Review

Interferon-stimulated GTPases in autoimmune and inflammatory diseases: promising role for the guanylate-binding protein (GBP) family

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Abstract

Human IFNs are secreted cytokines shown to stimulate the expression of over one thousand genes. These IFN-inducible genes primarily encode four major protein families, known as IFN-stimulated GTPases (ISGs), namely myxovirus-resistance proteins, guanylate-binding proteins (GBPs), p47 immunity-related GTPases and very large inducible guanosine triphosphate hydrolases (GTPases). These families respond specifically to type I or II IFNs and are well reported in coordinating immunity against some well known as well as newly discovered viral, bacterial and parasitic infections. A growing body of evidence highlights the potential contributory and regulatory roles of ISGs in dysregulated inflammation and autoimmune diseases. Our focus was to draw attention to studies that demonstrate increased expression of ISGs in the serum and affected tissues of patients with RA, SS, lupus, IBD and psoriasis. In this review, we analysed emerging literature describing the potential roles of ISGs, particularly the GBP family, in the context of autoimmunity. We also highlighted the promise and implications for therapeutically targeting IFNs and GBPs in the treatment of rheumatic diseases.

Key words: interferons, ISG, GBP, autoimmune, rheumatoid arthritis, IBD, psoriasis, Sjögren's syndrome, lupus

Rheumatology key messages

- Guanylate-binding proteins play an important role in immune defence mechanisms that are altered in autoimmune diseases.
- Emerging evidence points to their pivotal role in rheumatic diseases and in the function of IFN- γ .
- More studies are needed to validate their role in pro-inflammatory cytokine signalling in rheumatic diseases.

Introduction

Inflammation is an inherent response of the body in defending itself from infections and injury while also ensuring timely resolution and restoration of normalcy. This delicate balance is illustrated during viral infections,

wherein type I and type II IFNs coordinate unique but complementary arms of the body's immune response [1, 2]. Localized or systemic disruption in the critical balance of pro-inflammatory and anti-inflammatory factors underlie autoimmune diseases [3]. For several decades, IFNs have not only been characterized in innate and adaptive immunity, but also in cancer, inflammatory, and autoimmune diseases [3]. In this review, we outline emerging evidence that highlights the potential role of IFN-inducible GTPases (ISGs), and particularly the guanylate-binding protein (GBP) family, in the context of autoimmunity. We further discuss the implications and opportunities for pharmacologic interventions that directly or indirectly target the IFNs and their downstream products in the treatment of rheumatic diseases.

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Submitted 2 June 2020; accepted: 23 August 2020

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Interferons (IFNs)

Much of our fundamental knowledge of IFNs stems from viral studies. Type I IFNs (IFN-I, such as α and β) induce antiviral response within infected cells and their surrounding tissue by upregulating proteins that impede viral replication, and promote recruitment and activation of monocytes, dendritic cells (DCs) and NK cells [4]. Type II IFN (IFN- γ) similarly induces intracellular antiviral programs in tissues, but also enhances phagocytosis, antigen presentation and co-stimulatory signalling by macrophages and DCs to provoke an adaptive immune response [4]. Besides antiviral activity, the IFNs have immunomodulatory effects that restore homeostasis as part of the resolution and repair processes after infection.

Initially, the classification of IFNs was based on the cell type from which they are produced; however, more recently, their nomenclature was changed according to their receptor specificity. IFN- α is produced and secreted by almost all cell types, whereas IFN- β is mainly produced by fibroblasts. On the other hand, IFN- γ is produced by Th1 lymphocytes, NK cells, NK T cells, cytotoxic lymphocytes, B cells, and antigen-presenting cells [5]. IFN- γ does not exhibit any homology to type I IFN. IFN- λ , type III IFN, has also been characterized in autoimmune rheumatic diseases [6], but the role of this IFN is beyond the scope of this review article.

