Review

Type I interferon signature in Sjögren's syndrome: pathophysiological and clinical implications

N. Marketos[1], I. Cinoku[1], A. Rapti[1], C.P. Mavragani[1-3]

Departments of [1]Physiology,
[2]Pathophysiology,
[3]Joint Academic
Rheumatology Program,
National and Kapodistrian
University of Athens, Greece.

Nikolaos Marketos*, MD
Ilir Cinoku*, MD
Anna Rapti, MD
Clio P. Mavragani, MD

*These authors contributed equally to this paper.

Please address correspondence to:
Dr Clio P. Mavragani,
75, Mikras Asias St,
115277 Athens, Greece.
E-mail: kmauragan@med.uoa.gr

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ABSTRACT

Type I interferons (IFN) have long been recognised as mediators of innate immune defense mechanisms against viral threats. Robust evidence over the last 15 years revealed their significant role in the pathogenesis of systemic autoimmune diseases, including systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS). Despite the progress, methods of detection, initial triggers, biological functions and clinical associations in the setting of autoimmunity remain to be fully clarified. As therapeutic options for SS are currently limited, neutralising specific targets of the type I IFN pathway seems a promising option. In this review we summarise the current evidence regarding the role of type I IFN in SS.

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune entity affecting typically the exocrine glands of middle-aged women. Infiltration of immune cells in the salivary and lacrimal glands leads to loss of secretory function and eventually to desiccation of oral and ocular mucosa. In a significant proportion of patients, extraglandular manifestations are present involving mainly the joints, kidneys, lung, and peripheral nervous system (1, 2). Activation of innate immune pathways has long been considered as central pathogenetic contributor, with type I interferon (IFN) attracting particular interest over the last decade (3).

Type I IFNs, members of the class II family of α-helical cytokines, along with type II IFNs and numerous other cytokines, include 13 IFNα subtypes, β, ε (expressed in placenta, supposedly having a role in reproduction), ϰ (in keratinocytes), ω, δ, and τ (the two latter not found in humans). They mainly signal through IFNα/β receptor (IFNAR), consisting of two subunits IFNAR1 and IFNAR2. Upon binding with IFNs, autophosphorylation of the Janus protein kinases Tyk2 and Jak1 -attached to IFNAR1 and IFNAR2 subunits respectively-, occurs. As a result, signal transducer and activator of transcription (STAT) 1 and 2 are phosphorylated leading to the formation of a STAT2/STAT1 heterodimer, which following binding of interferon-regulatory factor 9 (IRF-9), leads to the formation of a complex namely IFN-stimulated gene factor 3 (ISGF3). The latter translocates to nucleus and following binding to specific elements (interferon stimulated response elements) on the promoters of IFN stimulated genes (ISGs) leads to their transcription. The encoded ISG proteins are characterised by a wide variety of antiviral properties including prevention of the assaulting virus from entering the cells and subsequently being replicated, translated, assembled and released (4, 5).

Though the main action is antiviral/antineoplastic, several immunomodulatory functions have also been attributed to type I IFNs including induction of B cell activating factor (BAFF) (6), immunoglobulin switching (7), increased antigen presentation, T-cell mediated and natural killer cell (NK) cytotoxicity (8). As a result of these immunostimulatory properties, a tight control of type I IFN activation is mandatory in order to ensure adequate host defenses avoiding collateral tissue damage by excessive responses. Thus, checkpoints at several levels of type I IFN pathway have been detected including mainly post-transcriptional modifications of the signalling pathway (phosphorylation) and regulation of type I IFN expression by epigenetic modifications including DNA methylation, histone modification and non-coding RNA effects (9).

