# The Role of Interferon in Premature Atherosclerosis in Systemic Lupus Erythematosus Patients

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**Abstract:** Systemic Lupus Erythematosus (SLE) is a chronic systemic autoimmune disease associated with significant cardiovascular morbidity and mortality. Studies have established that patients with SLE develop accelerated atherosclerosis related to endothelial cell dysfunction and acute vascular events not explained by Framingham risk score risk stratification. In this article, we closely explore the role of interferons in endothelial cell apoptosis and vascular dysfunction. Understanding the mechanisms responsible for the significant increase in atherosclerotic cardiovascular complications in patients with SLE, and the role of type I interferon may serve as the basis for developing target therapy with pharmacological agents.

**Keywords:** Interferon, Premature atherosclerosis, Systemic Lupus Erythematosus.

## 1. INTRODUCTION

The pathogenesis of atherosclerosis is multifactorial developing years prior to clinical symptoms. Risk factors include dyslipidemia, hypertension, diabetes mellitus, family history, age, smoking, systemic inflammation and obesity. Endothelial cell dysfunction and atherogenesis occurs as a result of hyperlipidemia, oxidative stress, hypertension or inflammatory insults [1]. Endothelial damage stimulates the oxidation and accumulation of LDL-C in the affected vessel; and monocytes migrate and transform to macrophages in the subendothelial intima which then accumulate to form a lipid core [2, 3]. The fibrous cap of the atherosclerotic plaque is formed when inflammatory mediators and cytokines stimulate migration and proliferation of smooth muscle cells of the vascular intima. Elastin and collagen expand to form the fibrous cap which can then rupture exposing the thrombogenic tissue below. Atherosclerotic development can continue to occur following plaque rupture resulting in further lumen narrowing and ischemia of tissues [4]. Patients with SLE have up to a 50-fold increase in the incidence of cardiovascular disease as a result of immune dysregulation [5, 6]. Interferons have been shown to play a central role in this inflammatory process of atherosclerosis especially in SLE patients.

## 2. TYPE 1 INTERFERON (IFN)

IFN's are part of a diverse family of cytokines produced by innate signaling pathways in response to viruses, nucleic acid-containing immune complexes, and necrotic debris associated with antimicrobial peptides [7]. Type 1 IFN's include interferon alpha (IFN-$\alpha$) and interferon beta (IFN-$\beta$) Both have been recognized to play a pathogenic role in SLE and serum levels of type I IFNs, predominantly IFN-$\alpha$, are elevated in ~50% of patients with SLE [1, 4, 5]. Type 1 IFN's are released from the host cell into the surrounding medium, then bind to receptors on target cells inducing transcription of approximately 20-30 genes in the target cells [8]. Gene expression profiling has revealed that the expression of IFN-inducible gene (IFIG) transcripts is also up-regulated [9].

### 2.1. Interferon Alpha

IFN-$\alpha$ is a potent antiangiogenic factor that inhibits angiogenesis by down-regulation of endothelial progenitor cells (EPC) through modulating molecules relevant to interleukin-1 functioning. It also skews myeloid precursor cells away from myeloid circulating angiogenic cells (CAC) towards non angiogenic phenotypes by inducing CAC apoptosis. The number and function of these cells is decreased in patients with SLE as a result of increased IFN levels. Exposure of EPC/CACs to IFN-$\alpha$ has been shown to result in upregulation of IL-18, caspase 1 and absent in melanoma-2 (AIM2). Inhibition of caspase 1 improves EPC/CAC differentiation suggesting that ongoing activation of this inflammasome- associated enzymes contributes to dysfunction of cells that are crucial in vascular repair [10]. AIM2 is activated by dsDNA found in lupus patients [10]. Increased caspase activity is driven by the formation of the inflammasome. Inflammasome scaffold AIM2 is responsive to

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Interferon alpha and activates caspase [10]. The components of inflammasome all represent an interplay of complex interactions. IL - 18 is a cytokine activated *via* cleavage by caspase -1 [11]. IL-18 prevents differentiation of EAC/CAC which is integral to maintenance of intact epithelium. The role of IL-18 in affecting differentiation of EAC/CAC cells is further supported by studies where neutralization of IL-18 restores EAC/CAC differentiation capacity specifically in lupus models [10]. Exogenous IL-18 has also been observed to induce EPC/CAC dysfunction in healthy controls [10]. These results support the contribution of IL-18 to EAC/CAC dysfunction. EAC/CAC dysfunction is key in the pathogenesis of atherosclerosis as it promotes endothelial damage and affects vascular repair. Elevated levels of IL-18 correlate with increased vascular stiffness and intima media thickness in men without evidence of coronary artery disease, indicating that this cytokine may play a role in early stages of atherosclerosis development [12]. There is an ever-increasing focus on the innate immune system and, in particular, IFNs in terms of pathogenesis, recent studies have drawn connections in both human and murine models between neutrophils, plasmacytoid dendritic cells, IFNs, and endothelial dysfunction [13].