IFN signalling

IFN signalling begins with two subunits of both IFN receptor classes, receptor 1 (IFNAR1 and IFNGR1) and receptor 2 (IFNAR2 and IFNGR2). Both of the IFN receptors signal through the Janus kinase – signal transducer and activator of transcription (JAK-STAT) pathway. For type I IFNs, the receptor IFNAR1 is associated with JAK-1, whereas IFNAR2 is associated with TYK-2. However, IFNGR1 and IFNGR2 are associated with JAK-1 and JAK-2, respectively. Binding of IFNs to both type I and II receptors causes the autophosphorylation of JAK-1 and JAK-2 kinases (JAK1 for both types I & II IFN, and JAK1 and JAK2 for IFN- γ). This autophosphorylation triggers the subsequent phosphorylation of STAT1 and STAT2. After activation, the STATs homodimerize or heterodimerize and translocate to the nucleus to bind to the promoter region of IFN-inducible genes [7]. IFNs coordinate key biological activities in immunity, cancer, inflammation, and autoimmune diseases by inducing the expression of hundreds of genes. The identifiable IFN-inducible gene expression profile encoding antimicrobial proteins, cytokines, chemokines, and growth factors is known as the IFN signature in various disease states [8].

For many years, IFNs were associated with multiple autoimmune diseases, including RA, SS and SSc [3, 9]. However, in this review we will focus on ISGs and their possible connection with autoimmune diseases.

ISGs

IFN-primed cells produce four classes of dynamin-like GTPases named myxovirus resistance proteins (Mx), GBPs, p47 immunity-related GTPases (IRGs), and very large inducible GTPases (VLIGs) [10]. The most highly studied of these is the Mx family of proteins. These were first characterized for their antiviral activity against influenza and influenza-like virus [11]. More recently, Mx family proteins have been characterized in mice and humans and were found to have the most potent antiviral activity of the ISGs [2]. The *IRG* gene family varies among species. For example, mice have >20 *IRG* genes, whereas humans have only two and chickens have none. The VLIG family proteins are the largest in terms of molecular weight and are the least characterized among the ISG family. Like IRGs, VLIGs vary significantly among species [11].

p47 IRG proteins

IFN- γ regulates the expression of over 1200 genes, a number of which encode effectors that function to eradicate pathogens from host cells [12–14]. One family of IFN- γ -regulated proteins that play an essential role in resistance to bacterial and protozoan infection was initially known as the p47 GTPases, recently termed IRGs [1, 15, 16]. *IRG* genes or related sequences have been described in mice, in which the expression of most genes is stimulated by IFN- γ via classical IFN-stimulated response elements (ISREs) and/or gamma-activated sequence sites (GASs) in the promoter regions of the genes. Expression of the IRG proteins occurs in most tissues, as well as in many leukocytes, including macrophages, neutrophils, and T- and B-lymphocytes. Recent studies have implicated p47 IRGs in autoimmune diseases. For example, in experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis (MS), LRG-47 (also known as *Irgm1*) promotes Th1/Th17 cell differentiation and T cell infiltration into the CNS in MS pathogenesis [17, 18]. Importantly, these effects were abolished by CCL20 small interfering RNA (siRNA), anti-TNF- α , or anti-IL-1 β mAb, suggesting a central role of these mediators in LRG-47 function. The specificity of the IFN response was illustrated using other cytokines, including IL-1 α , IL-1 β , IL-2, IL-4, IL-6, TNF- α and GM-CSF, all of which failed to activate *IRG-47* gene expression [19–21], suggesting that p47 GTPases are specific to IFN responses. In the realm of rheumatic diseases, a recent study by de Jong *et al.* showed the downregulation of IFN-responsive genes in response to immunosuppressive treatment in RA patients [22]. However, the role of p47 IRGs in RA pathogenesis remains inconclusive.

Mx proteins

Mx proteins are evolutionarily conserved dynamin-like large GTPases, and GTPase activity is required for their antiviral activity. *Mx* genes exist in nearly all vertebrate genomes, from fish to primates, and they are active

mainly against RNA viruses. The expression of *Mx* genes is controlled by type I and type III IFNs, which upon binding to their receptors (IFNAR1/2 and IL-28Ra/IL-10RB, respectively), induce antiviral activity within the cell by inducing the transcription of IFN-stimulated genes.