While virtually any cell is able to produce type I IFNs (10) following stimu-

lation by exogenous or endogenous nucleic acids either through toll-like receptors (TLRs) or cytoplasmic sensors, plasmacytoid dendritic cells (pDCs) are considered to be the main IFNα producing cells. They are characterised by increased constitutive expression of TLR7 and TLR9 -stimulated by ssRNA/endogenous RNA and unmethylated DNA respectively- and a rapid and robust response following triggering by nucleic acids (11). Upon ligation, they signal through the myeloid differentiation primary response 88 (MyD88) activating IRF7, which has a central role in type I IFN production (12).

The main cytoplasmic receptors able to detect endogenous nucleic acids include the DNA sensors absent in melanoma 2 (AIM2) and cyclic GMP-AMP synthetase (cGAS) sensing dsDNA as well as the retinoic acid-inducible gene I (RIG-I) like receptor (RLR) family comprising RIG-I and Melanoma differentiation associated gene 5 (MDA5) triggered by dsRNA (13). The downstream signalling pathway mediates the phosphorylation of IRF3, 7 and Nuclear Factor-kappa B (NFκB) and subsequent type I IFN and induction of proinflammatory cytokine genes (14).

Activation of type I IFN pathway in SS

The first indication of type I IFN activation in SS dates back to late seventies (15). Since then, a growing body of evidence supports a significant role for type I IFN in the pathogenesis of SS, similarly to what was observed in other systemic autoimmune diseases such as systemic lupus erythematosus (SLE) (16), dermatomyositis (17) and systemic sclerosis (18).

Using microarrays and real time PCR studies, upregulation of ISGs was initially detected in SS disease targets such as minor salivary gland (MSG) biopsies (19-21) and ocular epithelial cells (19) compared to healthy controls. After immunoblot testing using specific probes for either type I or type II IFN related proteins (IFIT-3 and GBP-2, respectively) the heterogeneity of IFN activity at the level of MSG tissues has been appreciated (22). In a follow-up study, using validated probes for type I

or type II IFN pathway, three IFN patterns in MSG biopsies were detected including either a predominantly type I or type II, or a mixed type I/II IFN; IFIT-3 (another type I IFN inducible gene) mainly stained salivary duct epithelial cells, while GBP-2 (a type II IFN related gene) was found in both lymphoid and duct epithelial cells surrounded by inflammatory cells by immunohistochemistry (23).

Beyond salivary gland tissues, the presence of type I IFN signature was next evaluated at systemic level. Thus, increased expression of type I IFN inducible genes or proteins has been revealed in SS derived peripheral blood mononuclear cells (PBMCs) (24, 25), whole blood (24, 26, 27), monocytes (28-30) and recently B-cells (31, 25). Based on previous work on lupus (32) and SS derived salivary gland tissues (23), Bodewes *et al.* revealed 3 distinct IFN patterns in peripheral blood from SS patients, a type I IFN predominant, a type I and II IFN mixed pattern as well as an inactive one (33). The presence of a heightened type I IFN signature in SS ranges from 53% to 81% in several gene expression studies (27, 33-35).

Measurement of IFNs in periphery has traditionally been elusive (36, 38). While initial reports failed to detect the presence of IFNα in serum (37), possibly due to the presence of many type I IFN subtypes currently undetectable by commercial ELISAs, the introduction of bioassays allowed the detection of systemic type I IFN activity in SS serum (28) or plasma (6), which seems to account for the upregulated type I IFN signature. Whether IFNα (39, 40) or IFNβ (28) account for the increased type I IFN activity in SS peripheral blood remains to be clarified. In a recent study an advanced ELISA with single molecule array (SIMOA) digital technology was used in order to measure attomolar IFNα levels in different groups, including adult and juvenile SLE, diabetes mellitus (DM), and IFNopathies (41). This technique, though promising, has not been applied in SS yet.

