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Abstract
Systemic lupus erythematosus (SLE) is a chronic autoimmune disease and its pathogenesis is unknown. SLE is regulated by complement receptors, proteins and antibodies such as complement receptor 2 (CR2/CD21), anti-dsDNA antibodies, Cysteine p Guanidine DNA (CpG DNA), toll-like receptor 9 (TLR9), interleukin-6 (IL-6), and interferon-α (IFN-α). Upon activation of plasmacytoid dendritic cells by bacterial CpG DNA or synthetic CpG ODN, these ligands binds to the cell surface CR2 and TLR9 to generate pro inflammatory cytokines via through NF-κB. In this, binding of these ligands induces releases of IFN-α from the plasmacytoid dendritic cells which further binds to IFN-α 1 & 2 receptors present on B cells. This binding was not completely blocked by an anti-IFNαR1 inhibitory antibody, indicating that the released IFN-α may partially binds to the CR2 present on the surface of B cells. IFN-α and IL-6 released from B cells was partially blocked by anti-CR2 inhibitory mAb171. These studies suggested that the cell surface CR2 partially involved in binding these ligands to generate pro inflammatory cytokines. More importantly these CpG DNA or CpG ODN predominantly binds to the cell surface/cellular TLR9 on B cells in order to induce the release of IL-6 and IFN-α, and other pro-inflammatory cytokines. This review describes how the bacterial CpG DNA/CpG motif/ CpG ODN regulate the innate immune system through B cell surface CR2 and TLR9 in B cell signaling.

Keywords: Complement receptor 2 (CR2/CD21), Cysteine p Guanidine DNA (CpG DNA), Cysteine p Guanidine-Oligodeoxynucleotide (CpG-ODN), Toll like receptor 9 (TLR9), Interleukin-6 (IL6) Interferon-α (IFN-α), Anti-dsDNA antibodies and Systemic Lupus Erythematosus (SLE)

Introduction
Role of complement receptor 2 (CR2/CD21) in SLE

Human complement receptor 2 (CR2/CD21) is a 145 kDa trans membrane glycoprotein comprised of 15 or 16 short consensus repeat domains (SCRs). Each SCR consists of 60-70 amino acids length, 28 amino acid trans membrane domains and 34 amino acid intracytoplasmic tails, connected by 4-8 amino acid linker peptides in solution for flexibility [1-8]. CR2 is a multi-functional glycoprotein receptor expressed on B cells, FDCs and a subset of peripheral and thymic T cells [9-11]. CR2 has five well characterized ligands, including the complement C3 derived products C3d, iC3b and C3dg [12,13], which become covalently attached to foreign antigens as well as the EBV gp350/220 viral coat protein [14,15] and CD23 [16,17]. The C3d binding site resides in the first two SCRs of CR2 [1,2,18], as well as the EBV gp350/220 viral coat protein [3,4,14,19], the low affinity IgE receptor CD23 [16], IFN-α [5], bacterial DNA [6] and CpG ODN 2006 [20]. Except for the low affinity IgE receptor CD23, which finds to an additional site located in SCR domain 5-8. In all these the ligands binding site present in SCR 1-2 was inhibited by the anti-CR2 inhibitory monoclonal antibody raised against the first 2 SCR, mAb 171 [21]. In terms of the co-crystal structure of the CR2 SCR 1-2-C3d complex [22] it has been reported that the interaction interface markedly differed and the structure was more consistent with the available biochemical data than the previous co-crystal structure published [18]. However, the CR2 interface in the new structure still contains a positively charged region [22].

In mice, CR2 is encoded along with the larger receptor CR1 by the Cr2 gene, which produces both proteins through alternative splicing by a common mRNA [23]. In humans, CR1 and CR2 differ not only in their ligand binding, but also in their cellular
distribution patterns and functions [24]. The expression and function of CR1 and CR2 in various B cell subsets has been reported in both healthy subjects and rheumatoid arthritis (RA) patients at various stages of the disease by FACS analysis, H3-thymidine incorporation and ELISA [25]. It was found that CD19+CD27+ naive B cells up-regulate the expression of the inhibitory CR1 during differentiation to CD19+CD27+ memory B cells in both healthy donors and RA patients, whereas the expression of activating CR2 is down-regulated. This study clearly demonstrated that the expression of these two antagonist, complement receptors are regulated differentially during the development of human B cells, a phenomenon which may influence the maintenance of peripheral B cell tolerance. These findings suggest mouse CR2/CR1 receptors may be in fact two separate complement receptors.

