

Type-III interferons in Sjögren's syndrome

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ABSTRACT

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterised by aberrant activation of innate and adaptive immune responses. Part of this hyper-activation is due to the interferon (IFN) system. Deregulated expression and activity of the type-I IFN system has been extensively studied in pSS. Type-III interferons (IFNs) are the latest addition to the IFN family, and exhibit potent anti-viral functions, similarly to type-I IFNs. More recently they have started to attract attention as key modulators in the interface of innate and adaptive immunity and chronic inflammation. Deregulated expression of type-III IFNs has been demonstrated in various autoimmune diseases over the last ten years. The scope of this review is to summarise recent findings regarding the biology of type-III IFNs in pSS. We highlight factors that regulate their induction, their downstream effects, their similarities and differences with type-I IFNs and their possible modes of action in Sjögren's syndrome. Finally, we discuss their potential benefits as targets for therapeutic intervention.

Introduction

Primary Sjögren's syndrome (pSS) is a chronic, systemic autoimmune disease characterised by lymphocytic infiltrations of the salivary and lachrymal glands resulting in the presentation of sicca symptoms (oral and ocular dryness). Extra-glandular manifestations include arthritis, Raynaud's phenomenon, vasculitis, neuropathy, multiple organ involvement, whereas approximately 5% of the patients may develop B cell lymphoma (1). The disease is characterised also by plethora of autoantibodies, with the most common being directed towards the ribonucleoproteins Ro/SSA and La/SSB (2). Associated comorbidities include cardiovascular and musculoskeletal degenerative diseases (3).

Although the precise pathogenetic mechanism of pSS remain unclear, it is believed that the disease is a result of complex interactions between genetic/epigenetic, hormonal, immune and environmental factors. Aberrant stimulation of the innate and acquired immune responses in genetically predisposed individuals is a key feature, arising probably from environmental triggers. Indeed, viral infections may account for epithelial cell activation in the salivary glands, since toll-like-receptor (TLR) signalling pathways (like TLR2-4 and TLR7-9), which are sentinel pathways for viruses, have been shown to participate in innate responses in pSS. TLR signalling results in the production of cytokines like type-I IFNs (3). Type-I and -II interferons (IFNs) have been reported to be activated in pSS patients as well. Approximately 30 years ago, type-I IFNs were first shown to be upregulated in the blood of pSS patients (5). Activation of IFNs was more recently evaluated and confirmed by the expression of interferon stimulating genes (ISGs) also termed the 'interferon signature'. SS patients exhibit an IFN signature in peripheral blood mononuclear cells (PBMCs), isolated monocytes, plasmacytoid dendritic cells (pDCs), B cells and the afflicted salivary gland (6, 7). Genome-wide association studies (GWAS) and epigenome wide association studies (EWAS) have also revealed single nucleotide polymorphisms and a hypomethylated status in many IFN-inducible genes respectively in pSS (3). Inappropriate stimulation or persistent IFN activity may entail detrimental effects and type-I IFN activity has been associated with disease activity in SS (8).

The IFN pathway, appears as a main pathogenetic mechanism in SS and recent studies implicate also the activation of type-III IFNs. We herein review the latest findings on type-III IFNs in

SS, the potential factors that may regulate their expression, downstream effector functions and discuss how they may correlate with the underlying disease mechanisms. Lastly, we explore the clinical value of the combined IFN activity and potential therapeutic targeting in pSS.

Type-III IFNs

IFNs are produced by host cells in response to infection by pathogens (viruses, bacteria and parasites), exhibiting also anti-proliferative and immunomodulatory roles (9). Type-III IFNs, also termed IFNs- λ encompass four members in humans, namely IFN- λ 1/IL-29, IFN- λ 2/IL-28A, IFN- λ 3/IL-28B and IFN- λ 4, (10, 11). Their expression is induced upon stimulation of pattern recognition receptors (PRRs) that activate interferon regulatory transcription factors (IRFs). The promoter regions of type-III IFNs genes contain binding sites for IRF3, IRF7 and nuclear factor-kappa B (NF- κ B) (12). The preferential expression of IFN- λ 1/IL-29 is attributed to both IRF3 and IRF7, whereas IFN- λ 2/IL-28A and IFN- λ 3/28B expression is mainly due to IRF7 preferential binding. Repressor molecules that limit their expression include ZEB-1 and BLIMP-1 (13, 14), the miR-548 (microRNA) family (15), the ubiquitin protease USP18 (16) and the suppressor of cytokine signalling (SOCS) (17). Type-III IFNs exert their effects by engaging a common IFN- λ receptor, composed of the specific IFN- λ receptor chain 1 (IFN- λ R1/IL28RA) and the shared IL-10 receptor chain 2 (IL-10R β) (10, 11). Subsequent signalling events include the activation of JAK-STAT-dependent and independent cascades (MAPK and ERK) (18).

