

Interferons, B Cells and Neutrophils: Innate and Adaptive Allies in Systemic Lupus Erythematosus

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Abstract

Systemic lupus erythematosus (SLE) is clinically and immunologically heterogeneous with variable organ involvement, severity and therapeutic responses. B cells are known as mediators of disease manifestations. Therefore, disease control is targeted by inhibiting proliferation and inducing apoptosis of B cells via conventional cytotoxic drugs or newly developing biologics. However, the outcome of therapy is sometimes heterogeneous which further attracts to understand other immune mechanisms influencing B cell functions such as interferons (IFNs) cytokines and the innate cells, neutrophils. Successful management of SLE requires an understanding of how these factors interact, taking into consideration the patients' variations in these factors which might confer heterogeneity in disease outcome. In this review, it will be focused on the existing data in literature on the mutual influence between interferons, B cells and neutrophils in disease pathogenesis and the clinical impact in disease assessment and their potential blocking.

Keywords: Systemic Lupus Erythematosus; B Cells; Neutrophils; Cytokines and Inflammatory Mediators; Autoantigens and Autoantibodies

Abbreviations

SLE: Systemic Lupus Erythematosus; **pDCs:** Plasmacytoid Dendritic Cells ; **TLRs:** Toll-Like Receptors; **IFNs:** Interferons; **BAFF:** B-Cell Activating Factor; **APRIL:** A Proliferation-Induced Ligand; **PBMCs:** Peripheral Blood Mononuclear Cells; **PB:** Plasma blast; **PCs:** Plasma Cells; **CSR:** Class Switch Recombination; **Ig:** Immunoglobulins; **BM:** Bone Marrow; **IL:** Interleukin; **BCR:** B Cell Receptor; **mAb:** Monoclonal Antibodies; **SHM:** Somatic Hyper Mutation; **AICDA:** Activation-Induced Cytidine Deaminase; **TNF- α :** Tumor Necrosis Factor- α ; **MyD88:** Myeloid Differentiation Primary Response 88; **IFNAR:** Interferon Alpha Receptor; **WT:** Wild Type; **TH:** T Helper; **IFNR:**IFN Receptors; **Ab:** Antibody; **ODN:** Oligo Deoxy Nucleotides; **NK:** Natural Killer; **NETs:** Neutrophil Extracellular Traps; **MZ:** Marginal Zone; **TACI:** Transmembrane Activator and Calcium Modulator and Cyclophilin Ligand Interactor; **LDGs:** Low-Density Granulocytes; **G-CSF:**

Granulocyte Colony Stimulating Factor; **GM-CSF:** Granulocyte Macrophage Colony Stimulating Factor; **PAD4:**Peptidyl Arginine Deiminase-IV; **ACPA:** Anti-Citrullinated Peptide Antibodies; **Siglec-1:**Sialic Acid-binding Ig-like Lectin-1; **UNGAL:** Urinary Neutrophil Gelatinase-Associated Lipocalin.

Introduction

Systemic lupus erythematosus (SLE) is a multi-system autoimmune disease characterized by involvement of all organs in the body. The clinical features of the disease are heterogeneous and run in a remitting-relapsing course with variable prognosis [1]. SLE is characterized by the important role of B cells and the wide range of autoantibodies targeting particularly the nuclear antigens and mediating the disease [1, 2]. B cells are targeted whether by standard cytotoxic drugs or by B-cell depletion therapy as biologics. However, the heterogeneity among patients in the clinical effects suggests the presence of underlying factors contributing to the development of B cells and their aberrant behaviour [3, 4]. Research has focused on the role of innate mechanisms such as plasmacytoid dendritic cells (pDCs), Toll-like receptors (TLRs), interferons (IFNs), neutrophils and gene mutations in the initiation and perpetuation of the disease [5-7].

IFNs are a big family composed of type-I that includes importantly IFN- α/β and type-II that includes IFN- γ [8]. IFN-like molecules have also been described as type-III IFN, (IFN- λ 1, λ 2, and λ 3) [9].IFNs play a physiologically important role in the defense mechanism against viral and bacterial infection. They are secreted in response to activation of pathogen recognition receptors on the antigen presenting cells [10].A persistent mimicked antiviral response is appreciated in SLE in which IFNs drive autoimmune reactions [11]. IFNs expression and signature, mainly type-I and -II, have been confirmed in SLE and also linked to disease activity in some studies [12-14]. IFN blocking therapies have demonstrated evidence of efficacy [15]. Of note, neutrophils have grasped attention as newly discovered partners in SLE pathogenesis via synthesizing IFNs and other pro-inflammatory

cytokines and enhancing autoantibodies production [16]. This review sheds the light on the interplay between IFNs, B cells and neutrophils for better elucidation of the comprehensive interaction between them.