In a recent study, Lambers *et al.* found an increase in IFN-score (a measurement of IFN type I expression and IFN-related mediators) in approximately half of the patients with incomplete or established SLE [23]. An increase in IFN-score strongly correlated with *MxA* levels in the blood and unaffected skin, which suggest *MxA* as a biomarker for IFN upregulation in SLE. Another study revealed a significant association between *MxA* expression levels in muscle fibres and clinical measures of muscular disease activity in JDM patients [24]. Among several ISGs, *MxA* was found to be one of the key downstream mediators of TANK-binding kinase 1 (TBK1), an important signalling hub downstream of retinoic acid-inducible gene-I-like receptors (RIG) and DNA-sensing receptors. This pathway is implicated in patients with autoimmune IFNopathies, including SLE, primary SS (pSS), and DM [25, 26].

Similarly, *MxA* and *MxB* genes were found to be significantly associated with the levels of anti-citrullinated protein antibodies and anti-carbamylated protein (Anti-CarP) antibodies in early as well as established RA [27]. Plasmacytoid dendritic cells (pDCs) isolated from synovial tissues of RA and PsA patients showed higher expression of *MxA* when compared with OA synovial tissues, and blood-derived pDCs from RA and PsA patients responded more strongly to CXCL-10-, CXCL-11- and CXCL-12-driven chemotaxis owing to higher CXCR3 and CXCR4 expression [28]. Furthermore, another study demonstrated the clinical utility of baseline IRGs, such as *MxA*, as predictive biomarkers for non-response to rituximab therapy in RA [29]. A study by Maria *et al.*, which aimed to examine the clinical applicability of IFN type I in a cohort of 35 pSS patients, found that IFN scores correlated best with monocyte *MxA* protein and whole-blood *MxA* levels, and offered a similar correlation with European League Against Rheumatism (EULAR) Sjögren's Syndrome Disease Activity Index (ESSDAI) and pSS clinical parameters, concluding that the *MxA* assays may serve as excellent tools for assessing IFN type I activity in pSS [30]. Immunological staining of skin biopsies for *MxA* has been a valuable tool for differentiating the new onset or severe exacerbation of psoriasis, as a rare complication arising from anti-TNF therapy, from psoriasis vulgaris [31].

VLIG family proteins

The VLIG GTPase family members are the largest in terms of their molecular weight (~280 kDa) and the most recent ISG family to be described [21, 32]. There are six VLIG genes encoded on chromosome 7 in the mouse, but only one has been discovered in humans (*VLIG-1*) [32]. The single article by Klamp *et al.* reporting on discovery of this family provided insights into VLIG-1

localization in cytoplasm and nucleus. However, the functional relevance to any diseased condition or to autoimmunity remains to be determined [32]. Its activation mechanism is mediated partly through IRF-1 TFBS (transcription factor binding sites), and the protein-protein interaction database search identified cullin-3, COP9 signalosome subunit 5, and protein kinase D2 as its interacting partners [BioGRID Database, www.thebiogrid.org], which needs to be further validated experimentally. *VLIG-1* can be activated in response to other cytokines, such as in TNF- α -treated fibroblasts or IFN- γ - and IFN- β -stimulated macrophages. While some follow-up studies suggest roles in adaptive antiviral protection and antibacterial actions [33], the functional significance of their expression and role in autoimmunity have not been studied yet.

GBPs

The GBP family proteins were first isolated and characterized from murine cells in response to IFN- γ activation. GBPs are ISGs with a molecular weight range of 65–73 kDa. This protein family is mainly known for its diverse activity against invading microbes and pathogens as a part of innate immune responses [10, 11]. In humans, there are seven *GBP* genes clustered on chromosome 5. In mice, 11 *GBPs* are distributed over two chromosomes. GBP1, the most highly characterized protein of the GBP family, has been shown to act against various viruses and other pathogens [34]. GBPs are dynamic GTPase enzymes known for their ability to bind to GTPs and GDPs with similar affinity [35].

Our understanding of the potential role of the GBPs in autoimmune diseases will hinge on an understanding of their structure, protein binding, and enzymatic functions. Experimentally manipulating the functional domains of GBPs has provided valuable information about the mode of action of GBPs in various disease states. One distinct property of GBPs is that they do not require GTPase-activating proteins or guanine nucleotide exchange factors to hydrolyze GTP. The effect of each GBP varies based on its structure and mode of enzymatic action [1], either by GTPase activity or through the helical domain [36–38].