Recently [6] reported that multiple forms of DNA, including bacterial, viral and mammalian DNA bind to human CR2 with moderately high affinity using ELISA and surface plasmon resonance techniques among which bacterial DNA binds at higher affinity. Bacterial DNA bound to the first two domains of CR2 and this binding was blocked by using the specific inhibitory anti-CR2 mAb 171. Further more recently [20] reported that bacterial DNA/CpG DNA/CpG motif/CpG ODN 2006 binds to CR2 by ELISA and surface plasmon resonance analysis and this binding was blocked by anti-CR2 inhibitory mAb 171. In addition CpG DNA acting as a birding molecule between plates bound TLR9 and solution base CR2. Furthermore this CpG DNA/CpG motif/CpG ODN 2006 binds to cell surface CR2 from primary B cell to induce to release IL6 and IFN-α and this release was partially blocked by anti CR2 inhibitory mAb 171. In addition recently [6] reported that DNA immunization in Cr2−/− mice revealed a specific defect in the immune response to bacterial DNA. CR2 can act as a receptor for DNA in the absence of complement C3 fixation to this ligand. These results suggest that CR2 plays a critical role in the recognition of foreign DNA during the host-immune response. This recognition function of CR2 may be a mechanism that influences the development of autoimmunity to DNA in SLE.

CR2 has been implicated in the pathogenesis of SLE in several studies [26,27]. In mice, CR2 is encoded along with the CR1 gene which produces both receptors as alternate splicing of common mRNA [23,28], while in humans these proteins are derived from two separate but closely linked genes on chromosome 1. CR2−/− mice are defective in antigen specific as well as T cell dependent and independent immune responses [29], whose effects are due to the absence of CR2 and CR1 receptors on the B cell and FDC surface [30]. Although the down regulation of CR2/CR1 has been reported in SLE, mice lacking CR2 do not develop the lupus phenotype, indicating that there is no role of CR2/CR1 in murine lupus. However, CR2−/− mice do exhibit defects in B cell memory and natural antibody development [31]. A recent study suggests that CR2 also plays a major role in maintaining tolerance to self-antigens such as dsDNA, chromatin and histone complex, but the relationship to the development of SLE and the molecular mechanism causes this function to be unknown. This study also suggests that CR2 may be a strong candidate gene in lupus susceptibility in the NZM2410 mouse model [32]. Furthermore, introducing a N-linked glycosylation site in the ligand binding domain of CR2 due to a single nucleotide polymorphism functionally alters the asparagine residue results in defective ligand-binding in lupus [33]. There is no report available that CR2 interacts with TLR9 on the cell surface. We propose a model that describes the interaction of CR2, CpG DNA,TLR9, IFN-α, IL6 and anti-dsDNA antibody in the pathogenesis of SLE (Figures 1 and 2).

**Figure 1:** The Toll-like receptors (TLRs) are a family of receptors involved in pathogen recognition that play crucial roles in initiating inflammation and the development of adaptive immunity. Schematic diagram showing activation pathway of the ligand-cell surface receptor (CR2, bacterial DNA and TLR9) interactions in B cell signaling to generate pro-inflammatory cytokines via NF-κB in autoimmune diseases such as Systemic Lupus Erythematosus.
breakdown, as well as DNA fragmentation by apoptotic cells during infection, leading to IC formation, deposition, and resulting in tissue damage in SLE. The anti-dsDNA antibody is used as a valid clinical biomarker for the diagnosis of SLE [35]. In healthy subjects, PBMCs, expressing the DNA binding receptor, rapidly clear the dead cell-DNA, nucleosomes, chromatin-immune complexes, anti-dsDNA antibodies and nucleosome antibodies, but this clearance mechanism is defective in lupus patients [37]. Furthermore, simultaneous positivity for anti-DNA, anti-nucleosome and anti-histone antibodies is a biomarker for lupus nephritis [38]. Recent studies also suggest that the membrane-bound complement regulatory proteins decay-accelerating factor (DAF), membrane cofactor protein (MCP), CR1 and CD59 serve as both biomarkers and also therapeutic targets for treatment of SLE [39]. The presence of anti-dsDNA antibodies by the Crithidia luciliae immunofluorescence test (CLIF) in newly diagnosed SLE is associated with certain markers of increased disease activity. It may have utility as a biomarker for a specific clinical phenotype suggestive of a more severe involvement at the time of the diagnosis [40]. This review describes the role of immune proteins and antibodies in the regulation of SLE, in particular, the mechanism by which the excess production of anti-dsDNA antibody together with the TLR9-CR2 interaction playing a role in the pathogenesis of SLE.