Interferons Enhance B cell Development and Immune Repertory

Interferons protect B cells at various stages of development. One mechanism is through inducing the secretion of growth factors, B-cell activating factor (BAFF) and A proliferation-induced ligand (APRIL). In vitro and in vivo studies on healthy donors or patients treated with IFNs showed that upon exposure to IFNs, BAFF and APRIL are up regulated in peripheral blood mononuclear cells (PBMCs), granulocytes and other cell types [17-20]. In BAFF transgenic mice model, excess BAFF acts as a rescuer to auto-reactive B cells that are normally deleted at the late T2 stage in the peripheral development; they mature and colonize the splenic follicles. Also, auto-reactive B cells normally deleted at the marginal zone are found to be rescued and repopulate on excess BAFF [21]. BAFF also enhances plasma blast (PB) survival and differentiation to plasma cells (PCs) [22]. BAFF/APRIL assist in the class switch recombination (CSR) of immunoglobulin's (Ig) from C (μ) to C (γ) and /or C (α) [18]. IFN type-I also up regulates the growth factors co-receptor, heparan sulphate, on murine splenic B cells. Heparan sulphate is thought to play a significant role in B cell immune response since its removal abrogates antibody production in response to APRIL [23].

Noticeably, the effect of IFNs also involves stages of B cell development in bone marrow (BM). In SLE BM, transitional B cells, particularly T2, are shown to be significantly higher in IFN-high SLE BM as compared to IFN-low SLE BM and healthy controls, which could be attributed to the high expression of BAFF induced by IFN- α in BM [24, 25]. Furthermore, the memory fraction in BM showed significant reduction in the un switched memory CD27/Ig D+ and expansion of the switched CD27/IgD- memory B cells in SLE high-IFN BM. This was linked to more autoantibodies and chemokine expression in the high-IFN BM and was associated with a parallel response in the memory B cells in the peripheral blood [24].

Contrarily, IFN type-I showed a selective inhibition of interleukin (IL)-7 dependent growths and proliferation of pre-B cell lines suggesting its regulatory role that allows the selective survival of immuno competent B lineage cells [26]

PCs are also thought to be influenced by factors conferring longevity. PCs reside in a BM niche that is composed of stoma cells and multipotent hematopoietic precursors as shown previously in the BM of animal models [27]. There has been great belief that long-lived PCs derived from memory B cells have the potential to re-enter the cell cycle if the niche environment changes [28]. Notably, the high expression of type-I IFN was confirmed in BM of SLE patients and was associated with increase in BAFF and APRIL levels [24]. However, modification of niche environment by high cytokines levels that might confer the longevity to PCs in BM warrants investigations.

IFNs also enhance the production of immunoglobulin's. In mice models, it was shown that TLR ligand that result in type-I IFN production cause T cell-independent antibody response. This response was associated with high titers of all IgG subclasses [29, 30]. Moreover, it was noticed that the secondary immune response is preserved with type-I IFN priming indicating that type-I IFN induces long-term antibody production and memory function [30]. On the other hand, lack of type-I IFN was associated with absence of IgG's in pDCs-depleted human PBMCs after culturing with live influenza virus despite the presence of B cells at the end of culture where as virus-triggered pDCs induced B cell differentiation into PCs [31].

Interferons Improve Proficiency of Autoimmune B Cells

IFNs not only affect the development of B cells but also affect B cell functions as summarized in Table 1. Type-I IFN lowers the threshold of stimulation of B cell receptor (BCR) and induces the expression of MHC-II, CD69, CD86 and CD25, thus potentiating the antigen presenting capacity of B cells as shown in splenic and BM B cells of the murine models. Additionally, it reduces the concentration and duration needed by anti- μ monoclonal antibodies (mAb) to produce maximum BCR internalization probably via enhancing calcium influx following BCR stimulation [32]. (Table 1)

The influence of type-I IFNs encompasses the TLRs of B cells. CpG-mediated TLR-9 activation of healthy donors 'naive B cells increases the antigen presenting capacity. This results in IgM secretion with CSR and somatic hyper mutation (SHM), via activation-induced cytidine deaminase (AICDA) up regulation, and ultimately IgG production [33]. It also induces the differentiation of CpG-activated naive B cells into memory cells and increases the production of IL-1 β , IL-10, IL-6 and tumor necrosis factor- α (TNF- α) and myeloid differentiation primary response 88 (MyD88) expression with further enhancement of CpG function [34].