Type-III IFNs: how similar are they to type-I and -II IFNs?

Type-III IFNs are induced at barrier surfaces by cells of epithelial and hematopoietic origin in response to signals of innate immunity (12). Even though the stimuli that induce the expression of type-III IFNs are common with the other IFN families, some of the fundamental differences rely largely upon their transcriptional requirements. (19).

Genes encoding type-I IFNs lack introns, whereas type-III IFN genes entail a 5-exon gene structure typically found in the IL-10 cytokine family members (9), allowing the possibility for additional, regulatory post-transcriptional open mechanisms. Concerning their kinetics of expression, the induction of type-III IFN is more delayed and prolonged, over time, compared to the early peak and decline of type-I IFNs (9, 12). This feature is probably attributed to the different intrinsic properties of their signalling pathways and not the expression levels of their receptors (22). In terms of structure, type-III IFNs exhibit homology with type-I and type-II-IFNs, as well as members of the IL-10 superfamily. In terms of gene organisation and protein structure, type-III IFNs have more similarities to the IL-10 superfamily, even though functionally they share more features with type-I IFNs (23, 24). An important difference of type-III IFNs is their restrained/local action owing to the limited spectrum of cells expressing their receptor (mainly epithelial cells of mucosal/barrier surfaces). This provides a contained protection at barrier surfaces, that are mostly vulnerable to infections without the collateral damage of the highly potent type-I IFN response (23, 25). Interestingly, although type-I and type-III IFNs provide their signal through distinct receptor complexes, the downstream effector genes (ISGs) are to a large extent the same as those induced by type-I IFNs (IFN α/β), making the initial trigger of an IFN signature difficult to discern (26). This pronounced overlap is probably attributed to the cross-talk between the common components of downstream signalling pathways (27). Distinct IFN signatures among type-I, -II and -III IFNs may provide valuable information regarding their functional roles.

Cross-regulation of type-III IFNs with other IFN members

Evidence from *in vitro* and *in vivo* studies suggest a cross-regulation activity between type-I, -II and -III IFNs. Type-I IFNs (IFN- α) can induce the expression of type-III IFNs in type-2 myeloid DCs (dendritic cells) and PBMCs following

TLR stimulation (30), whereas the ablation of type-I IFNR signalling in mice (IFNAR^{-/-}), decreased the expression of type-III IFNs following viral infections (31). On the other hand, type-III IFNs are able to modulate the production of type-I and type-II IFNs. However, studies have shown both inducing (32, 33) and inhibitory effects (34) depending on the experimental settings.

Type-III IFNs in immunity and autoimmunity

Type-III IFNs have been postulated to contribute to the effectiveness of immune responses via their action at the interface of innate and adaptive immunity (35). One of the major functions of type-III IFNs is their effect on pDCs, which both produce and respond to type-III IFNs (36). *In vitro* studies have shown that type-III IFNs on human pDCs result in the induction of CD80 and ICOS-L expression (37), whereas on conventional DCs they lead to the proliferation of Foxp3+ suppressor T cells (38). Recent studies have provided evidence for an effect of type-III IFNs on neutrophils, where type-III IFNs have been shown to inhibit neutrophil recruitment in an experimental model of rheumatoid arthritis (RA) (39), downregulate oxidative stress (40) and inhibit the formation of neutrophil extracellular traps (NETs) (41). The potential contribution of type-III IFNs in skewing towards Th1 polarisation has only recently started to be investigated, with the results so far suggesting that type-III IFNs favour Th1 polarisation via increasing Th1 and/or reducing Th2 cytokines, decreasing Tregs and/or upregulating CD8 T cells (9). Genome analysis has revealed that an SNP (single nucleotide polymorphism) (rs8099917) in the *IFNL3* locus is associated with increased IFN- λ 3/IL-28B production by PBMCs and Th1 dominant responses (42). Also, in various experimental settings and in the context of vaccination as adjuvants, type-III IFNs have been found to downregulate Th2 cytokines (43, 44). Regarding humoral immune responses, the effect of type-III IFNs is still conflicting with some studies demonstrating an inducing effect on B cell activation and IgG

Table I. Type-III IFNs in various autoimmune diseases and proposed associations with clinical and/or laboratory parameters.