**Role of anti-dsDNA antibody in SLE**

SLE is a chronic complex rheumatic autoimmune disease regulated by immune proteins and antibodies. In the United States more than one million people suffer from this disease, 8 females and for every male (an 8:1 ratio) in an age range of 18-35, mainly affecting connective tissue such as the skin, joints, renal, cardiovascular and nervous manifestation by genetic modification in the lupus susceptible genes and to a lesser extent, environmental factors [35]. Upon activation or pathogenic infection of the host immune system by bacterial, unmethylated CpG rich DNA, B cells secrete an excess of anti-dsDNA antibodies that form small immune-complexes (ICs). These ICs become deposited in the kidneys, joints and blood vessels, causing inflammation and tissue damage [35,36]. In addition to this, nucleosomes are the building blocks of DNA, and chromatin

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**Figure 2:** A schematic representation of IFN-α induction in the pathogenesis of Systemic Lupus Erythematosus. Here we show that IFN-α binds to CR2 and that B cells respond to this by an increase in interferon-α inducible gene expression (IFαIG), leading to immune complex deposition and end organ damage.

and 2). The CR2 domain of TT30 (65 kDa recombinant human fusion protein consisting of the iC3b/C3d-binding region of complement receptor 2) specifically targets the fusion protein to sites of complement activation that are marked by the deposition of C3 fragments, including iC3b and C3d. Thus, TT30 is designed to selectively inhibit complement alternative pathway (CAP) activation specifically at the sites of complement activation, which in the case of Paroxysmal Nocturnal Hemoglobinuria (PNH), is the surface of (Glucose phosphate isomerase) GPI-deficient RBCs [34]. This review mainly focuses on human complement receptor 2 (CR2/CD21) and its role in SLE.

**Role of bacterial DNA in SLE**

Bacterial DNA plays a major role as the potential factor
in the innate immune system activation through an apoptotic process of host immune cells by virulent pathogenic attack. In this context, the bacterial cell wall, containing lipopolysaccharide (LPS) or bacterial-DNA containing an unmethylated CpG motif, is the prime structural molecule that activates immune cells. In bacteria, the CpG motif is at least a 20 fold greater frequency than in mammalian DNA. However, these CpG motifs are suppressed and methylated in eukaryotic DNA [41]. Mammalian-DNA is generally non-immunogenic to mammals and contains reduced numbers of CpG dinucleotides, which are mostly methylated. In addition, mammalian-DNA has potential inhibitory activity on the innate immune activation processes through the activity of TLR9. The ineffective clearance of the apoptotic dead cell DNA results in an increased level of nuclear antigen as a form of biological garbage in the body; the result of retaining most of the hypomethylated DNA fragments and host cell chromatin in the circulation that is recognized by the TLR9 [42] as well as by B cells being present on their surface linked via a DNA binding receptor. Presently, there is no report of the presence of any such DNA binding receptor on the surface of B cells; leading to the hypothesis that the B cell surface receptor, human CR2/CD21, mediates the clearing of biological garbage in vivo [6,20]. These DNA fragments are effectively cleared in healthy subjects by PBMCs, but this process is defective in SLE patients. Recent studies have also suggested that a synthetic CpG ODN retains a stimulating capacity similar to bacterial methylated DNA; hence, researchers used an identical synthetic CpG ODN to study the innate immune system activation in B cells [43]. This was also used as a therapeutic drug for treatment of a variety of diseases such as cancer, allergies, asthma and infectious diseases [44,45]. In addition, studies have suggested that the hypomethylated mammalian genomic self-DNA containing CpG motif mimics bacterial DNA in stimulating anti-DNA antibody production in the pathogenesis of SLE, but the mechanism of action is not known [46]. Ultimately, these CpG rich DNA recognize and bind the TLR9 receptor for subsequent activation, leading to a range of biological functions.