In this context, pristane treated interferon alpha receptor (IFNAR) 2-/- mice failed to produce IgG antibodies against immune complexes compared with pristane-treated wild type (WT) with no significant difference in IgM in both groups. Furthermore, IFNAR2-/- B cells failed to up regulate TLR7 and TLR9 and the responses to TLR7 and TLR9 stimulation were reduced after type-I IFN treatment [35]. IFNAR1-/- B cells also suffered a significant reduction in proliferation after TLR7 stimulation compared to WT B cells. Besides, the ability of B cells to produce cytokines after TLRs stimulation was markedly diminished in IFNAR1-/- B cells [36]. Deletion of IFN type-I receptors was associated with marked reduction in anti-erythrocyte antibodies, hemolytic anemia, anti-ds DNA antibodies, kidney disease and mortality in the knockout mice [37]. However, these findings were contradictory to earlier findings in congenic lupus-prone mice lacking type-I IFN receptors which exhibited surprisingly worsening of lymph proliferation and end-organ damage [38]. This might be attributed to different responses to IFNs in different strains of murine models [39]

It was observed that activated pDCs isolated from healthy donors, through their high IFN-I production, are the principal mediators of B cell differentiation into IgM, IgG and IgA secreting cells and up regulation of transcription factors Blimp-1 and XBP-1 [6]. B cells adopt a B cell effector-1 function, similar to T helper (TH)-1 function, which is facilitated by IFN- α . It was noted that IFN- α increases STAT4 activation and T-bet expression in healthy donors 'resting B cells which in turn leads to enhanced IFN- γ expression in B cells in response to IL-12 or BCR and TLR-2 stimulation [40].

Whilst type-I IFN exerts their function as anti-viral, anti-cancer and anti-angiogenic through mediating apoptosis and up regulating proapoptotic genes [41], it protects B cells from apoptosis by PI3-kinase-Akt pathway which is downstream from IFN receptors (IFNR) and this function was abrogated by IFNR blockade [42]. In other words, IFN type-I works in favor of B cells by providing accumulated self-antigens and protecting them against apoptosis. In vitro studies showed that type-I IFN up regulates the surface expression of the adhesion molecule L-selectin in human B lymphoid Daudi cell line. L-selectin is essential in the migration of B and T cells to peripheral lymphoid tissues [43-45]. Therefore, type-I IFN boosts trafficking of B cells to peripheral lymph nodes.

Noticeably, B cell responses to IFN- γ were shown to be more complex. While IFN- γ plays an important role in T cell-mediated activation of B cells during initiation of primary antibody response, it targets stimulated B cells inhibiting further stimulation and reducing IgM secretion [46, 47]

B cells as Innate Proponents Preserve IFNs

In certain circumstances, B cells play a role in the innate defense mechanism and cause increased production of IFNs by a way or another. Interestingly, CD20- antibody (Ab) was found to affect human B cell biology in vitro and enhances type-I IFNs production upon viral infection. IFNs production was confirmed to be not related to viral replication and specific for B cells with CD20-Ab dose dependent manner particularly early in treatment [48]. B cells were also reported to produce type-I IFN in response to TLR7 and TLR9 stimulation [36].

B cells, induced by RNA-immune complexes, Oligo Deoxy Nucleotides (ODN) or herpes simplex virus, were noticed to increase IFN- α production when they are co-cultured with pDC's in a dose dependent manner compared with pDCs-only cultures. Clustering of pDCs and B cells in the co-cultures indicates cell to cell contact stimulation [49]. Also, CpG-stimulated B cells enhance IFN- α production by pDCs, though they cause inhibition of activated myeloid DCs and enhance apoptosis of this subset [50].

AB-cell subset, CD11a high FC γ R III high CD19+, was reported to be induced in the central and peripheral lymph organs of mice challenged with TLR ligand such as lip polysaccharide and CpG-ODN. This subset lacks high expression of MHC-II but rich in cytokines and cytokine receptors, and upon CD40 activation, they produce IL-1, IL-6 and IFN- γ . The production of IFN- γ was high

and comparable to natural killer (NK) cells [51].

