Type I Interferon Signature in Systemic Lupus Erythematosus

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ABSTRACT: Type I interferons (IFN) are primarily regarded as an inhibitor of viral replication. However, type I IFN, mainly IFNα, plays a major role in activation of both the innate and adaptive immune systems. Systemic lupus erythematosus (SLE) is a chronic, multi-systemic, inflammatory autoimmune disease with undefined etiology. SLE is characterized by dysregulation of both the innate and the adaptive immune systems. An increased expression of type I IFN-regulated genes, termed IFN signature, has been reported in patients with SLE. We review here the role of IFNα in the pathogenesis and course of SLE and the possible role of IFNα inhibition as a novel treatment for lupus patients.

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Systemic lupus erythematosus is a chronic, remitting and relapsing, multi-systemic, inflammatory autoimmune disease. The prevalence of the disease worldwide is approximately 20–150 cases per 100,000 [1]. Higher disease rates are found among African-Americans, Hispanics and Asians. SLE is more prevalent in women, particularly during the childbearing years, and has a female to male ratio of 9:1 [1]. The clinical manifestations of the disease are diverse, ranging from fatigue and oral ulcers to life-threatening renal and neurological disorders. The precise etiology of SLE has yet to be defined, although many studies suggest a role for genetic, hormonal, immunological and environmental factors [2].

SLE is characterized by dysregulation of both the innate and the adaptive immune systems, with a breakdown of tolerance and the production of various autoantibodies and inflammatory cytokines. Genetic deficiencies of complement proteins may result in defective clearance of immune complexes and of apoptotic cells and failure of B cell tolerance. Apart from the generation of autoantibodies, immune complex deposition and T cell abnormalities, cytokines appear to play a major role in the pathogenesis of SLE [3]. An increased expression of type I interferon-regulated genes, termed IFN signature, was recently reported in blood and tissues of patients with SLE. We review here the significance of IFN signature in the pathogenesis, course and treatment of SLE.

IFNα inhibition is considered a novel treatment modality for systemic lupus erythematosus

THE INTERFERON SYSTEM

Interferons are cytokines with the capacity to interfere (hence their name) and to suppress viral replication. There are three types of interferons: type I IFN (IFNα, IFNβ and other less explored members), type II IFN (IFNγ) and type III IFN (IFNλ). This review will focus on type I IFN. IFNγ (type II IFN) is produced predominantly by natural killer cells and natural killer T cells as part of the innate immune response, and by CD4 and CD8 T lymphocytes. Apart from antiviral activity, IFNγ acts against intracellular bacterial infections and takes part in tumor control. IFNγ is an important activator of macrophages [4]. The subset of IFNλ (type III IFN) includes three cytokines. Virtually any cell type can express IFNλ following viral infection, and presumably infection by most viruses induces IFNλ expression. IFNλ can activate host antitumor mechanisms that inhibit the growth of certain tumors [5]. The terms 'Type I IFN signature' and 'IFNα signature' are used to distinguish this signature from the one induced by IFN type II and IFN type III. Type I IFN is a multi-gene family of cytokines that consists mainly of IFNα and IFNβ [6]. Type I IFN includes 13 genes of IFNα, one IFNβ gene and several other members. All type I IFN cytokines bind the same receptor, type I IFNα receptor. IFNβ can be produced by almost any cell, whereas IFNα is produced mainly by plasmacytoid dendritic cells. Plasmacytoid dendritic cells are a rare cell population, constituting only 0.2–0.8% of the peripheral blood mononuclear cells, but their capacity to produce high levels of IFNα is unique (100–200 times more than other cells) [7].

The endosomal toll-like receptor reacts with nucleic acids. Thus, dsRNA reacts with TLR3, ssRNA with TLR7/8 and cpGDNA with TLR9 [7]. As a result of microbial binding to TLR, signaling through several pathways occurs and ultimately various transcription factors are activated. The engaged TLR7 and TLR9 bind to the adaptor protein MyD88 and activate IFN regulatory factor 7, which is the key regulator of type I IFN transcription. TLR3 binds to the adaptor protein to activate IFN regulatory factor 3, the transcription factor for *IFNβ* gene. The cytosolic retinoic acid-inducible gene I (*RIG-I*)-like receptor detects

SLE = systemic lupus erythematosus
IFN = interferon

TLR = toll-like receptor

viral RNA and stimulates the transcription of IFNβ. pDCs constitutively express TLR7 and TLR9, which might explain the increased ability of these cells to release high amounts of IFNα. The secreted IFNα binds to its receptor on target cells, and signaling occurs mainly through the JAK/STAT pathways [8]. By this, IFNα induces the transcription of more than 300 different genes [9]. The induced genes are named interferon-regulated genes. Those genes amplify IFN signaling, activate the adaptive immune response, and produce factors that directly inhibit viral replication. The consequences of IFNα stimulation include up-regulation of major histocompatability complex as well as co-stimulatory molecules that increase survival and activation of dendritic, B and T cells [10]. IFNα regulates the functions of natural killer cells by enhancing the ability of NK cells to kill target cells. Furthermore, IFNα promotes accumulation and survival of proliferating NK cells and enhances production and secretion of other cytokines by NK cells. IFNα enhances the antibody response to soluble antigens and stimulates production of all immunoglobulin subtypes. Moreover, IFNα induces production of memory B cells. The direct effect of IFNα on naive CD4 T cells favors their differentiation into T helper 1 cells, which secrete INFγ. IFNα also stimulates CD8 T cells, enhancing their cytotoxic activity [10] [Table 1].