Autoimmune disease	Findings	Associations
Systemic lupus erythematosus (45, 67)	Increased levels of IFN- λ 1/IL-29 in serum and PBMCs	Correlated with SLEDAI, renal involvement, arthritis, anti-dsDNA antibody, CRP, and inversely correlated with C3 levels
Cutaneous lupus erythematosus (47)	Increased serum levels of IFN- λ 1/IL-29 and IFN λ R1 in skin lesions	Correlated with disease activity
Psoriasis (68)	Elevated production of IFN- λ 1/IL-29 in psoriatic lesions (mainly Th17 cells)	IFN- λ 1/IL-29 responsible for the elevated ISG levels on psoriatic lesions
Systemic sclerosis (69)	Increased serum levels of IFN- λ 1/IL-29	Correlated with levels of IFN- γ
Rheumatoid arthritis (70)	Increased levels of IFN- λ s in serum, PBMCs and synovial fluid	Associated with synovitis
Hashimoto's thyroiditis (71)	Increased serum levels of IFN- λ 1/IL-29 and IFN- λ 2/IL-28B	-
Inflammatory bowel disease (72)	Increased IFN λ and IFN λ R1 transcript levels in intestinal epithelial cells	-
Anti-phospholipid syndrome (73)	Decreased IFN- λ 1/IL-29 in PBMCs	Levels associated with obstetric APS
Primary Sjögren's syndrome (55, 56)	Elevated expression of IFN- λ 2/IL-28A (and possibly other IFN- λ s) in SGs; Increased levels of IFN- λ 1/IL-29 in serum	Related to intermediate grade inflammatory lesion severity

PBMCs: peripheral blood monocytes; SLEDAI: systemic lupus erythematosus disease activity index; dsDNA: double stranded DNA; CRP: C-reactive protein; C3: complement 3; ISG: interferon stimulated genes; IFN- γ : interferon gamma; SGs: salivary glands.

Table II. Genetic polymorphisms of type-III IFNs and of their signalling receptor found in autoimmune diseases and suggested associations.

Autoimmune disease	Locus	Associations
Systemic lupus erythematosus (74)	<i>IFNL1</i>	rs4649203, G correlates with exacerbated disease
Psoriasis (74)	<i>IFNL1</i>	rs4649203, G acts as a protective allele
Hashimoto's thyroiditis (71)	<i>IL-28B</i>	rs8099917, T acts as a protective allele

production and others showing an inhibitory effect (9).

Type-III IFNs in autoimmune diseases

Type-III IFNs have been found to be dysregulated in various autoimmune diseases, and in the context of some of them they have been associated with disease activity (Table I). They may contribute to the IFN signature, which encompasses deregulated expression of ISGs, some common with type-I and -II IFNs and most probably some yet unknown, unique for type-III IFNs. In diseases where type-III interferons have been found to be deregulated, this deregulation is observed mainly within the afflicted tissues, highlighting the ability of type-III IFN to act as local effectors. Type-III IFN expression is found to be significantly induced in several autoimmune lesions, including the skin in

psoriasis and cutaneous lupus erythematosus (CLE), salivary glands (SGs) in pSS and the intestinal epithelium in inflammatory bowel syndrome (IBD) (Table I). The consistent upregulation of IFN- λ 1/IL-29 in the blood of patients with different autoimmune diseases (Table I) suggests that IFN- λ 1/IL-29 might also have a systemic role. GWAS studies have also disclosed polymorphisms in type-III IFN and IFNLR loci that were correlated with autoimmune diseases (Table II). The assessment of type-III IFN expression levels in rheumatic disease, their specific 'IFN signature' and potential associations with clinical indices is still poorly explored in the literature. However, taking as an example the paradigm of type-I IFNs, the implementation of novel methodologies along with clinical grade tests for type-III IFNs would be important for diagnosis, assessment and better

understanding of their contribution in autoimmune diseases (8).