The Interplay between Neutrophils and the Mononuclear Cells

Neutrophils have long been known for their roles as the first line of defense against microbial infection. They are the first cells recruit at sites of inflammation. They function as phagocytes and cause intracellular degradation of the microbes. Additionally, they release antimicrobial agents and form neutrophil extracellular traps (NETs) that limit and eliminate different microbes [52, 53].

The role of neutrophils extends to the enhancement and regulation of other immune cells. For example, LL-37 and heparin-binding protein, products of neutrophils, mediate recruitment of monocytes and subsequent augmentation of the immune response [54]. Moreover, heparin-binding protein and human neutrophil peptides 1-3 enhance the phagocytic activity of macrophages and trigger them to release TNF- α and IFN- γ [55]. Also, DCs are attracted and activated by alarmins to produce augmentation of the immune response [56]. The story is more complex with T cells where neutrophils appear to activate or suppress T cells according to different contexts. The cross talk between both types of cells occurs via cytokines from both sides that affect the function of each other and via cell-to-cell contact [57].

Neutrophils Outfit B Cells in the Lymphoid Tissues and Bone Marrow

Neutrophils colonize physiologically around the marginal zone (MZ) areas of the spleen and in the perifollicular areas of the mesenteric lymph nodes in healthy donors where they interact with MZ B cells; therefore known as B cell-helper neutrophils [58]. However, in the pathological conditions such as SLE, the MZ is lost [59] and the neutrophils are noticed extensively infiltrating the germinal-center areas activating MZ B cells via contact-dependent and contact-independent mechanisms and their efficacy in B cell activation was comparable to that of CD4 T cells [58]. Similar findings in WT and lupus prone mice, neutrophils reside in the perifollicular areas of the spleen in WT with more extension to the T cell zones in lupus prone mice [60]. The interaction between MZ B cells and B cell-helper neutrophils results in up regulation of AICDA gene which is responsible for inducing CSR and SHM [58, 61].

Immunoglobulin diversification and SHM are also the result of the contact independent effect of neutrophils on B cells. Neutrophils are reported to secrete BAFF, APRIL and IL-21. B cell-helper neutrophils tend to express higher surface BAFF and release more soluble BAFF, APRIL and IL-21 than do conventional neutrophils [58]. Correspondingly, MZ B cells express mRNA for transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) higher than naive B cells but comparable expression of BAFF receptors. Blocking of BAFF, APRIL or IL-21 receptors results in impaired IgM, IgG and IgA production and poor development of effectors B cells [58, 62, 63].

Since neutrophils also enhance CD4 T cells proliferation and IFN- γ production via BAFF, depletion of neutrophils resulted in

dramatic reduction of BAFF and IFN- γ levels, the numbers of CD4 T cells, germinal center B cells and PCs together with diminished serum anti-ds DNA and immune complexes deposition in the murine kidneys [60].

The link between neutrophils and B cells was also prominent in the BM. The mature neutrophil fraction in SLE BM exhibits significantly higher IFN- α , BAFF and APRIL expression which could not be attributed to DCs fractions and this finding was also noticed in the BM neutrophils of lupus prone mice [24].

Neutrophils Are an Additional Source of IFN

In vitro analysis of type-I IFN production in different peripheral blood leucocytes from healthy donors in response to different stimuli showed that neutrophils were the strongest producer of IFN- α in response to chromatin in a dose dependent manner. Of note, this response was shown to be variable among different individuals of SLE and healthy controls and higher percentage of IFN-producing neutrophils were obtained when neutrophils were cultured with the whole blood. As regarding CpG-ODN stimulation, pDCs produce much more IFNs but neutrophils' production is still comparable owing to their markedly higher abundance [64].

There has been also other evidence in the peripheral blood confirming that neutrophils are producers of IFNs. Low-density granulocytes (LDGs), a subset of neutrophils, have been identified in the mononuclear cell fraction in the peripheral blood of SLE patients and represent activated phenotype of neutrophils although they lack the phagocytic activity. They are characterized by their capacity to synthesize high levels of type-I IFNs and IFN- γ [65, 16]. Stimulation of IFN- α production from neutrophils is enhanced by the additive effect of other cytokines such as granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF) or IFN- α and results in up regulation of TLRs and DNA sensors. Moreover, it results in induction of NETosis that will be discussed below [64].