While only modest basal expression of GBPs has been observed in the liver, lung, kidney, and digestive tract, and GBPs can be induced by both type I and II IFNs, this family of proteins is primarily induced by IFN- γ [34]. Other inflammatory cytokines such as IL-1 α , IL-1 β and TNF- α can also induce GBP1 in epithelial cells, where it was suggested as a potential activation maker in these cells [39]. The induction of the GBPs by inflammatory cytokines points towards their potential roles in the pathogenesis of inflammatory diseases (summarized in Table 1).

Unfortunately, only a few studies have demonstrated the effect of GBPs in the cytokine signalling cascades. p50 was shown to increase the promoter activity of GBP5, and NF- κ B was found to be important in activating the promoter activity of GBP5 during Influenza-A

TABLE 1 List of autoimmune diseases and associated guanylate-binding proteins

Diseases	Cell type involved	GBPs	Cytokine stimulation	Species	Detection Method	Ref.	Year
RA	Serum	GBP1		Human	ELISA	[40]	2011
	Synovial fibroblast	GBP1, GBP5	IFN- γ , IL-1 β , TNF- α	Human	Western blot	Unpublished	
	Synovial tissues	GBP1, GBP5		Human	scRNA-seq, RNA-seq, mass cytometry, flow cytometry	[41]	2019
Psoriasis	Psoriatic skin	GBP1, GBP2		Human	Affymetrix oligonucleotide arrays	[42]	2001
	HUVEC	GBP1	IFN- γ , IL-1 β , TNF- α , IL-1 α	Human	Western blot	[39]	2002
	Endothelial cells of uterus, lung, heart, spleen	GBP1		Human	Immunohistochemistry	[39]	2002
	Mononuclear cells in the bladder, lung, stomach, colon and liver	GBP1		Human	Immunohistochemistry	[39]	2002
	Epithelium in, lung, colon, thyroid, prostate and stomach						
	CD31 in the tissue of bladder, endometrium, heart and lung	GBP1		Human	Immunohistochemistry	[39]	2002
AIDS-associated KS LE	Diseased skin	GBP1		Human	Immunohistochemistry	[39]	2002
	Serum of patients with SLE	GBP1		Human	ELISA	[40, 43]	2011, 2011
	Skin of patients with Cutaneous LE	GBP1		Human	Immunohistochemistry	[43]	2011
SSc IBD	Primary keratinocytes	GBP1	IFN- α & IFN- γ	Human	Western blot		
	Serum	GBP1		Human		[40]	2011
	Primary mouse intestinal endothelial cells	GBP1	IFN- γ	Mouse	Western blot	[44]	2015
	Non-transformed colon epithelial cells		IFN- γ , IL-1 β , TNF- α	Human	Western blot	[45]	2013
	Blood vessel endothelial cells of IBD patients			Human	Western blot	[44]	2015
	Colon mucosa of patients with ulcerative colitis or Crohn's disease			Human	Immunohistochemistry	[45]	2013
	Epithelial cell lines		IFN- γ	Human		[46]	2009

CD31; cluster of differentiation 31; GBP (1/2/5); guanylate-binding protein (1/2/5); HUVEC; human umbilical vein endothelial cell; KS; Kaposi's sarcoma; LE; Lupus Erythematosus; scRNA; small conditional RNA; seq; sequencing;

viral infection. In 293T cells, IFNs such as IFN- β and IFN- λ and other downstream effectors such as IFN-Stimulated Response Element (IRSE) were activated by GBP5 during influenza-A virus induction [47]. Also, GBP1 was found to be upregulated by the NF- κ B pathway in Kaposi's sarcoma-associated herpesvirus (KSHV) infection in human umbilical vein endothelial cells (HUVECs) [48].

Similarly, the NF- κ B pathway is essential in TNF- α -mediated MMP-9 production by murine GBP2 (mGBP2) in NIH 3T3 cells. Interestingly, mGBP2 did not inhibit TNF- α -mediated degradation of I κ B α ; instead, mGBP2 inhibited the binding of p65 to the promoter region, inhibiting TNF- α -mediated Rac activation [49]. Also, both in T84 and HT29 cell lines, overexpression of GBP2 repressed the Wnt signalling pathway [50].

