb can then activate C3 to generate the alternative pathway C3 convertase. Furthermore, the alternative pathway is critical for enhancing complement effector functions after classical or lectin pathway activation via an amplification loop. Effector functions of activated complement include direct lysis of cells and opsonization of complement-coated antigens, both of which are important in SLE, and active disease is often accompanied by complement activation with C3 and C4 deposits being found in inflamed tissue [96]. Complement activation is regulated by both membrane-bound and fluid-phase proteins and occurs at all levels: from preventing the initiation of the classical pathway to inhibiting the membrane attack complex. Regulation occurs as a series of proteolytic steps with disruption of macromolecular structures (such as C3 convertases) occurring first, followed by sequential degradation of active components (C3b to iC3b to C3d, etc.). Complement also plays a role in bridging innate and adaptive immunity by

influencing both humoral and cellular immune responses [97, 98]. In normal conditions, the complement system functions to recognize/ kill foreign pathogens and modulate adaptive immunity. However, it also has a pathogenic role in various diseases including SLE (Table 1) and other autoimmune and chronic inflammatory conditions (reviewed in [99]).

Paradox of the complement system in the pathogenesis of SLE

The complement system plays a dual role in the pathogenesis of SLE: complement proteins are key components in tissue damage and inflammation, but genetic deficiencies of the early components of the classical pathway of complement, including C1q, C1r/C1s, and C4, are strongly associated with the development of lupus [100–102]. Additionally, low levels of circulating MBL were associated with SLE, but no association at the genetic level was reported [103]. The seemingly paradoxical observation that complement is required for both protection and pathogenesis has been explained by the ability of complement to participate in the removal of apoptotic debris. Complement-opsonized apoptotic debris is transported to the reticuloendothelial system by erythrocytes via complement receptor type 1 (CR1), where it is engulfed by spleen and liver macrophages via complement receptors type 3 and 4 (CR3 and CR4) and Fc receptors [104, 105]. The importance of this process has been demonstrated in C1q-deficient mice, in which apoptotic debris is not efficiently cleared and ~25 % develop glomerulonephritis associated with multiple apoptotic bodies [106]. The classical pathway also inhibits precipitation of immune complexes, and deficiencies in C1q or C4 lead to rapid immune complex aggregation [107]. Therefore, the classical pathway of complement appears to play a protective role in lupus because of its ability to opsonize the apoptotic debris that can drive the development of autoantibodies and prevent precipitation of immune complexes that can lead to kidney deposition and nephritis.

In contrast, the alternative pathway of complement has been shown in a number of recent studies to play a key role in the pathogenesis of lupus. This was first suggested by studies demonstrating that MRL/*lpr* mice deficient in alternative pathway components factor B and factor D were protected from renal disease [108, 109]. These results suggest that, although the classical pathway is responsible for initiating complement activation, alternative pathway amplification of the initial classical pathway activation is required to drive disease. This is further supported by the finding that 80 % of the post-C3 convertase complement effectors (C5a and the membrane attack complex) are generated by alternative pathway amplification [110]. Interestingly, factor B-deficient mice, but not factor D-deficient animals, had significantly decreased levels of anti-dsDNA antibodies at earlier time points compared to wild-type controls [108, 109]. These differences became less marked as the mice aged [108, 109], perhaps because of renal deposition of anti-dsDNA-containing immune complexes. These data suggest that alternative pathway activation may also drive anti-dsDNA antibody production.

Taken together, these observations indicate that the classical pathway is essential for removing potentially immunogenic self-antigens and preventing autoantibody development, but the pathogenic consequences of classical pathway deficiencies are mediated by the alternative pathway. The spontaneous activation of the alternative pathway requires constant regulation to prevent inappropriate activation on self-tissues. The importance of the

alternative pathway in complement-mediated damage suggests that defective complement regulation could be involved in SLE. One important fluid-phase regulator, factor H, accelerates the decay of the alternative pathway C3 convertase and acts as a cofactor for factor I-mediated C3b cleavage. It is active both in solution and on cell surfaces, where it works with other regulators (e.g., CD46 and CD59) to form a protective surface zone [111]. Deficiency of factor H may result in increased complement activation by pathogenic immune complexes and lead to kidney disease. In support of this, MRL/*lpr* mice deficient in factor H had accelerated lupus nephritis, despite having similar levels of anti-dsDNA antibodies compared to wild-type controls [112].