Neutrophil Nuclear and Cytoplasmic Weapons

NETosis is another way whereby neutrophils can influence B cells. NETosis is fundamentally neutrophil suicide committed

to capture organisms through formation of NETs composed of non-condensed chromatin DNA in association with histones and granular protein. Neutrophils can be activated to produce NETs by ligands of TLRs, antibodies, chromatin and immune complexes [66, 67]. NETs in SLE serve as type-I IFN inducer by activating pDCs as well as neutrophils [68, 64].

Furthermore, NETosis provide a novel source of autoantigens. The extrusion of intracellular components renders these autoantigens triggers of B cells to produce autoantibodies [7]. Moreover, NETs formation involves reactive oxygen species that modify DNA and proteins making them more immunogenic [69]. These modified proteins have been shown to produce more pathogenic antibodies in a variety of diseases such as SLE [70]. Of note, the pathogenicity of NETs depends on the integrity of their components to produce the damaging effects on the tissues [7]. Failure to degrade NETs was also found to be associated with higher levels of anti-NETs and anti-ds DNA autoantibodies and higher frequency of lupus nephritis [71]. This effect could be partially reversed by DNase-I that degrade NETs whereas C1q and antibodies that bind NETs serve as protectors for NETs against degradation [71, 72].

NETosis requires posttranslational modification of histones to release chromatin by peptidyl arginine deiminase-IV (PAD4). Those citrullinated histones are thought to increase the load of citrullinated antigens and result in further increase in anti-citrullinated histone antibodies [73]. The role of NETosis immunogenicity can be exemplified by a model proposed by Dwivedi and RadicforFelyt's syndrome pathogenesis, in which neutrophils are responsible for increasing the pathogenicity of the disease, as compared to RA. Neutrophils extrude modified histones and chromatin that, in complex with bacteria, trigger autoantibodies formation which further target neutrophils antigens causing neutropenia and subsequent increase in the modified histones and autoantibodies [74]. It has been shown that citrullinated histones are targets of anti-citrullinated peptide antibodies (ACPA) and ACPA are capable of inducing NETosis in RA neutrophils [75-77]. Notably, histone modification particularly acetylation was noticed to enhance the immunostimulatory effect of NETs in SLE [78].

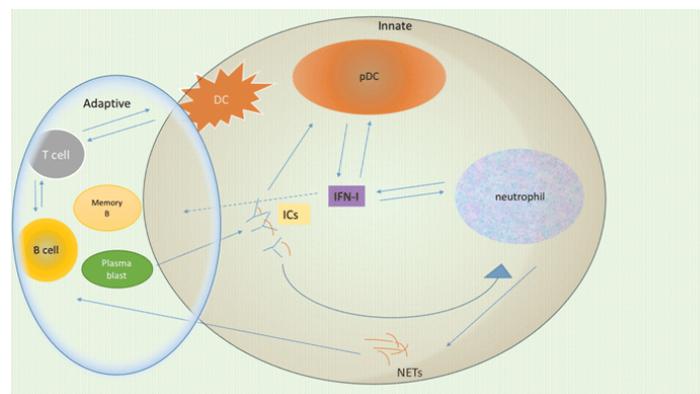


Figure 1: The integration between the innate and adaptive immune cells in SLE
IFN-I, interferon type I; pDC, plasmacytoid dendritic cell; DC, dendritic cell, ICs, immune complexes; NETs, neutrophil extracellular traps.

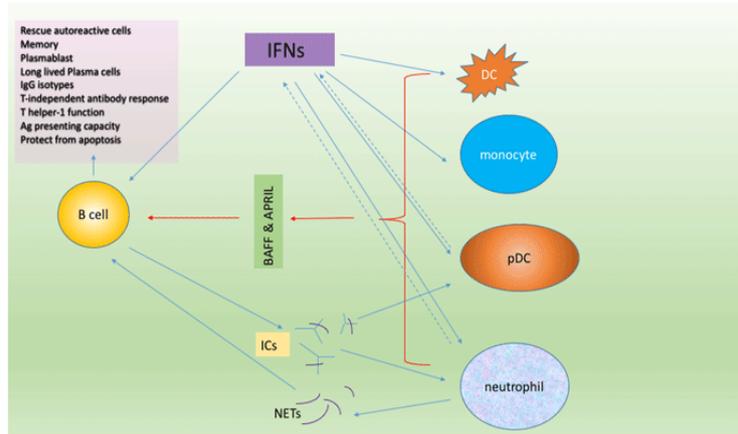


Figure 2: The interplay between neutrophils, B cells and IFNs
IFNs, interferons; BAFF, B-cell activating factor; APRIL, A proliferation induced ligand; DC, dendritic cell; ICs, immune complexes; NETs, neutrophil extracellular traps; IgG, immunoglobulin G; Ag, antigen.