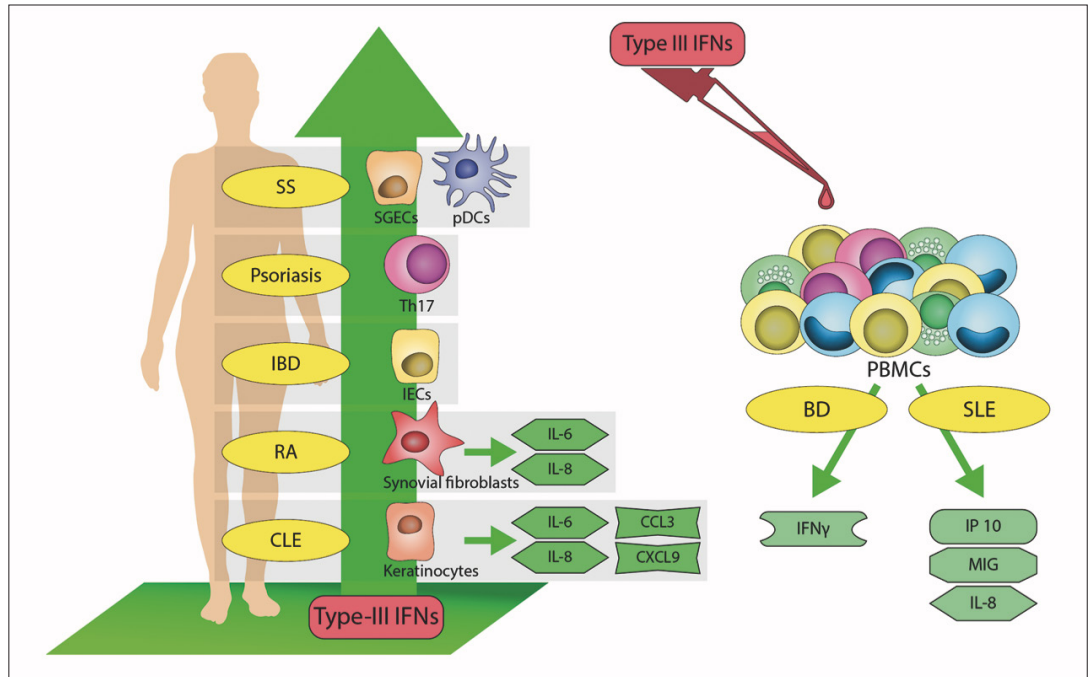
Potential downstream effects of type-III IFNs in autoimmune diseases have been demonstrated by various *in vitro* studies. In PBMCs from SLE patients, IFN- λ 1/IL-29 induced the production of chemokines CXCL10, IL-8 and MIG (45), whilst on synovial fibroblasts from RA patients they induced the production of IL-6 and IL-8, following TLR-4 stimulation (46). IFN- λ 1/IL-29 treatment of keratinocytes *in vitro* induced the expression of IL-6, IL-8, CCL3, and CXCL9 (47) and in human skin cells induced the CXCR3A binding chemokines (CXCL9, CXCL10, and CXCL11) (48). In PBMCs from Behçet's disease (BD) patients, IFN- λ 2/IL-28A induced IFN- γ production (49). The reported induction of type-III IFNs and potential downstream actions are illustrated in Figure 1.

Type-III IFNs in primary Sjögren's syndrome

Innate immune pathways in primary Sjögren's syndrome

Self-recognition and protection from non-self or harmless stimuli (commensals or allergens) relies on a set of in-

Fig. 1. Elevated expression/signalling of type-III IFNs in the afflicted tissues of various autoimmune diseases, their cellular sources and their proposed downstream properties. The *in vitro* effects of type-III IFNs in isolated peripheral blood monocytes (PBMCs) from SSc, BD and SLE patients. Arrows indicate induction.