Though the GBP family has not yet been well characterized in the context of the spectrum of autoimmune diseases, emerging evidence as summarized in this

review provides an argument for rigorous research to decipher the potential role of GBPs in autoimmunity.

GBPs in rheumatic diseases

IFN- γ and GBPs have shown altered expression in rheumatic disorders such as RA, SLE and SS [3, 51]. Fig. 1 provides an overview of the signalling pathways affected by GBPs in response to IFN stimulation. However, the reason for the altered expression of these GBPs and underlying mechanism(s) is not well characterized, which provides an opportunity in this review to examine the emerging relevance of the GBP family in rheumatic diseases as discussed below.

RA

RA is a chronic autoimmune disease characterized by erosive polyarthritis in the joints. RA can impact other organs and can result in extra-articular complications, progressive disability, increased morbidity, and early

Fig. 1 The signalling pathways affected by guanylate-binding proteins in response to stimulation

Activated signal transducers and activators of transcription (STATs) translocate to the nucleus and bind to the IFN-stimulated response elements (ISREs) (for Type 1 IFN) and gamma-activated sequence sites (GASs) (for Type 2 IFN) promoter region to induce the transcription of IFN-stimulated (guanosine triphosphate hydrolases (GTPases)) (ISGs). Induction of guanylate-binding proteins (GBPs), one of the IFN-induced GTPases, further influences various signalling pathways. Studies suggested that GBP1 and GBP5 cause activation of the NF- κ B signalling pathway. mGBP2 causes the suppression of NF- κ B and Wnt signalling [47–50].

mortality [52]. The exact aetiology of RA remains unknown; however, genetic predisposition, environmental factors (especially smoking) and infection have been found to contribute to disease pathogenesis.

Soluble GBP1 was found to be higher in the serum of patients with RA compared with normal healthy donors [40]. In comparison with other rheumatic diseases such as SLE or SSc, the levels of GBP1 in the serum of RA patients was found to be higher. The study further tried to provide evidence for a role of GBP1 in endothelial dysfunction and the regulation of endothelial progenitor cell (EPC) activity. The findings suggested that GBP1-induced premature differentiation of EPCs, as evident from increased expression of the cell differentiation markers Flk-1 and vWF, is a major reason for their impaired vasculogenic ability, proliferation, and migration.

EPCs are released from bone marrow and play an important role in the formation of blood vessels in the embryo and also in adults [53]. A reduction in the number of circulating EPCs is associated with coronary artery disease and is a marker for increased risk of

cardiovascular disease [54]. RA is not only associated with bone destruction but also with increased risk of cardiovascular complications. On the other hand, several studies suggest active recruitment of EPCs to the inflamed joints, where they contribute to the synovial neovascularization, is critical in RA pathogenesis [55, 56]. RA patients with active disease have reduced peripheral blood EPC counts, which increased upon initiation of anti-TNF- α therapy [57]. While Hammon *et al.* provided circumstantial evidence for using GBP1 as a novel biomarker for inflammatory vessel activation in RA [40], it is still unclear how synovial tissue expression of GBP1 relates to the increased EPC counts in the synovial microenvironment that are important for neovascularization of RA joints. Furthermore, a correlation of synovial tissue GBP1 with its circulating levels in RA vs healthy donors may provide a more precise understanding of whether GBP1 contributes to or helps in downregulating inflammatory vessel formation and unwanted joint remodelling in RA pathogenesis.

Among interacting protein partners of GBPs, we found that GBP5 interacts with the anti-inflammatory protein