The TLR9 receptors are expressed on many different cells, but predominately on plasmacytoid precursors, dendritic cells, B cells and, to a lesser extent, monocytes. CpG DNA not only directly activates PDCs, but in humans the autoantibody-DNA complex activates dendritic cells in cooperation with CD32 and TLR9 [47]. CpG DNA does not stimulate B cells, but it can synergize with certain antigens and lead to B cell proliferation [44,48]. Bacterial DNA, as well as CpG ODN 2006 containing a human-specific CpG motif binds to CR2/CD21, by ELISA and surface plasmon resonance analysis [20]. Although the majority of the available reports suggests that the CR2/CD21 receptor is expressed as a trans membrane glycoprotein, there is evidence that it is also expressed inside B cells and FDCs (Karp and Holers et al., unpublished data). In addition, the rapidly internalized unmethylated bacterial CpG DNA or CpG ODN binds to TLR9 in the endocytotic vesicle at acidic pH, a special characteristic of the conditions that exist in the endosomes, which is not same when it binds to human CR2, in which case it binds at physiological pH 7.4. This evidence is consistent with the literature and strongly suggests that upon the binding of unmethylated CpG DNA or CpG ODN with TLR9 in the endosomes, these CpGs further bind to human CR2 outside the acidic pocket in cells to activate cell signaling cascades such as NF-kB activation in the nucleus followed by pro-inflammatory cytokine and chemokine release. Consequently interactions between TLR9 and CR2 may further play a role in signaling cascades in the innate immune system. In addition, a limited body of literature supports TLR9 being present on the cell surface, where it acts.
to bind, deliver and activate the TLR9 dependent signaling cascade to release pro-inflammatory cytokines and chemokines, particularly IL-6 from B cells and IFN-α from PDCs, which may be CR2-dependent processes. On the other hand B cell surface CR2 may play coordinated role in delivers captured foreign ligands such as bacterial DNA/CpG motif/CpG DNA to cell surface TLR9 to engage binding, internalize and further cell signaling. In this, Chloroquine have been used to block the CpG ODN-TLR9 binding in endosomes, which particularly blocks the interaction between CR2-TLR9 in vitro, whereas solvent did not [20]. Recent studies also have suggested that exacerbated autoimmunity in the absence of TLR9 in lupus-prone MRL Faslpnr mice depends on IFNαR1. Recent research has focused on the genes associated with Toll-like receptors, type I interferons, immune regulation pathways and immune-complex clearing. TLR7 and TLR9 have been extensively studied using lupus-prone mouse models [60]. CpG and TLR9 play a protective immune response in lung fibrosis in mice [61].

### The role of IFN-α and Cytokines in SLE

IFN-α is a subtype of the Type I family of interferons expressed by PDCs, lymphocytes and fibroblasts [62,63]. Type I interferons are involved in viral pathogenesis and cancer; and it also exhibits anti-viral and anti-proliferative effects on cells. IFN-α is used for the treatment of several diseases, such as hepatitis C and various types of lymphoid cancers [64], and it also plays an important role in the pathogenesis of SLE. Bacterial-CpG DNA/CpG-ODN/CpG motif binds to TLR9 to stimulate the release of pro-inflammatory cytokines and chemokines, including IFN-α. IFN-α in turn binds to the IFNαR1 & 2 receptors present on B cells for subsequent activation [65]. On the other hand [5] published data suggests that in addition to IFNαR1 & 2 binding, this released IFN-α also binds to soluble human recombinant CR2 (human rCR2), as demonstrated by surface plasmon resonance analysis and ELISA. In addition, the IFN-α inducible gene expression (IFNαG) of myxovirus resistance (MX1) and protein kinase RNA regulated protein (PRKR) is partially blocked by the inhibitory anti-CR2 mAb known as “171”, shown by quantitative RT-PCR analysis [5]. Furthermore, it was shown using flow cytometry that an anti-IFNαR1 antibody did not block IFN-α binding on the cell surface. This further supports our hypothesis that in addition to IFNαR1 & 2 binding, the released IFN-α binds to the cell surface CR2.

The role and importance of the interaction between CR2-IFN-α in SLE are poorly understood. It was reported in the early 1990’s that IFN-α blocked the Epstein barr virus (EBV) binding and capping in Burkitt lymphoma’s Raji cells expressing the CR2 receptor, suggesting that IFN-α contains a sequence motif similar to the binding site that exists on the complement fragment C3d, which is blocked by the anti-CR2, C3d and IFN-α peptide antibody, but not by anti-IFN-α antibody [66]. Another previous study suggested that IFN-α directly blocks EBV infection of B cells through receptor competition [67] or blocks the binding of other ligands to CR2. There is no data available, at present, to show whether IFN-α blocks CD23 when it binds to B cells in vivo. Additionally, the functional significance of CR2-IFN-α interactions in SLE are unknown. The biological role of these CR2 interactions with IFN-α and DNA-containing complexes is not well understood, but may be important in the development of the type of SLE that is characterized by the enhanced IFN-α levels and loss of the tolerance to DNA-containing self-antigens [68].