nate immune receptors that recognise structures specific to pathogens and danger/tissue damage (pathogen and danger-associated molecular patterns, or PAMPs and DAMPs, respectively) (50). These include TLRs, Retinoic acid inducible gene-1 (RIG-I)-like receptors (RLRs) and nucleotide oligomerisation domain (NOD)-like receptors (NLRs). Patients with pSS exhibit upregulated levels of both those sentinel pathways and their ligands (7). Following their activation, IFNs are upregulated and remain uncontrolled in pSS patients. Subsequently, by acting at the interface between innate and adaptive immunity, they contribute towards an over-activation of the immune system that entails increased autoantigen expression, expansion of autoreactive T cells, differentiation of B cells and enhanced antibody production (26). The initial triggering of the aforementioned pathways is not clear. However, based on our current knowledge on their function, it is hypothesised that it could be either extrinsic factors like infection with pathogens, viruses or intracellular bacteria, or intrinsic factors like apoptotic/necrotic cells (51, 52). Interestingly, endogenous triggers of IFNs also include ribonucleoprotein complexes such as Ro52 (53) and endogenous virus-like retro-elements (LINE-1). The latter are normally found to be silent and have

been shown to be activated/upregulated in pSS patients (54). Moreover, inflammasome activation which has been linked to TLRs and the induction of inflammation following exposure to both exogenous and endogenous danger signals, has been found activated in pSS (3).

Upregulation of type-III IFNs in primary Sjögren's syndrome

All three members of type-III IFNs have been reported to be expressed in the salivary glands of pSS patients and non-SS controls in two independent studies. In the study by Apostolou *et al.*, all individual members of the type-III IFN family were found to be expressed in the SGs. Interestingly, a differential upregulation of IFN- λ 2/IL-28A only in the afflicted SG of pSS patients with intermediate inflammatory lesions, compared to *sicca* controls was noticed (55). This difference was confined to the epithelial compartment of the SGs and specifically in the ductal epithelium. In the study by Ha *et al.*, the presence of type-III IFNs was additionally confirmed in the SGs of pSS patients. Similarly, the proportion of positive for type-III IFNs acinar epithelial cells was higher in pSS patient SGs compared to non-SS controls whereas the highest intensity was observed in the ductal epithelium (56). The correlation of type-III IFN levels

in pSS patients with the inflammatory lesion severity is important. The grade of inflammatory lesion correlates with a distinct composition of cells (predominantly T cells in mild and B cells in severe lesions) in the inflammatory foci indicating most probably distinct underlying mechanisms (57). A differential pattern of expression of type-I and -II IFNs has also been documented in the SGs of pSS patients. Three distinct phenotypes have been recognised among pSS patients: Type-I IFN predominant, type-II IFN-predominant and mixed type-I/II. In the type-I/II phenotype, type-I IFNs were found predominantly in ductal epithelium and were associated with the grade of inflammatory lesion severity, type-II IFNs were found in infiltrating immune cells (6) and the ratio of type-II to type-I was associated with the prediction of lymphoma development (58). It would be interesting to investigate the relative expression of all three types of IFNs simultaneously in the SGs of pSS patients and the potential associations with histological, laboratory and clinical features.

The expression of the common receptor IFNLR has been found to be expressed in the SGs (55, 56). The expression levels were comparable in both pSS and non-SS controls with a similar pattern; it is expressed predominantly in the epithelial compartment of the SGs and

in some infiltrating mononuclear cells (MNCs) in pSS lesions. In the study by Apostolou *et al.*, the strong expression of IFNLR in MNCs was mainly attributed to pDCs, as revealed by double immunofluorescent analysis. pDCs constitute the "professional" IFN-producing cells and were among the first cell types found to express IFNLR. Type-III IFNs have been shown to act on pDCs and induce the production of type-I IFNs, TNF- α and the upregulation of co-stimulatory molecules like CD80 and CD86, that trigger the maturation of pDCs (36). These actions appear to have a central role in the immune responses in pSS, since the salivary tissue constitutes the initial site of autoimmune responses. The high expression of the IFNLR in pDCs along with the local expression of type-III IFNs by the salivary gland epithelial cells suggests a potential novel link between pDC activation by the functionally altered epithelium in pSS.