The interaction between the innate and adaptive immunity is shown in Figure 1 and the interplay between neutrophils, B cells and IFNs is highlighted in Figure 2. (Figure 1, 2)

Clinical Utility

The unabated secretion of IFNs has direct and indirect toxic effects on different body systems as summarized in Figure 3. Owing to the fact that IFNs, particularly IFN- α , are pivotal cytokines in the pathogenesis of SLE, they have an indispensable

importance in aiding the diagnosis and follow up of disease activity, and a potential to antagonize to reverse disease progression. IFN- α level were first measured in 1980's and were found in SLE correlating with disease activity [79, 80]. However, the measurement of IFN induced genes is considered more sensitive than direct measurements of IFNs [81, 12, 13]. IFN-induced genes were observed correlating with disease activity [82]. Nevertheless, they fail to longitudinally correlate with changes in disease activity [83.] (Figure 3)

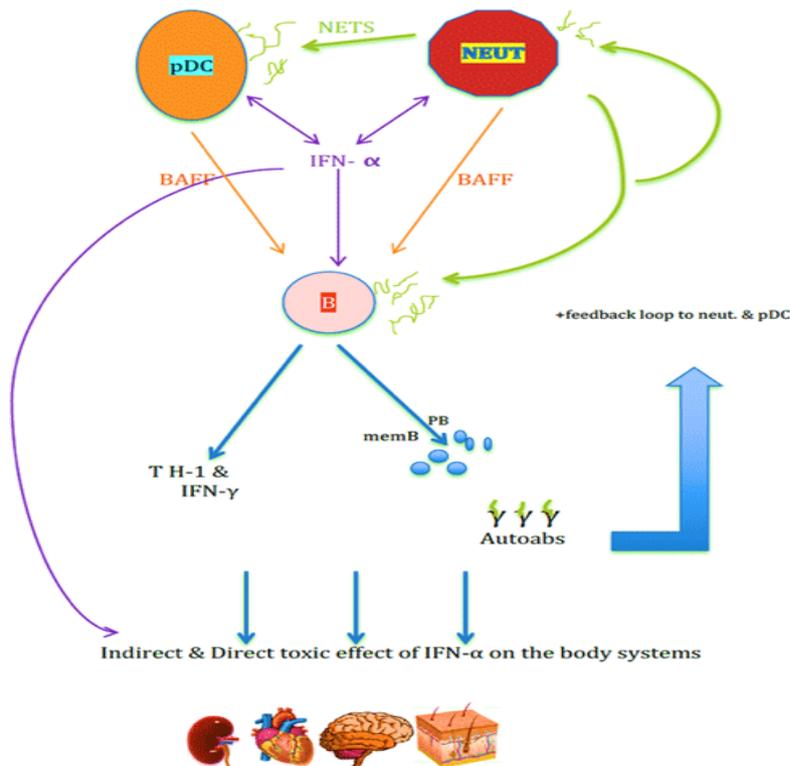


Figure 3: Summary of the direct and indirect toxic effects of IFNs on different body systems⁴

Table 1: Possible effects of IFNs on B cell development and function

IFNs effects	B cell Development	B cell Functionality
↑BAFF & APRIL(15, 18, 20) ↑ IL-10, IL-1β, IL-6 & TNF-α(32)	Rescue autoreactive B cells from deletion (19)	Lowering BCR -stimulation threshold (30)
↑ Heparansulphate(21)	Enhance plasmablast cells survival and differentiation (20)	↑ Antigen presenting capacity (30, 31)
↑ MHC-II, CD69, CD86 & CD25 (30)	Class switch recombination (16)	B cell effector-1 function and IFN-γ expression (38)
↑ AICDA (16)	Increase responsiveness to APRIL (21)	Trafficking of B cells to the lymph nodes (41)
↑ L-selectin (41)	Reduction in pro/pre-B and ↑ in transitional and follicular B cells in BM (22)	↑ Apoptosis & accumulation of self-antigens (39)
↑ PI3-kinase-Akt pathway via IFNR (40)	↑ Switched CD27/IgD- memory B in BM and peripheral blood (22)	Protect B cells from apoptosis (40)
	? Conferring longevity to PCs (26)	
	Acquire plasma cell phenotype and immunoglobulins secretion (6)	
	↑ Primary and secondary antibody response (27, 28)	

IFNs, interferons; BAFF, B-cell activating factor; APRIL, A proliferation induced ligand; IL-10, interleukin 10; TNF-α, tumour necrosis factor alpha; BCR, B-cell receptor; AICDA, activation-induced cytidine deaminase; BM, bone marrow; PCs, plasma cells; IFNR, interferon receptors.