The type I interferon (type 1 IFN) system is persistently activated in systemic lupus erythematosus (SLE) and many other systemic autoimmune diseases. Studies have shown an association between SLE and several gene variants within the type I IFN system [69]. Cytokines regulate control the immune system. In SLE, several of the following cytokines are over expressed and contribute to the disease pathogenesis, i.e. TNF-α, IL-1, IL-6, IL-10, IL-15, IL-17, IL-10 and IL-23. Cytokine inhibition has been successfully used to treat other rheumatic and autoimmune diseases, and several cytokines are currently being investigated to determine whether inhibition would be therapeutic in lupus [70]. It was also reported that a variety of cytokines, including BLyS, interleukin-6, interleukin-17, interleukin-18, type I interferon and TNF-α play a major role in the pathogenesis of SLE [71].

### The mechanisms of CR2- bacterial DNA, TLR9, IL-6, IFN-α and anti-dsDNA antibody interaction in the pathogenesis of SLE

TLR9 plays a critical role in recognizing and binding bacterial CpG DNA/CpG ODN/CpG motif, and activating B cell signaling. All of the TLRs so far identified in the innate immune system competitively recognize different foreign ligands present via pathogenic infection. The peptidoglycans and bacterial lipopolysaccharides present in the cell wall of gram-positive bacteria are recognized by TLR2. The dsDNA recognized by TLR3. The LPS present in the gram-negative bacterial cell wall components is recognized by TLR4. The ssRNA recognized by TLR7 and 8. All of the TLR signals in the cell are transferred through a common signaling pathway which involves myeloid differentiation factor (MyD88), IL-1R associated kinase (IRAK), Tumor necrosis factor associated factor 6 (TRAF6), Tumor growth factor-B activated kinase1 (TAK1), and the kinase complex of IkB (IKK) and nuclear factor-kappa B (NF-kB) [72]. In this review, we address, in addition to this activation of the innate immune response, the internalized bacterial CpG DNA binding to TLR9 that may binds to intracellular CR2 for further cell signaling. On the other hand the cell surface CR2 and TLR9 play a coordinated role in binding, delivering and internalizing the foreign bacterial CpG DNA/CpG ODN/CpG motif in order to activate the B cell signaling. Since CR2 ligation is independently involved in NF kB activation, it is possible that in addition to bacterial CpG DNA binding to TLR9 and subsequent activation of IFN-α and IL6 release is through the TLR9 dependent fashion [20]. Furthermore, a DEC-205, a multilectin receptor present on the surface of B cells binds to CpG-ODN by ELISA and Surface plasmon resonance analysis, which is B cell [73] dependent TLR9 signaling. Bacterial CpG DNA-TLR9 binding may promote TLR9-CR2 dependent NF kB activation in the nucleus. It is shown in figures 1 and 2 that bacterial CpG DNA-TLR9-IFN-α binds to the IFNAR1 & 2 receptors as well as B cell surface CR2. On the other hand, CpG DNA-TLR9-CR2 and NF kB activation are CR2 dependent processes causing overlapping signaling to take place during this B cell activation. These activation mechanisms may take place in a complement-dependent or complement-independent fashion.

### Conclusion

In summary, CR2 generally interacts with many ligands. Bacterial CpG DNA/CpG motif/ CpG ODN in B cell activation in particular largely depends upon TLR9 signaling. B cell surface CR2 and cell surface/ cellular TLR9 play a coordinated role in...
binding, delivering and internalizing, subsequent co-localization may be a vital process that leads pro-inflammatory cytokines generations via NF-kB mediated signaling. Importantly, IFN-α and IL6 a major cytokines play a role in SLE is partially blocked by anti CR2 inhibitory mAb 171 indicated that CR2 partially involved in recognizing this foreign CpG DNA/CpG motif/ CpG ODN. TLR9 predominately play a role which is known to be involved in multiple cell signaling systems in recognizing pathogenic foreign substance in B cell activation.

References

to the positivity of anti-dsDNA by the Crithidia luciliae method. Lupus. 2015;11:1198-1203.


57. Pogge SL, Preston BT, Stalder J, et al. The receptor for type I IFNs is highly expressed on peripheral blood B cells and monocytes and mediates a distinct profile of differentiation and activation of these cells. J Interferon Cytokine Res. 2004;24(2):131-139.