The presence of type-III IFNs has been also evaluated in the peripheral blood. In contrary to other autoimmune diseases, such as SLE and RA (Table I), type-III IFNs could not be detected in circulating PBMCs from pSS patients (55). The investigation of sera, however, revealed that IFN- λ 1/IL-29 was found to be higher in patients with intermediate inflammatory lesions in the SGs (55). The importance of this finding remains unaddressed; it follows the pattern of IFN- λ 2/IL-28A in the salivary gland and probably suggests a cross-regulation between the individual members of type-III IFN family. Even though no differences were found in the blood regarding the levels of IFN- λ 1/IL-29 in the study by Ha *et al.*, the authors do address the low number of patients analysed and the lower disease activity index, as evaluated by the ESSDAI score of their cohort (56). Whether IFN- λ 1/IL-29 has a systemic role in pSS remains an open issue and further analysis is required with larger cohorts of patients.

Factors that could drive the activation of type-III IFNs in primary Sjögren's syndrome

The exaggerated expression of type-III IFNs in afflicted salivary glands in pSS

is probably induced by tissue micro-environmental factors such as viral infections, apoptotic/necrotic cell death etc. Salivary gland epithelial cells (SGECs) from pSS patients and salivary gland cell lines *in vitro* do not constitutively express type-III IFNs. However, TLR3 activation *in vitro* in both cases induces strong expression of type-III IFNs (55, 56). TLR3-induced type-III IFN activation was evident within a few hours, and particularly for SGECs, the pattern was similar to that previously observed for IFN- β (4, 55). In both SGECs and salivary gland epithelial cell lines, the effect on IFN- λ 1-IL-29 was more pronounced, compared to that for IFN- λ 2/3-IL-28AB. However, regarding IFN- λ 2/3-IL-28AB, the time-course analysis of TLR3 stimulation in SGECs from pSS displayed an interesting pattern for biphasic expression which was not observed in *sicca* controls (55). The former observation suggests that additional factors acting downstream of type-III IFN signalling may augment a second wave of expression in a positive feedback manner. The nature of the inducing factor is still unknown, however, given the cross-regulatory properties among the IFN families it would be plausible to hypothesise that this factor could be a type-I IFN. In this scenario, type-III IFNs could potentially be employed and contribute in both early and later lymphocyte-mediated stages of the underlying disease mechanisms. Type-III IFNs in the salivary gland cell lines *in vitro* upregulated also the expression of TLR3, suggesting a positive feedback mechanism that may affect the availability of both type-I and type-III IFNs locally in the salivary gland (56). In this case, a chronic stimulation of the salivary gland epithelium by environmental (*i.e.* viruses) or intrinsic (*i.e.* cell death) factors could contribute in a vicious circle of sustained IFN expression.

Effects of type-III IFNs in salivary gland epithelial cells

The local effects of type-III IFNs in the salivary gland are not yet established. Previous studies have demonstrated the capacity of type-III IFNs for inducing immunomodulatory cytokines from

epithelial cells at mucosal surfaces, hence modulating both innate and adaptive immune responses (59). However, the effect of type-III IFN treatment on the expression of type-I and or type-II IFNs in SG epithelial cells has not been studied yet. Regarding factors that act downstream of type-III IFNs, treatment with IFN- λ 1/IL-29 in a salivary gland epithelial cell line (NS-SV-DC) was shown to induce the upregulation of B-cell activating factor (BAFF), CXC-motif chemokine 10 (CXCL10) and TLR3, in synergy with IFN α (56). BAFF is found elevated in the serum, saliva, mononuclear cells and in the SGs of pSS patients (60) and constitutes a key mediator of B cell activation. The levels of BAFF in pSS have been suggested to correlate with disease activity and with clonal B cell expansion (60). CXCL10 is upregulated in the saliva, tears and the SGs of pSS patients and the CXCR3 receptor is expressed in the majority of T cells in salivary gland inflammatory lesions (61). Both cytokines have been associated with IFNs. BAFF production has been previously shown to be dependent on IFN- α (58) whereas CXCL10 can be induced by type-I IFNs (62). The synergistic effect of IFN- λ 1/IL-29 on CXCL10 production in a salivary gland cell line in the study by Ha *et al.* is most probably indirect. A recent study has shown that CXCL10 transcription relies completely on IRF-1. STAT-1 dependent transcription of IRF-1 is mediated by type-I but not type-III IFNs (62). Consequently, the synergistic upregulation of CXCL10 is most probably mediated by a type-III IFN-induced factor (probably type-I IFNs) rather than IFN- λ 1/IL-29 *per se*. The synergistic effect of type-III IFNs in upregulating BAFF and CXCL10 could actually be attributed to a positive feedback mechanism acting on type-I IFNs or other unknown mediating factors.