Furthermore, polymorphisms in the genes encoding factor H (*CFH*) and the factor H-related proteins (*CFHR*), located telomeric to *CFH* on human chromosome 1, have been associated with SLE susceptibility in humans [113]. The causal variant was tagged by rs6677604 located in intron 11 of *CFH* and rs16840639 located in the intergenic region between *CFHR1* and *CFHR4*. The gene products of both *CFHR3* and *CFHR1*, the two genes most 5' in the *CFHR* cluster, regulate complement activation, albeit less effectively than factor H, and both compete with factor H for binding at the cellular surface [114, 115]. In contrast, *CFHR4* activates the classical pathway of complement by binding C-reactive protein and the alternative pathway of complement by binding C3b and forming an alternative pathway convertase that is resistant to factor H-mediated decay [116], thus potentially enhancing opsonization of apoptotic debris and its subsequent phagocytosis. The effect of the associated *CFH/CFHR* variant in lupus is not known, but the tight linkage of rs16840639 to deletion of *CFHR3* and *CFHR1*, which can result in development of anti-CFH autoantibodies that block CFH binding to cell surfaces [117, 118], suggests one possible mechanism. Whether rs16840639 also alters the expression of *CFHR4* and its potential effects on clearance of apoptotic debris is not known.

Effects of complement on immune complex processing and handling

Efficient clearance of immune complexes is essential to preventing both anti-dsDNA development, by removing the antigenic structures, and pathogenesis, by inhibiting immune complex-mediated tissue targeting and IFN- α release. In vitro, complement enhances the solubility of immune complexes with the classical pathway being required to inhibit initial precipitation at the time of complex formation and the alternative pathway essential for solubilization of preformed immune complexes [107]. However, in vivo, it is likely that complement proteins primarily function to opsonize these structures rather than alter their solubility. A recent study has demonstrated that SLE patients had increased levels of microparticles that were associated with both antibodies and complement components, suggesting that microparticles could be an important antigenic scaffold for the formation of pathogenic immune complexes [119]. Neutrophil-derived NETs have also been shown to activate complement via the classical pathway in an autoantibody-dependent manner, implicating these structures as another source of the antigen component of pathogenic immune complexes [120]. Since both contain nuclear material that can drive anti-dsDNA antibody development, ineffective complement-mediated clearance of these complexes could precipitate the onset or exacerbation of lupus.

Complement receptors 1 (CR1/CD35) and 3 (CR3/ CD11b) are involved in clearing immune complexes, and both receptors have been associated with SLE. CR1 is expressed on many cell types including neutrophils, monocytes, follicular dendritic cells, B cells, and erythrocytes. CR1 on erythrocytes is involved in clearance of circulating immune complexes [121]. SLE patients have decreased levels of erythrocyte CR1, suggesting impaired immune complex handling [122], although the reduction in erythrocyte CR1 expression could be a reflection of the erythrocytes being overwhelmed by increased immune complex levels rather than a causative phenotype. CR3 (also known as Mac-1) is found mainly on monocytes and neutrophils but has also been described on T cells, natural killer cells, and B-1 cells [123]. CR3 is an integrin ($\alpha_M\beta_2$), but is also able to bind a wide variety of proteins including iC3b of the complement system [124]. A SNP in the α chain of CR3 was associated with SLE [125] and was later shown to associate with renal disease [126], although the exact functional mechanisms of the altered gene product remain unclear. It is possible that CR3 is structurally or functionally altered in SLE resulting in impaired binding of iC3b. Consistent with this is the description of an SLE patient with an α_M polymorphism in the iC3b binding site that impairs phagocytosis [127]. On neutrophils, CR3 contributes to the clearance of complement-containing immune complexes. Interestingly, neutrophil CR3 levels are increased in SLE patients, yet a clearance defect remains [128]. Neutrophils stimulated with tumor necrosis factor upregulate surface expression of properdin and activate complement via the alternative pathway directly on their surface [129]. Complement anaphylactic fragments C3a and/or C5a then lead to further activation of neutrophils including increased expression of CR3. These data suggest that increased alternative pathway complement activation contributes to elevated surface levels of CR3 on neutrophils, which in patients with lupus may still not function appropriately with respect to clearance of immune complexes.