Given the apparently conflicting data between studies in regard to whether type I IFN or II predominate in SS and taken into account SS phenotypic

heterogeneity, the overlapping regulation of many ISGs by both type I and II IFNs and the type of biological sample implemented, we proceeded to quantitation of transcripts predominantly induced either by type I or type II IFN in both peripheral blood and MSG tissue from well characterised SS patients. Thus, while in peripheral blood a type I IFN predominant signal was observed, in salivary glands type II IFN related genes were mainly overexpressed. Moreover, increased IFNγ but low IFNα transcripts were detected in MSG tissue derived from SS patients with lymphoma compared to SS with no lymphoma and sicca controls; as a result the IFNγ/IFNα ratio has been shown to be a potential biomarker for identification of lymphoma among SS patients with high area under the curve values in the Receiver-operating characteristic analysis (ROC) (27).

Additionally, recent data support a contributing role for type III IFN – namely IFNλ – in the pathogenesis of SS. All three subtypes IFNλ1/interlekin (IL)-29, IFNλ2/IL-28A and IFNλ3/IL-28B signal through the heterodimeric IFNλ R1/IL-28Ra receptor predominantly expressed in pDCs. SS patients with intermediate lesions in MSGB tissue were found to have increased both IFN-λ2/IL-28A epithelial expression and IFN-λ1/IL-29 levels in the periphery in comparison to sicca controls (42). Furthermore, in another study the addition of IFNλ1/IL-29 to IFNα led to even more enhanced stimulation of the ISGs BAFF and CXCL10 as well as the prolongation of phosphorylation of STAT in the immortalised human salivary gland ductal cell line NS-SV-DC, implying a synergistic effect of IFNα and IFNλ in the pathogenesis of SS (43), although studies in a larger scale are needed to confirm these results.

Of interest, proteins induced by type I IFNs, such as sialic acid binding Ig like lectin 1 (SIGLEC1), a cell surface protein on monocytes and macrophages detected by flow cytometry in peripheral blood, was shown to correlate with EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) score and discriminate SS patients with glandular and extraglandular manifestations (30).

In a recent report, soluble SIGLEC5, a transmembrane member of immunoglobulin superfamily expressed in neutrophils (44), was found to be elevated in saliva but not serum in SS patients compared to controls, in association with impaired salivary secretion, increased ocular damage and higher serum IgG levels (45).

Though the source of systemic and local IFN production has not been yet fully elucidated, several lines of evidence point toward pDCs, the professional type I IFN producing cells as chief contributors of IFN signature in SS. Thus, reduced (28, 46) but activated pDCs (28) in peripheral blood of SS patients together with their identification at the level of salivary gland tissue (19, 27, 37), imply a potentially significant role in both systemic (28) and local IFN activity (19, 27, 37).

Contributors of exaggerated type I interferon production in SS

The initial trigger of type I IFN production in the setting of autoimmunity remains an area of intensive research, a growing body of data so far strongly support endogenous nucleic acids as a potential source for the intrinsic activation of type I IFN system in the absence of exogenous viruses. In SS, similarly to studies in lupus (47), immunocomplexes consisting of RNA-containing apoptotic bodies and antibodies against ribonucleoproteins derived from SS sera were shown to induce type I IFN production, following pDC stimulation (37).

Previous studies revealed that DNA derived from SLE patients was characterised by repetitive sequences (48), was enriched in CpG nucleotides and displayed high homology to retroviruses (49). Moreover, Perl *et al.* (50) noted the presence of antibodies to endogenous retroviruses in sera of patients with several autoimmune diseases including SS. A potential source of endogenous retroviral material include Long interspersed nuclear elements (LINEs; L1), which comprise 17% of the human genome and are able to translocate within the genome, once they are fully transcribed (51). The typical structure of L1 elements includes open reading frames (ORF) that can encode an endonuclease