Implications of therapeutic targeting of IFNs in primary Sjögren's syndrome

Since pSS patients exhibit a prominent IFN signature that has also been proposed as a biomarker for active disease and disease-affected tissue (63, 64), the

therapeutic targeting has focused so far either on inhibition of type-I IFNs production or targeting cells or molecules that are induced in response to them. Hydroxychloroquine impairs type-I IFN production from pDCs, possibly through the restriction of TLR7 and TLR9 ligation by nucleic acids (65). In a randomised clinical trial in SS patients, hydroxychloroquine failed to improve symptoms, even though type-I IFN inducible genes had been downregulated. RSLV-132 (an RNase fused to the Fc domain of IgG1) dampens type-I IFN production indirectly by degrading circulating immuno-stimulatory RNA-immune complexes. It is currently evaluated in a phase II clinical trial in SS patients with upregulated type-I IFN responses. An ILT7 (Immunoglobulin-like transcript 7) receptor antibody (MEDI7734/VIB7734) that dampens type-I IFN production from pDCs is also currently in a phase-I clinical trial in SS patients, whereas an ongoing trial with BX795 (TANK inhibition) dampened type-I IFN ISGs in PBMCs from SS patients. Kinase inhibitors including lanraplenib (Tyk2 inhibition) and filgotinib (JAK1 inhibition) interfere with type-I IFN signalling and have been tested in a phase-II trial for efficacy and safety in SS patients (66). Targeting IFN signalling must be based on the IFN signature of selected patients that could potentially benefit from such interventions. This is important and may partly explain the limited efficacy of such treatments in specific patient subsets. Since the involvement of type-III IFNs has only recently started to be explored in the biology of SS, it would be important in future studies and clinical trials to take into consideration their patterns of expression and potential downstream effects. Furthermore, the largely overlapping functions of type-I and type-III IFNs, along with the nature of restricted expression of type-III IFNR in certain cell subsets, suggest that targeting them instead of type-I IFNs might be favourable and safer. Moreover, since the knowledge of the biology of type-III IFNs in pSS is yet limited, currently unknown, non-redundant roles may suggest that a combined modulation of type-I and type-III IFNs might offer a benefit.

Perspectives of type-III IFNs in primary Sjögren's syndrome

The proposed functions of type-III IFNs are rather complex, diverse and can be mediated either independently or in combination with the other IFNs. Their immunomodulatory function in pSS seems to exhibit mainly a local role. This confined upregulation of type-III IFNs in the salivary gland may result from chronic activation of innate immune pathways in an effort to restrict either viral infections or intrinsic defects stemming from aberrant cell death. The type-III IFN expression by the salivary gland epithelial cells could exert both an autocrine and a paracrine effect. Type-III IFNs can signal to pDCs and to epithelial cells and regulate their function. Sustained expression and/or over-activation of the type-III IFN system could potentially result in the altered antigen presentation properties of the former cells within the salivary gland, Th1 skewing and auto-antibody production. Recent studies identify type-III IFNs as a first line defence mechanism that compared to type-I IFNs is lower in magnitude, less inflammatory and concentrated at the epithelial compartment. There, they balance the necessity of the more potent type-I IFNs to be activated, which would also have a systemic effect. In this scenario, they can act by tempering the activation of systemic responses by type-I IFN which can be associated with severe consequences like the induction of pro-inflammatory cytokines, collateral tissue damage and hindering of adaptive immune responses that would eventually resolve inflammation and promote protective immunity. Similarly, in pSS type-III IFNs may act as modulating factors in maintaining epithelial cell integrity in the salivary gland. When this integrity is compromised, they initiate more drastic measures by promoting activation of the type-I IFNs. Lastly, the identification of type-III IFN signature in pSS could be a valuable tool for the distinction between diverse subsets of patients, aiming to clarify the heterogeneity underlying this complex disease. Given the similarities and differences they present with type-I IFNs in terms of function, induction and downstream

effects, it would be interesting to further investigate their mechanistic role in the aberrant immune responses in pSS.

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