IFN has a pleiotropic effect on angiogenesis genes. IFN-$\alpha$ inhibits angiogenesis through repression of interleukin 1 pathways, up regulation of interleukin 1 receptor antagonists, down regulation of the proangiogenic molecule Il-1b and down regulation of vascular endothelial growth factor (VEGF) [10, 14]. The pleiotropic effects on angiogenesis impairment has been used as adjuvant antiangiogenic therapy in neoplasias, diabetic retinopathy, hemangiomas, and Kaposi sarcoma. IFN-$\alpha$ can decrease colony formation of hematopoietic progenitor cells further suggesting its implication in EPC decreases in SLE [5]. Therefore, following vascular injury increased levels of type I IFNs may lead to periods of endothelial damage, followed by aberrant repair due to decreased levels of IL-1 and VEGF-A [10].

Amongst the many sources of IFN-$\alpha$ production are the dendritic cells found within atheromatous plaques which sensitizes antigen-presenting cells toward pathogen-derived Toll-like receptor 4 and toll 9 ligands [15]. Thus, local production of IFN-$\alpha$ leads to enhanced synthesis of the proinflammatory cytokines and matrix metalloproteinases implicated in atherosclerotic plaque destabilization. IFN-$\alpha$ expressed within the plaque stimulates cytotoxic T cells in blood vessels, augmenting vascular damage. Studies indicate that pathogens or nucleosome-containing immune complexes that induce synthesis of IFN-$\alpha$ contribute to instability of inflamed atherosclerotic plaques [16-18].

All of the above highlight the importance of IFN-$\alpha$ in the progression of atherosclerosis. A recent study by Li *et al*. highlighted the role of interferon alpha in the initiation of atherosclerosis. IFN-$\alpha$ priming has been shown to upregulate the expression of SR-A in human monocytes/macrophages, leading to increased lipid uptake and foam cell activation [2, 9]. Macrophage derived foam cells have been identified as one of the main components of early atherosclerotic lesions [19].

### 2.2. Interferon Beta

IFN-$\beta$ may promote atherosclerosis by promoting macrophage recruitment to arteries [20]. IFN-$\beta$ enhances macrophage- endothelial cell adhesion and promotes leukocyte attraction to atherosclerosis prone site in mice and increases macrophage accumulation in plaques [20]. Goosens *et al*. demonstrated that IFN-$\beta$ treatment induced chemokine dependant adhesion and migration of leukocytes and atherosclerosis development *in vivo*. Leukocyte adhesion was attributed through the action of CCL5 and CCR5 dependant mechanisms. They also showed that there is a strong increase in atherosclerotic lesions in mice treated with IFN-$\beta$. Upregulation of IFN-$\beta$ signaling was shown to be increased in plaque rupture in atherosclerotic lesions [20].

IFN-$\beta$ also has been shown to upregulate production of IL-10 which is a classical anti inflammatory cytokine [20]. This anti-inflammatory cytokine activity contrasts the pro-atherosclerotic effect observed with IFN-$\beta$ due to the pro-atherosclerotic mechanisms that regulate cells affinity to atherosclerotic lesions [20]. Although IL-10 production is enhanced, Goossens *et al*. demonstrates that through induction of the CCL5-CCR5 axis leukocyte attraction to lesions is increased [20]. Further analysis is needed to demonstrate the cardiovascular risk of type 1 IFN treatment. Targeting of IFN signaling may prove to be a therapeutic option to prevent and treat atherosclerosis.

## 3. EMERGING THERAPY TARGETING ALPHA INTERFERON

### 3.1. Alpha Interferon Antibody

Rontalizumab is a recombinant humanized monoclonal antibody to IFN-$\alpha$. McBride *et al.*

demonstrated that it was generally safe and well tolerated in their phase I trial in patients with mildly active SLE [21]. With regard to viral infections, it was demonstrated that the exposure-adjusted rate of infections was similar between treatment groups, and no dose-related increase in infections was observed [21]. In contrast to Rontalizumab, which was studied in patients with mildly active SLE, sifalimumab is an anti–IFN-α monoclonal antibody which was studied on patients with moderate to severe disease. A Phase I Randomized, Controlled, Dose-Escalation Study done to evaluate the safety and tolerability of multiple intravenous (IV) doses exhibited a dose-dependent target neutralization [22]. In addition the study also reported decreased SLE flares at higher sifalimumab doses further suggesting that the efficacy of sifalimumab is dose dependent. Inhibition of the interferon pathway is concerning for possible viral reactivation, however the study demonstrated an acceptable safety and tolerability profile, with a low level of viral and other infections [22]. AGS-009 is anti-IFN-α monoclonal antibody. In the Phase 1a study, all patients who received AGS-009 trended toward normal IFN-α signatures after only a single dose, whereas none of the patients receiving placebo showed a similar shift [23]. Furthermore, the magnitude of the shift toward normal IFN-alpha signatures was dose-proportional, suggesting that AGS-009 neutralizes IFN-alpha effectively [23].