TNF- α -stimulated gene 6 (TSG-6) (our unpublished data). TSG-6 is a 35 kDa secretory protein initially identified by Lee and colleagues in fibroblasts stimulated with TNF- α [58]. TSG-6 is produced in a wide variety of cells upon stimulation with cytokines such as IL-1 β , TNF- α and TGF- β [59] and is upregulated in the SF of RA patients [60]. Two essential roles of TSG-6 are ECM remodelling and cell proliferation. TSG-6 expression is lower in serum as compared with SF, indicating that this protein is upregulated in the local inflammatory environment [61]. TSG-6 knockout mice showed severe joint damage in inflammatory arthritis [62], whereas TSG-6 transgenic mice exhibited reduced susceptibility to collagen-induced arthritis [63]. While these findings point to the potential role of GBP5 in the regulation of TSG6, the mechanisms by which GBP5 controls the expression of TSG-6 and mediates its anti-inflammatory actions have not been characterized yet. Important to note here is the study by Glant *et al.*, in which transgenic mice constitutively expressing TSG-6 protein in cartilage were only protected from cartilage destruction, but not severe inflammation, in the antigen-induced arthritis model [64]. This finding, in conjunction with our unpublished observation in which GBP5 not only maintained TSG-6 expression, but also suppressed RA synovial fibroblast-mediated inflammation, provides a reason to further study how GBP5 can serve as a potential therapeutic gene that concomitantly regulates inflammation and tissue destruction in RA.

IFN- γ -induced GBP1 and GBP2 are the most abundantly expressed proteins in the ISG family [65], and studies suggest their involvement in processes central to RA pathogenesis. Angiogenesis in RA is essential for RA synovitis and is driven by pro-inflammatory cytokines, chemokines, and growth factors [66–68]. Angiogenesis differs from vasculogenesis in that the new blood vessels are formed from the existing blood vessels rather than differentiating from stem cells. Angiogenesis is also associated with wound healing, metastasis, and tumor growth. GBP1 has been shown to mediate the anti-proliferative effect of pro-inflammatory cytokines such as IL-1 α , IL-1 β and TNF- α on endothelial cells without affecting their cell adhesiveness. In contrast, angiogenic growth factors such as basic fibroblast growth factor and VEGF inhibited the expression of GBP1 to revert this process [37]. A follow-up study by the same group showed that pro-inflammatory cytokine-activated GBP1 inhibited endothelial cell proliferation by suppressing MMP-1 production in HUVECs [69]. Guenzi *et al.* showed that retroviral vector-mediated expression of GBP1 in HUVECs lowered the expression of MMP-1, with no change in the expression of MMP-2 and MMP-14, while MMP-9 was undetected in endothelial cells [69]. The expression of GBP1 inversely correlated with MMP-1 expression, which further defined the potential role of GBP1 in angiogenesis induced by growth factors and pro-inflammatory cytokines in HUVECs [69]. The expression of GBP1 inversely correlated with the production/

expression of MMP-1. Through mutation studies, the authors further showed that inhibition of MMP-1 production in HUVECs was governed by the enzymatic activity of GBP1. These two studies from the same group suggest a role for GBP1 as an anti-angiogenic protein in endothelial cells.

Interestingly, both the anti-viral and putative anti-rheumatic activity of GBP2 and GBP5 may pivot on the cellular proprotein convertase furin. It has been shown that GBP2 and GBP5 together inhibit furin [70]. The processing activity of host cell furin is exploited by a number of bacterial and viral pathogens to maximize their infectivity. An array of viruses and virus families rely on host furin to convert viral proteins to their mature form, including Herpesviridae, Papillomaviridae, Coronaviridae, Flaviviridae, Togaviridae, Bornaviridae, Bunyaviridae, Filoviridae, Orthomyxoviridae, Paramyxoviridae, Pneumoviridae and Retroviridae (including HIV-1) [70, 71]. Many bacterial toxins depend on furin processing, including anthrax toxin, diphtheria toxin, pseudomonas exotoxin A, shiga toxin and shiga-like toxins [71]. Thus, inhibition of furin activity confers broad anti-viral and anti-bacterial effects to GBP2 and GBP5. However, furin is also important for the processing of host cell proteins, including growth factors, receptors, cytokines and enzymes. Furin directly or indirectly activates key proteins that contribute to the pathogenesis of rheumatic diseases. Matrix proteinases such as A disintegrin and metalloproteinase with thrombospondin motifs and membrane-type 1 MMP, as well as angiogenic factors such as VEGF-C/-D and PDGF rely on furin for their biological activity [72, 73]. TNF- α is processed by TNF- α -converting enzyme, which is a furin substrate [74]. Furin has been explored as a direct therapeutic target; patents for furin and its inhibitors were reviewed by Couture *et al.* [72].