and a reverse transcriptase. L1 promoter methylation is one of the major mechanisms that keep it suppressed, though other controlling mechanisms have been also described (52, 53). We have previously shown that L1 transcripts were found to be overexpressed in SS patients compared to sicca controls in strong correlation with both IFNα and β at the level of MSG tissues. Failure of upregulation of L1 expression following stimulation of healthy PBMC and other cell lines with IFNα and TLR7/TLR9 ligands excluded the possibility of Type I IFN-induced L1 overexpression. On the other hand, transfection of pDCs or CD14+ monocytes with L1-carrying plasmids or L1-RNA led to type I IFN pathway activation through both TLR dependent and independent pathways, evidenced by the abrogation of type I IFN production following incubation with a TLR7/TLR8 or a TNF receptor associated factor NF-κB activator (TBK1)/IKKε inhibitor (54). Of interest, L1 expression was increased in patients with uncomplicated local SS which usually occurs in patients with advanced age (55). In line with these observations, a recent study has shown that L1 can drive type I IFN production in senescent cells (56).

Compatible with these findings, SS patients with heightened type I IFN scores in peripheral monocytes were shown to display increased transcript levels of both endosomal and cytoplasmic nucleic acid receptors such as TLR7, MDA5, RIG-I and protein kinase R (PKR) in both pDCs and monocytes, implying their contributory role in type I IFN production (57). Moreover, increased basal phosphorylation levels either in B cells (58) or T and Natural Killer (NK) cells together with enhanced B cell signalling through TLR7 and TLR9 ligation (25), have been proposed as potential drivers of augmented IFN responses in these patients.

Possible mechanisms accounting for L1 derepression remain of particular interest. A negative correlation between L1 expression and methylation levels of the L1 promoter were highly suggestive of impaired methylation mechanisms in SS patients (54). In order to further explore underlying abnormalities ac-

counting for the decreased methylation levels, we measured gene expression of several members of the methylation machinery and found a positive correlation between DNMT1, DNMT3B and MeCP2 and L1 expression in SS salivary glands and a negative correlation with lymphoid specific helicase (LSH). These observations imply a potential compensatory role for DNMT1, DNMT3B and MeCP2 in controlling inappropriate L1 overexpression with decreased LSH production potentially being responsible for the hypomethylation of L1 promoter (59).

Another restricting mechanism implicated in both viral infections and L1 control include members of the APOBEC family (60, 61). We have recently shown upregulation of APOBEC3A transcripts in SS MSG tissues in strong correlation with both L1 and IFNα mRNA, reflecting a potentially compensatory role against endogenous retroelements (62).

Finally, genetic influences have been shown to have an impact on type I IFN responses in the setting of autoimmunity. Thus, similarly to lupus (63), the rs10774671 variant of the 2'-5'-oligoadenylate synthetase 1 (OAS1), a viral RNA degrading enzyme, previously shown to be a risk allele for SS, has been shown to be related to a dysfunctional transcript. Thus, upon viral infection, a defective clearing mechanism of virus possibly occurs, leading to perpetuation of type I IFN responses due to the ongoing activation by viral remnants. In view of the implication of several viral triggers in SS pathogenesis (64), this mechanism might provide a functional explanation in the pathogenesis of SS. Moreover, in another study we have shown that protein tyrosine phosphatase non-receptor 22 (PTPN22), an allele associated with other autoimmune diseases such as SLE, DM and Grave's disease, is increasingly found in SS patients in comparison to healthy individuals, especially those with low type I IFN signature (65).

Type I IFN activation in SS clinical associations

Ocular and oral dryness are considered to be the most characteristic clini-

cal features of SS, but extraglandular manifestations can occur in at least one third of patients. The effort to associate specific clinical phenotypes with high or low IFN signature score follows the more well-established correlation between autoantibody/serological profiling and histopathological information from MSG biopsies, on the one hand and disease severity or even lymphomagenesis probability, on the other (66, 67).