Effects of complement on autoreactive B cells

In addition to the direct effector functions, the complement system also serves as a bridge connecting the innate and adaptive immune system. Complement degradation products covalently linked to antigen can simultaneously engage complement receptor 2 (CR2/CD21) and the antigen-specific B cell receptor (BCR), which can lower the threshold for B cell activation 1,000-fold [130]. Indeed, enhancement of B cell signaling and activation is the hypothesis for why vaccine antigens containing complement degradation products are more immunogenic [131]. However, excess complement activation can have the opposite effect and “desensitize” B cells by inhibiting initial signaling events via the sequestration of CD19 and its associated signaling molecules away from the BCR [132, 133]. The complement system therefore is uniquely positioned to serve as both a positive and negative regulator of B cell responses [134]. Autoantibody production results from aberrant B cell activation to self-antigens and complement could be involved in this process by either helping to activate or failing to inhibit.

Deficiency of *Cr2* in murine models of lupus resulted in increased anti-dsDNA antibodies and renal disease, but these effects were dependent on background genetics of the mice [135, 136]. *Cr2* has also been implicated as a lupus susceptibility gene in the NZM2410 mouse model of lupus as it encodes a structurally altered and dysfunctional protein [137].

Inclusion of the genetic interval containing the dysfunctional *Cr2* gene on a NZB background augmented autoimmunity, including autoantibody development and kidney disease [138]. However, the interval used in these studies contains other genes that may contribute to the observations of enhanced disease. In addition, *Cr2* encodes both CR2 and CR1 in the mouse by alternative splicing of a single gene product, and therefore, the specific role of CR2 in these phenotypes cannot be proven from these studies.

In humans, CR1 and CR2 are transcribed from separate genes, allowing their distinct functions to be more clearly defined. The first patient to be described with complete *CR2* deficiency had hypogammaglobulinemia and decreased class-switched memory B cells [139], demonstrating the importance of CR2 in activating B cells to low doses of antigen. Furthermore, human studies have shown associations between polymorphisms in *CR2* and the risk of developing SLE [140, 141]. Here, the alleles associated with increased risk of SLE resulted in elevated transcripts of the longer isoform of the CR2 protein, which is expressed exclusively on the follicular dendritic cell (FDC) [142], suggesting a possible FDC effect in lupus pathogenesis [141]. FDC CR2 could trap complement-coated apoptotic debris in the germinal center and present auto-antigens, such as dsDNA, to B cells.

Using CR2 to target complement inhibitors to sites of inflammation has also been tested in mouse models [143, 144]. Interestingly, soluble CR2 alone decreased immune complex formation and anti-dsDNA antibody production. This finding suggests that sCR2 can scavenge excess complement degradation products and prevent activation of autoreactive B cells [144].

Anti-dsDNA antibodies can arise by a variety of mechanisms, but activation of autoreactive B cells is likely an essential step in autoantibody development. Complement products are uniquely positioned to modulate B cell signaling and activation in response to antigenic stimulation via interactions with CR2. The associations of *CR2* with SLE suggest that complement activation directly regulates anti-dsDNA antibody production, in addition to its well-established roles in debris clearance and tissue damage.