Sialic acid-binding Ig-like lectin-1 (Siglec-1) is a type-I IFN induced gene. Measuring of the Siglec-1 protein on monocytes was found to correlate with disease activity and remission after therapy [84, 85].FCγR1 (CD64) was also thought to be a surrogate marker reflecting type-I IFN activity in the peripheral blood of SLE patients and its expression parallels renal disease[86, 87]. Lately, the hypomethylation of two CpG sites within the promoter region of IFFI44L is considered a highly sensitive and specific diagnostic marker for SLE as it was seen significantly higher in SLE compared to healthy controls and other autoimmune diseases. Additionally, the methylation levels were significantly lower in SLE with renal damage as opposed to those without and conversely, methylation increased in remission [88].

IFN type-I, and its cytotoxic-driven reaction, is responsible for what is known as interface dermatitis [89].MxA, a surrogate marker for local production of type-I IFN, was shown significantly higher in sub acute and chronic forms of lupus skin lesions compared to healthy controls, however, the chronic discoid lupus exhibited tremendously more MxA, which implies that type-I IFN together with the cytotoxic lymphocytes are responsible for the scarring events in these lesions[90].Additionally, MxA distribution in different cutaneous lupus lesions reflects the histological heterogeneity of the inflammatory reactions in these forms [91, 92].

The most important type of IFNs implicated in the pathogenesis SLE appears to be IFN-α, this was evident by the neutralization of IFN signature in serum assays and clinical trials by anti-IFNα strategies [15]. Anti-IFNα strategies are directed towards blocking IFN-α by neutralizing antibodies or blocking its receptors.

Rontalizumab and Sifalimumab are anti-IFNα monoclonal antibodies that bind to and specifically neutralize most IFNα subtypes. Results of phase 1 trials with Rontalizumab in mildly active SLE showed a rapid decline in the expression of interferon regulated genes but not in the levels of IFN-inducible proteins or levels of anti-ds-DNA and anti-extractable nuclear antibodies[93]. Results of phase 2 trials in patients with moderately to severely active extra-renal SLE did not achieve reduction in disease activity, however, the pre-specified group of low-IFN signature patients had a significant decline in SLE response index compared to placebo and achieved more frequently prednisone dose reduction [15]. Results of phase 1 clinical trials with Sifalimumab in moderately active SLE showed dose-dependent inhibition of type-I IFN signature in whole blood and corresponding changes in related proteins in the affected skin. There was also a trend towards improvement in disease activity in Sifalimumab-treated versus placebo-treated subjects [94]. Furthermore, Sifalimumab showed previously a trend towards suppression of the expression of BAFF, TNFα, IL-10, IL-1β and GM-CSF [95], and a trend towards normal complement (C3 or C4) levels [96].

Blocking IFNAR inhibits the activity of all IFN-I types. Therefore, both efficacy and toxicity might be higher compared to anti-IFNα mAb's. Phase 2 clinical trials to evaluate MEDI-546 (Anifrolumab) -IFNAR blocker-in SLE have shown consistent efficacy with reduction in all global and organ specific measures particularly in patients with high baseline IFN-signature [97].

Immunizing SLE patients with IFNα -Kinoid, recombinant conjugated human IFNα, produces polyclonal neutralizing anti-IFNα antibodies. This approach was effective in a lupus murine model to prevent disease flare [98]. Results of phase I/II clinical

trials have shown significant negative correlation between the neutralizing anti-IFN α titers and IFN scores. There was also associated reduction in B-cell transcripts [99].

TLR 7 and 9-stimulation on pDCs is claimed to be responsible for the reduced ability of glucocorticoids to suppress IFN pathways in SLE. Blocking of these receptors is expected to establish the sensitivity of pDCs to the blocking effects of glucocorticoids. The blocking strategies of potential use in SLE are the TLR 7/8/9 antagonists - CPG-52364 and IMO-8400 -as well as the TLR7/9 antagonists [15, 100-102].