A high type I IFN signature in CD14 monocytes was previously shown to identify SS patients with higher ESS-DAI scores (34). However, total ESS-DAI was not linked to high IFN activity (type I or mixed I/II) in a more recent study (33). SS individuals characterised by high type I IFN score have also exhibited higher anti-Ro52, anti-Ro60, anti-La, rheumatoid factor (RF) and serum IgG, as well as lower C3 levels and lower lymphocyte and neutrophil counts (34). Given that anti-Ro, anti-La and RF positivity has been associated with earlier disease onset, heavier glandular involvement and extraglandular manifestations, it is clear that there are clinical implications behind this association (68).

Despite the extensive knowledge on IFNα administration adverse effects, with flu-like symptoms and fatigue being the main complaints of patients receiving this treatment for various conditions, fatigue in the context of SS does not seem to correlate with IFN related genes (33, 69). As for IFN-induced protein expression on glandular tissue level, results from immunoblotting and immunohistochemistry showed a correlation between high IFN type I, II or mixed type I/II scores and more severe glandular secretory insufficiency, leukopenia and high ANA, anti-Ro/SSA, IgG and IgA titres. Last but not least, MSG biopsies focus score was remarkably higher in the high IFN type II group compared to low IFN altogether or high IFN type I groups (23).

All of the above lead to the impression that while both IFN types share some common pathways in the pathogenesis of SS, such as BAFF upregulation, it is the predominance of type II over type I IFN in the glandular tissue that tips the balance and promotes MALT lymphomagenesis. High focus score and low C4, seen more frequently in type II IFN-predominant MSG tissue group, have previously been identified as risk factors for lymphomagenesis in SS (23). Furthermore, IFNγ/IFNα mRNA ratio in MSG tissue has emerged as a histopathological biomarker for the prediction of in situ lymphoma development (27).

Finally, the presence of autoantibodies to interferon inducible protein-16 (IFI16) has been proven in various autoimmune diseases, including SS, in which it seems to be quite common. Anti-IFI16 antibody positivity characterises more frequently patients with abnormal Schirmer's test, elevated IgG levels and high ANA titres, as well as higher focus scores and germinal centre-like structures in their MSG biopsies (70). To be noted that IFI16 is an IFNγ-inducible protein which acts as a DNA detector in case of infection and regulates IFN type I transcription, with IFI16-knockout cells producing dramatically reduced IFNα (71).

A recent study revealed higher EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) total and ESSPRI sicca and pain domain scores among patients with the highest cumulative smoking consumption. Interestingly, type I IFN signature showed an inverse correlation with these domains. However, former or current smokers did not ever show remarkable controversy regarding type I IFN positivity or ESSDAI total score (72). Finally, in a recent study of medication-free SS patients receiving vaccination against H1N1, increased levels of protective antibodies were observed mainly as a result of IFNα induced B cell hyper responsiveness (73).

Treatment

Given that type I IFN overexpression seems to play a central role in disease initiation and progression in both SLE and SS, therapeutic attempts to downregulate its effect are gaining increasing attention over the last years (74).

As endogenous RNAs have been considered to be primary drivers of type I IFN in systemic autoimmunity, RNA degradation seems to be a logical tar-

get. RSLV-132, a fully human biologic Fc fusion protein consisting of an RNase fused to the Fc domain of IgG1, has been shown to inhibit type I IFN production. This agent is presently being tested in a phase II, double-blind, placebo-controlled study of 28 SS patients in light of promising results in a recently published clinical study of 32 SLE patients (75). The SS study is complete but no results have been as yet published (ClinicalTrials.gov Identifier: NCT03247686).

Of interest, administration of the antiviral agent lamivudine in an experimental aged model has been shown to downregulate type I IFN production through inhibition of L1 reverse transcriptase (56). These data might be relevant in SS patients, in which an upregulation of L1 retroelements have been observed at the level of salivary gland tissue (54).