MMP-9 is one of the downstream products produced in the synovial microenvironment known to play roles in osteoclastogenesis and metastasis, which are important pathological events in diseases such as RA and cancer [49, 75]. In a study by Balasubramanian *et al.* [49], mGBP2, the putative murine ortholog of human GBP1 was shown to inhibit MMP-9 expression. In NIH 3T3 fibroblasts, either IFN- γ or forced expression of mGBP2 inhibited basal and TNF- α -induced extracellular and intracellular MMP-9 production, which was attributed to the ability of mGBP2 to inhibit the binding activity of NF- κ Bp65 in these cells. On the contrary, disruption of the activity of mGBP2 by transient knockdown caused an increase in TNF- α -induced MMP-9 expression. Given the fact that IFNs can inhibit the expression of MMP-9 [76, 77], this study sheds light on the emerging role of mGBP2 (or human GBP1) as one of the ISGs responsible for modulating MMP expression and activity. Broadening the scope of such research to other GBPs and dissecting the mechanism in this regard can further strengthen our understanding of the IFN-inducible factors that play a specific role in beneficial or disease-promoting effects.

SS

SS is an autoimmune disease characterized by the inflammation of exocrine glands, particularly the salivary and lacrimal glands. The correct diagnosis of SS is complicated by the variety of symptoms manifested in patients, the similarity of symptoms elicited in other autoimmune disorders, and a complex presentation as secondary SS associated with other conditions such as RA or SLE [78]. Hu *et al.* conducted a study that screened for SS-associated biomarkers by comparing subjects with SS, SLE and healthy controls, which resulted in the identification of three salivary proteins and three mRNA biomarkers with predictive value for pSS detection. Interestingly, one of the mRNA biomarkers identified was GBP2, which was found to be significantly elevated in the saliva of pSS patients compared with both SLE and healthy controls [79]. Another study by Hjelmervik *et al.* analyzed minor salivary glands from patients with pSS and compared them with those of healthy controls to identify genes that contribute to the development of pSS [80]. Gene array analysis of submucosal salivary gland biopsies showed that among the top 200 highly differentiated genes in pSS, *GBP2* was identified among the top dysregulated genes. Interesting to note, *IFN- γ* , *IFN- β* , and *IFN- α* genes were not highly differentially expressed in pSS patients. However, IL-1 β -activated factors that contribute to inflammation (IL-6, leukotriene B, and IL-7) were highly expressed, and genes protecting glandular/acinar cells from apoptosis and disturbance of the cholinergic nerve signals (Bax inhibitor 1 and *BCL2L2*) were downregulated in pSS samples. These findings suggest a potential role of IL-1 β in *GBP2* gene expression, which needs to be experimentally validated.

Apoptosis in salivary glandular epithelial cells is a characteristic feature of SS [81]. A study by Schnoor *et al.* showed that *GBP1* expression was upregulated in epithelial cells of individuals with IBD, and siRNA-mediated knockdown of *GBP1* resulted in an enhanced permeability and epithelial cell apoptosis induced by inflammatory cytokines [46]. This finding is further supported by another study suggesting an important role of *GBP1* in the barrier function of normal human salivary gland duct epithelium and may prevent epithelial cell damage in IgG4-related disease [82]. Among other findings, *GBP1* promoted Toxoplasma detection by AIM2 to induce caspase-8-dependent macrophage apoptosis [83], while *GBP5* fostered selective NLRP3 inflammasome responses to pathogenic bacteria through caspase-1-dependent pathways [84]. In support of this result, the Gene Ontology analysis of the array data identified *immune response* and *cellular defence response* among highly ranked functions affected. Furthermore, given the role of MMP-9 in pSS pathogenesis [85–87] and the ability of GBPs to inhibit it as discussed in the earlier RA section, additional studies are needed that focus on differences in the expression profile of GBPs and whether experimental

manipulation of their expression alters the central pathogenic mechanisms governing epithelial functions in pSS.