Table 1. The effect of IFNα on the immune system

Healthy population [7,8,10]
Dendritic cells maturation ↑
NK cells activity ↑
Th1 cells ↑
CD8 cells ↑
Memory B cells ↑
BLyS ↑
Ig responses ↑
SLE patients [17,18,21,22]
IFN-α mRNA ↑
IFN-α serum levels ↑
BLyS ↑
CD4+FoxP3 cells ↓

NK = natural killer, TH1 = T helper 1, BLyS = B lymphocyte stimulator, Ig = immunoglobulin

INTERFERON IN SLE

Hooks et al. [11] reported high IFN levels in sera of patients with autoimmune disorders. In that study, increased IFN levels were more frequent in patients with active lupus disease. Initially, INFγ was thought to be increased in those patients. However, the high IFN levels were later found to be IFNα [12]. The fact that patients with systemic autoimmune diseases have increased production of IFNα received further attention following the observation that IFNα therapy (given to patients with malignancy or hepatitis C) could induce autoimmune disorders [13,14]. In 2003, when genome-wide gene expression profiling became available, several investigators reported the increased expression of IFNα in peripheral blood cells of lupus patients [15,16]. In patients with SLE this phenomenon was called IFN signature or IFNα signature [15]. Currently, IFNα levels can be determined either directly with antibodies (by enzyme-linked immunosorbent assay) or indirectly by mRNA detection of IFN-induced transcripts or proteins. The ELISA method for IFNα detection is considered to be specific but not sensitive, whereas the indirect IFN assessment by IFN-induced transcripts is much more sensitive.

High levels of IFNα are measured in the serum of active lupus patients

Pediatric lupus patients almost invariably display IFN signature at early stages of their disease. This suggests the importance of IFNα in the pathogenesis and the initiation of the disease [16]. Several mechanisms have been suggested for the role of IFNα in the pathogenesis of SLE. One of the hallmarks of SLE is the formation of immune complexes that activate dendritic cells, thus increasing the ability of antigen presentation which up-regulates IFNα secretion. On the other hand, IFNα promotes dendritic cell maturation as well as the up-regulation of several cell surface molecules. The latter effects promote the development of a T helper 1 response. In addition, IFNα also enhances antibody production and immunoglobulin class switching [17]. It is worth noting that the number of pDCs in peripheral blood of lupus patients is reduced as compared to healthy volunteers [18]. The decreased number of pDCs is most probably due to migration of these cells to tissues such as skin, lymph nodes and kidneys [19,20]. Nevertheless, peripheral blood pDCs of lupus patients are capable of producing large amounts of IFNα. The lack of inhibitory effects of lupus monocytes on pDCs may also contribute to the high IFNα production of pDCs in lupus patients. Apart from IFNα production, pDCs have other immunomodulatory effects. It was reported that pDCs from lupus patients have a reduced capacity to induce regulatory T cells and an increased capacity to induce Th-17 cells [21]. Those changes in T cell subpopulations were previously shown to contribute to the development of autoimmune disorders including SLE [22] [Table 1].

GENETICS

Several studies have demonstrated the effects of genetic background on the IFNα pathway in lupus patients. SLE patients with the transcription factor IRF5 and IRF7 genotype (e.g., rs2004640 T allele) were shown to have high IFNα serum levels as compared to lupus patients lacking those haplotypes [23,24]. Similarly, variants of signal transducer and activator of transcription 4, which interacts with the cytoplasmatic part of type I IFNα receptor, were also reported to be associated with

pDC = plasmacytoid dendritic cell
NK = natural killer
ELISA = enzyme-linked immunosorbent assay

Th = T helper
IRF = interferon regulatory factor

high IFNα serum levels in lupus patients [25]. On the other hand, loss of function polymorphism in TLR-independent pathways was shown to be associated with low IFNα levels in SLE patients [26].