Although these studies showed promising results, the end point was not cardiovascular outcomes. It can be however hypothesized that since premature atherosclerosis is related to general disease activity, that a decrease in general activity may play a role in reducing cardiovascular risk. There is also a theoretical risk of viral related malignancy in the future, however this risk can only be properly evaluated with phase III clinical trials.

### 3.2. Toll Like Receptor

Production of interferon alpha is stimulated *via* TLR7 and TLR9, both of which also serve as priming signals for inflammasome activation [24-26]. Komastuda *et al.* showed that there is a correlation between expression level of IFN-α RNA in PBMCs and those of *TLR7* and *TLR9* mRNAs suggesting that in lupus patients this is the source of interferon stimulation [27]. An approach towards therapy involves antibodies against TLR. IMO - 3100, produced by IMERA is a dual antagonist of TLR 7/ 9. It has been shown to correct lupus associated dyslipidemia by reducing serum cholesterol levels [28].

### 3.3. Interferon Alpha Vaccine

Zagury *et al*. prepared an IFN-α derivative, termed IFN-α kinoid, and demonstrated it to be an effective immunogen to mount a neutralizing anti-IFN-α Ab response [29].

### 3.4. Proteasome Inhibitors

Ichikawa demonstrated that proteasome inhibitors, including those that selectively target the immunoproteasome, are efficacious in the treatment of murine lupus *via* dual inhibition of pathogenic IFN-α production and autoreactive PCs [30]. They demonstrated that TLR-induced IFN-α production is completely abrogated by proteasome inhibition [30]. Carfilzomib is a non selective proteasome inhibitors and ONX 0914 is an immunoproteasome-specific inhibitor. Ichikawa *et al.* demonstrated that inhibition of the immunoproteasome is equally efficacious as dual targeting agents in preventing lupus disease progression [30].

Dynavax is working to characterize the role of phosphoinositide 3-kinase (PI3K). Scientists have shown that PI3K is required for the production of IFN by plasmacytoid dendritic cells (PDCs) in response to TLR7 or TLR9 stimulation [31].

## 4. CLINICAL IMPLICATIONS

With the knowledge of the role of IFN in the development of atherosclerosis, further research is needed to be able to clinically treat patients targeting IFN. Understanding the mechanisms of SLE can offer better drug targets for treatment. Future research needs to categorize patients by disease phase, pathogenetic mechanisms and genetic susceptibility to maximize the success of treatments [32]. Clinical trials involving emerging therapies are currently underway involving target therapies against IFN. These clinical trials may also help to further guide clinical management and the possibility of monitoring type-1 IFN levels to monitor disease progress and further disease management [33]. Ideally treatment should be targeted at the type 1 IFN pathway leading to disease leaving the pathways of type 1 IFN which contribute to host cell defenses in response to viruses intact [34]. Studies have shown that monitoring serum IFN-related chemokine levels can be used as biomarkers of disease activity in patients with SLE. These markers were also shown to be useful in monitoring patients for disease flares. Traditional laboratory values have also

been shown to correlate to disease process such as complement levels, ESR values and anti-dsDNA antibody titers; however serum levels of chemokines outperformed these traditional laboratory values longitudinally in correlating with disease activity [35]. Monitoring these serum levels can help to improve disease management and prevent future flare-ups. Data has shown that increased chemokine levels may predict a future disease flare-up within one year. These results indicate that patients with high levels of chemokines should be monitored more frequently [35]. Although these disease flare-ups do not directly correlate to atherosclerotic activity, knowing the mechanism of IFN in promoting atherosclerotic development it can be hypothesized that atherosclerosis activity may be increased in those patients with rising IFN-regulated serum chemokine levels and increased disease activity.

## 5. CONCLUSIONS

IFN plays an important role in the development of premature atherosclerosis of SLE patients. An imbalance between endothelial cell damage and repair develops as a result of alterations in EPC's and CACs which is mediated by IFN-$\alpha$. IFN-$\beta$ promotes atherosclerosis by various mechanisms by affecting adhesion and migration of leucocytes to plaques and by promoting plaque rupture. Understanding the role of IFN in promoting premature atherosclerosis is critical to the development of appropriate target therapy.

## DISCLOSURE POLICY

The authors declare that there is no conflict of interests regarding the publication of this article.

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