Conclusions

SLE is a complex autoimmune disease characterized by production of autoantibodies against dsDNA. These antibodies can arise by various mechanisms, all of which center around defective clearance of DNA-containing debris. Despite DNA being an intracellular target, anti-dsDNA antibodies can exert pathogenic effects directly in the cell or indirectly via complement and/or Fc receptor-mediated mechanisms. Complement proteins have a paradoxical affect in SLE: they are often found at the sites of tissue damage and can mediate inflammation, but deficiency in these proteins leads to increased risk of disease, at least in part due to decreased clearance of immune complexes and apoptotic debris. Furthermore, complement receptors may influence the development of anti-dsDNA antibodies by participating in clearance of immune complexes and/or modulating B cell activation in response to antigen. Because of the association of anti-dsDNA antibodies with severe disease, therapeutic interventions that can prevent development or decrease circulating levels of these autoantibodies could have a significant impact on disease outcomes in SLE.

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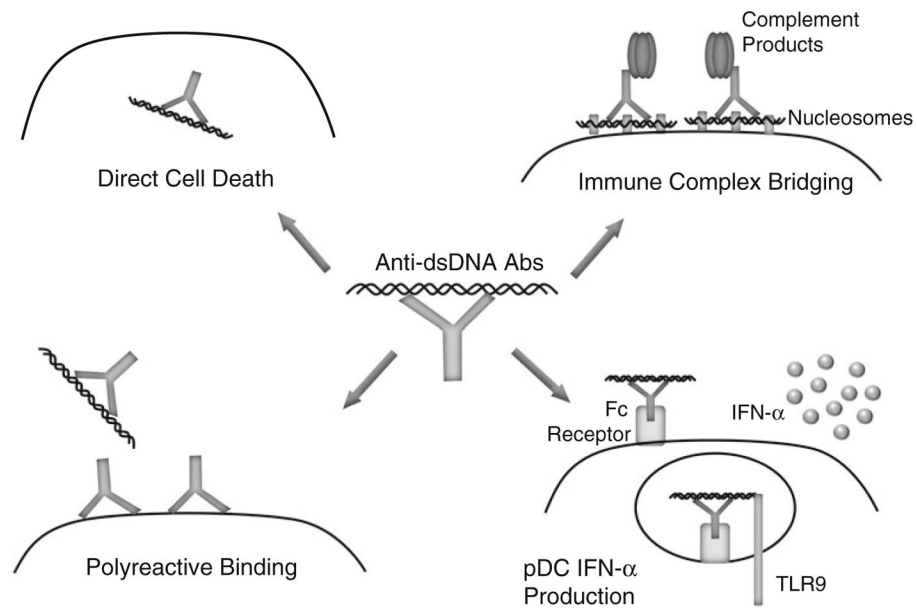


Fig. 1. Pathogenic mechanisms of anti-dsDNA antibodies. Anti-dsDNA antibodies can cause pathology by a variety of non-exclusive mechanisms: directly enter cells and induce apoptosis by binding cellular DNA (*top left*); bind cell surface, non-dsDNA antigens in a polyreactive manner (*bottom left*); bind to the DNA component of nucleosomes that electrostatically bridge the immune complex to the cell surface (*top right*); and induce IFN- α secretion after Fc-mediated uptake by delivering dsDNA ligand to TLR9 (*bottom right*)

Table 1

Human complement proteins associated with SLE

Complement protein	Function	Potential role in SLE	References
C1q/C1r/C1s	Initiate classical pathway activation	Debris clearance deficiency	[100, 101]
C4	Part of classical pathway C3 convertase	Deficient classical pathway activation	[102]
MBL	Initiates lectin pathway activation	Debris clearance deficiency	[103]
Factor H	Binds C3b, decays alternative pathway C3 convertase cofactor for factor I	Increased complement activation	[113]
Factor H-related proteins	Bind C-reactive protein and C3b; activate classical and alternative pathway	Unknown	[113]
CR1	Binds C3b/iC3b/C4b, cofactor for inactivation, clearance	Impaired immune complex clearance	[122]
CR2	Binds iC3b/C3d, modulates B cell activation	Increased autoantibody titers	[140, 141]
CR3	Binds iC3b, clearance	Impaired immune complex clearance	[125, 126]