The neutrophil products have also had clinical implications. The most prominent example is α -defensin. It was found significantly higher in SLE patients compared to healthy controls, and the high serum levels correlated significantly with disease activity [103]. However, other study described α -defensins as indicators of lupus nephritis per se [104]. Although anti-defensin antibodies were found in the sera of SLE with active and inactive disease, they were significantly higher in active SLE [103].

NETs were seen in specimens of lupus nephritis and the percentage of the glomeruli infiltrated by netting neutrophils were higher in class IV than class III lupus nephritis and also correlated with the activity index according to the WHO classification. Contrarily, no NETs were observed in renal specimen of fulminant Henoch-Schonlein purpura [16]. Additional evidence, supporting that neutrophil components are contributing to renal damage and lupus nephritis, comes from many studies undertaking the investigation of urinary neutrophil gelatinase-associated lipocalin (UNGAL) in lupus nephritis. UNGAL is proposed to be a marker of SLE and an indicator of renal activity, moreover, the UNGAL levels were seen correlating to the lupus nephritis grading [105-110]. Anti-lipocalin IgG has also been reported as a new marker in SLE that can assist in the diagnosis of SLE with high sensitivity and specificity in disease distinguishes [111].

Neutrophils and their production of high amounts of type-I IFNs have shown a clinical impact and an increase in the burden of SLE as regarding the vascular cytotoxicity since LDGs correlated with the development of premature cardiovascular disease through their potential to induce vascular damage and to inhibit vascular repair [65].

It is also important to note that some cutaneous forms of the disease involve an auto-inflammatory process in which neutrophils represent the major aetiologic factors, for example, bullous lupus erythematosus, urticarial vasculitis and early stages of specific cutaneous lupus, in addition to some neutrophilic dermatosis reported to occur in lupus erythematosus [112, 113].

The analysis of skin biopsies from different cutaneous lupus forms revealed the presence of NETs particularly in the papillary dermis, around the blood vessels and adnexae including hair follicles and the eccrine glands. In lupus panniculitis, the infiltration of NETs was seen in the lobular adipose tissue and the reticular dermis. NETs aggregate in the skin express LL-37-ds-DNA complexes, which are mostly the triggering for the formation of increased levels of anti-ds DNA in the sera [16]. Herein, we note

to the effect of Antimalarial drugs in decreasing the oxidative loads of NETs and modifying disease manifestations [114]

As IL-17 is proposed to have a role in the pathogenesis of lupus, it is important to report that, besides Th17, neutrophils are an additional source of IL-17 and LDGs were externalizing IL-17 in high proportions during NETs formation. Neutrophils are reported to be the most IL-17 expressing cells in lupus skin [16, 115, 116].

PAD activity, which initiates the formation of NETs, is of a clinical importance as an epigenetic marker for autoantibody stimulation [117, 74]. There is a potential to inhibit this enzyme to ameliorate the disease as was shown in various lupus models [118, 119].

Most of the biologics used in SLE are mainly targeting B cells. The mechanism by which they combat the auto reactive B cells is diverse. Some of B-cell targeted therapies are directed towards the surface molecules such as anti-CD20 (Rituximab and Ocrelizumab) and anti-CD22 (Epratuzumab) [120-122]. Others target the growth factors, BAFF and APRIL, such as Belimumab, Atacicept, Blisibimod and Tabalumab [123, 124]. Also, immunoglobulin receptor antagonist (Abetimus sodium), anti-CD40L and proteasome antagonist (Bortezomib) have been tried in SLE [125-127]. The heterogeneity in the efficacy of anti-B cell therapies reflects the heterogeneity among lupus patients in the pathogenesis of the disease which in turn warrants understanding the magnitude of interplay between multiple pillars in the pathogenesis of SLE.

Concluding Remarks

This review aims to deepen the understanding of the innate immunity role, exemplified in IFNs and neutrophils, in the development of SLE by direct and indirect effects on enhancing the adaptive immunity, particularly B cells. The heterogeneity in disease outcomes with different therapeutic modalities warrants to pinpoint and abate the immunological deficit with the major aberrations. Therefore, we hope that disease progression is limited by the comprehensive development in the assessment and monitoring of the innate and adaptive allies together, hence, targeted therapies are individualized for patients with different clinical and immunological phenotypes.

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