IFNα AS MARKER FOR LUPUS DISEASE ACTIVITY

Several studies have reported the association between high levels of IFNα (mRNA gene expression or sera levels) and SLE disease activity. Ytterberg and Schnitzer [12] were the first to demonstrate high serum levels of IFNα in patients with active SLE. The high serum levels of IFNα correlated with lupus activity index and with the titer of anti-dsDNA autoantibodies [12]. Increased levels of IFN-inducible genes were also reported to correlate with disease activity and with the presence of lupus nephritis [27]. Rose and co-authors [38] reported that 32% of patients with active lupus had high IFNα levels in their sera. In that study, IFNα levels correlated with disease activity better than other variables including anti-dsDNA autoantibody titers [28]. Recently, we were able to show high levels (threefold) of IFNα gene expression in unstimulated peripheral blood lymphocytes obtained from lupus patients, compared to the levels observed in peripheral blood lymphocytes of healthy volunteers. The high mRNA IFNα levels decreased concomitantly with the clinical improvement in those patients [29]. It should be noted that some studies failed to demonstrate significant correlation between IFNα signature and SLE disease activity [30]. In recent studies it was reported that INFα up-regulated levels of B lymphocyte stimulator, suggesting an additional mechanism by which INFα contributes to the pathogenesis of SLE [31,32].

IFNα produced by plasmacytoid dendritic cells stimulates TH1, CD8, NK and B cells

IFNα AS A TARGET FOR SLE THERAPY

Since IFNα was shown to play a role in the pathogenesis and course of SLE, several studies investigated the possibility of targeting IFNα as a novel treatment for lupus. Treatment modalities include monoclonal antibodies against IFNα, anti-IFNα antibodies-inducing vaccines and inhibitors of toll-like receptors which stimulate and promote IFNα production [33] [Table 2].

Zagury et al. [34] demonstrated that the injection of an adjuvant IFNα (IFNα-kinoid) to (NZBXNZW)F1 lupus-prone mice induced transient neutralizing anti-IFNα antibodies concomitantly with significant amelioration of lupus-related manifestations, including proteinuria, kidney damage and survival. More recently, Mathian et al. [35] immunized mice (HuIFN-α2b transgenic FVB/N mice) with human IFNα (conjugated to KLH, INF-K). The mice produced anti-human IFNα antibodies that neutralize all 13 subtypes of human IFNα as well as IFNα obtained from sera of active lupus patients [33].

Sifalimumab is a fully humanized anti-IFNα monoclonal antibody. In a phase I randomized controlled trial of lupus patients with moderately active disease, sifalimumab treatment proved to be safe and well tolerated. In addition, it also appears to be effective, thus supporting further clinical development of this monoclonal antibody for the treatment of SLE [36]. In a recent study, 25% of the SLE patients revealed endogenous anti-IFNα autoantibodies in their sera. Sera from anti-IFNα autoantibody-positive patients neutralized, in vitro, IFNα activity. Moreover, the presence of anti-IFNα autoantibodies was associated with lower levels of IFNα bioactivity, reduced downstream IFN-pathway activity, and lower disease activity [37]. Recently, an IFNα kinoid vaccine was developed for the treatment of SLE patients. Results from the phase I-II double blind, placebo-controlled dose escalation study in 28 SLE patients with mild-to-moderate disease demonstrated a dose-related anti-IFNα response with down-regulation of the IFN signature. Further studies of its clinical efficacy are warranted [38].

TLR inhibition is another approach to reduce IFNα production. Antimalarials (such as hydroxychloroquine, Plaquenil®, Sanofi-Aventis, Canada) are thought to be beneficial in the treatment of lupus patients, at least partially, by inhibition of the endosomal TLR9 [33]. Idera Pharmaceuticals (Cambridge, MA) developed a synthetic oligonucleotide-based inhibitor of TLR7 and TLR9. The latter drug was reported to inhibit the development of lupus in SLE-prone mice and to suppress TLR7 and TLR9-induced cytokine production in lupus patients in a phase I clinical trial [39]. Dynavax Technologies Corp. also developed an oligonucleotide-based TLR inhibitor for autoimmune diseases such as SLE. A phase I clinical trial of bifunctional TLR7 and TLR9 has begun. Pfizer Inc. developed another TLR inhibitor with activity against TLR7/8/9. The drug was reported to be effective in a murine model of lupus and is currently in a phase I trial in lupus patients [40].

Table 2. SLE therapeutic options based on IFNα inhibition

Monoclonal antibodies against IFNα [34,35]
Anti-IFNα antibodies vaccines [36]
Inhibition of TLR which promote IFNα production [37-39]

CONCLUSIONS

IFNα was shown to play a role in the pathogenesis and course of SLE. Thus, lupus patients, especially those with active disease, demonstrated high serum levels of IFNα as well as IFN signature, emphasizing the role of the innate immune system in SLE. Based on those observations, novel therapeutic modalities aimed to decrease IFNα activity (by specific monoclonal antibodies, IFNα vaccination or TLR suppressors) in SLE patients are currently in progress. Careful evaluation of the efficacy and safety of those novel therapeutic modalities are mandatory prior to their clinical use.

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