Given that pDCs are viewed as a major cellular source of type I IFN, targeting of this cell population is also a promising anti-IFN strategy. The humanised monoclonal antibody 24F4A binds to the C-type lectin BDCA2, which is a specific receptor for pDCs, thus inhibiting IFN production. Clinical trials of BII059, another BDCA2 ligand, showed encouraging results in SLE, but not in SS patients yet. Immunoglobulin-like transcript 7 (ILT7)/LILRA4/CD85g -a member of the immunoglobulin-like transcripts (ILTs) or leucocyte immunoglobulin-like receptors (LIR) gene family- is a molecule predominantly expressed on the surface of pDCs (76). It has been shown that activation of ILT7 leads to differentiation of pDCs from an IFN producing to an antigen presenting phenotype via downregulation of TLR7/TLR9 mediated IFN production (77-79). Stimulation of ILT7 by an antibody (MEDI7734/VIB7734) was tested in 36 patients of type I IFN-mediated autoimmune diseases including SS. Although the study was completed, no results have as yet been published (ClinicalTrials.gov Identifier: NCT02780674).

TANK is another promising target in view of the significant role it holds in type I IFN production through IRF-3 and IRF-7 ligation. Administration of BX795 -a TANK inhibitor- in PBMCs

derived from SS patients resulted in downregulation of type I IFN inducible genes (80).

The idea to use monoclonal antibodies (mAbs) against IFNα in a way similar to that of TNFα inhibition in RA or even to produce anti-IFNα antibodies through vaccination has been proven in SLE with moderate results, failing to downregulate fully the IFN signatures. This might occur either because the antibodies fail to target all IFNα variants or do not affect other types of IFNs (81, 82). Targeting IFNAR has shown encouraging results in SLE patients with high IFN signature, but at the cost of upper respiratory tract infections and herpes zoster reactivation (83).

As discussed earlier, JAK kinases have been shown to mediate type I IFN effects following ligation of IFNAR. Small molecule JAK inhibitors are in clinical trials against SLE and SS (ClinicalTrials.gov Identifier: NCT03100942), with filgotinib showing encouraging results (84) while tofacitinib, already approved for rheumatoid arthritis (RA), has shown efficacy in murine NZB/NZW F1 and MRL/lpr SLE models (85). No data in SS are currently available.

BAFF levels are heightened in SS and associate positively with both type I and II IFN signatures at both peripheral blood and salivary gland tissue (27). Belimumab, a fully humanised monoclonal antibody towards the soluble B lymphocyte stimulator (BLyS), was tested in SS in two European centres, Paris, France and Udine, Italy (ClinicalTrials.gov Identifier: NCT01160666 and NCT01008982 respectively), with promising results. In a follow-up assessment of SS patients participating in the initial BELISS study a clinical and immunological deterioration was observed 6 and 12 months after cessation of treatment (ClinicalTrials.gov Identifier: NCT01008982). Interestingly, in a subgroup of the patients recruited for the BELISS study increased blood and salivary NK cell numbers in association with a worse response to treatment with belimumab was reported (86), while increased type I IFN scores at baseline were associated with improved outcomes, such as reduced IgG, IgM and RF serum levels (87). Based on these findings, the authors proposed the existence of two distinct subsets of SS: one with a predominant type I IFN-BAFF-B cell axis, representing good responders to belimumab; and one with a predominant type II IFN-NK cell axis, representing non-responders (86, 87).

Despite the major success in many rheumatic diseases, TNF inhibition failed to demonstrate efficacy in patients with SS (88, 89). Augmentation of the already upregulated type I IFN activity and the ensuing increased BAFF levels has been postulated as a potential reason for TNF failure in these patients (40).

Conclusion

With regard to SS, it seems that type I IFN dysregulation and overexpression is implicated in disease pathogenesis making its blockade an attractive therapeutic target. Although no concrete data exist as yet on medications against type I IFN currently on clinical trials, current evidence indicate that inhibition of this pathway at various stages could alter the course of the disease for SS patients. In the future, as our knowledge on its pathogenetic role in SS expands, more advanced agents will be targeting this pathway in an attempt to restore the balance of the immune system in these patients.

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