Lupus Erythematosus (LE)

Cutaneous lupus erythematosus (CLE) is a chronic autoimmune disease which, being generally confined to the skin, is distinguished from SLE. Recent studies showed that type I IFNs, IFN- α and IFN- β play an important role in the pathogenesis of CLE [43, 88, 89]. Naschberger *et al.* [43] found that the protein MxA (induced by type I IFNs) is detected in the skin lesion sections obtained from CLE patients. Another study identified a critical role of IFN- κ in CLE pathology via enhanced IFN responses and photosensitivity [90]. Also, IFN- α promotes the activation and differentiation of B cells in peripheral blood mononuclear cells of SLE patients. Inhibition of IFN- α -mediated phosphorylation of STAT3 by STAT3 inhibitor also decreased the differentiation of B cells [91]. These studies showed the correlation of type I IFN in the disease pathogenesis. Notably, the study showed that *GBP1* was upregulated in almost all CLE subtypes, but not in atopic dermatitis or healthy controls. Interestingly, the expression was only detected in the lesional region, not in non-lesional skin biopsies even in the same patients, and the expression could be activated in primary keratinocytes from healthy donors by both type I (IFN- α) and type II (IFN- γ) [43]. Another study mentioned previously confirmed higher expression of *GBP1* in SLE patients [40]. In *S. aureus*-induced cutaneous inflammation in SLE, IFN- γ -inhibited the colonization and invasion of the pathogen [88]. The authors of this study observed a strong correlation of IFN- γ with DLE, a subtype of CLE that leads to alopecia and scar formation. It is important to note, in the transcription factor analysis to identify STAT1 and IRF1 binding sites predicted to be present in the promoters of top differentially expressed genes, *GBP1*, *GBP4* and *GBP5* were among the observed genes with higher binding affinity in the DLE subtype compared with in subacute SLE. Further studies in this area will help understanding of the unique role that these GBPs may play in DLE, which may be an important step towards novel targeted therapeutic approaches in lupus.

Psoriatic disease

Psoriasis is a chronic inflammatory skin disease characterized by erythematous scaly plaques [92]. This disease shares clinical, genetic and pathogenic factors with PsA, an inflammatory arthritis, and may be grouped with it as psoriatic disease. Like other autoimmune diseases, psoriasis is linked to genetic and environmental triggers leading to a dysregulated immune response. While early data suggested the primary role of IL-1, TNF- α and IFN- γ in psoriatic disease, recent studies point to a major role of the Th17 cytokine family (IL-17, IL-23 or IL-12) in disease pathogenesis [93, 94]. The overlapping role of these cytokines is implicated in other autoimmune and

inflammatory diseases. This has prompted rigorous clinical testing of biologics aimed against these target cytokines for the treatment of psoriasis and PsA [42, 94, 95]. Notably, a study by Bowcock *et al.* comparing 15 psoriatic involved skin, 11 uninvolved skin, and 6 normal skin samples detected increased expression of GBP1 and GBP2, 2-fold and 8-fold respectively, in the skin of psoriasis patients compared with healthy controls [42]. By using K-means clustering, which identifies clusters of genes with similar expression patterns in individual samples while taking all samples into account, authors identified one of the six clusters of highly upregulated genes in which GBP1 and GBP2 transcripts were highly upregulated. While no direct correlation of any GBP with psoriasis or PsA beyond these studies has been identified in the literature, other studies provide direction in this regard. IFN regulatory factor 8 (IRF8) is required for the production of IL-12 in myeloid cells' response to IFN- γ activation and protection against infections. A study by Marquis *et al.* identified 368 genes upregulated by IRF8 in response to IFN- γ /CpG stimulation in macrophages [96]. Further transcriptome analysis showed several murine and human GBPs among the targets of IRF8. However, the connection of those results to T cells and its direct relevance to the IL-12-GBP family nexus needs further extensive research. One study suggested that the expression of GBP1 in T cells remained unchanged when challenged with IFN- γ , in contrast with activation in other cell types, including B-cells, fibroblasts, keratinocytes, and endothelial cells [39]. Recently, a simple, yet an interesting study by Noack *et al.* examined the additive or synergistic interactions between IL-17, TNF- α and/or IL-1 β in different cell types, including psoriasis skin fibroblasts. They concluded that the 3-cytokine combination produced a synergistic effect in skin fibroblasts compared with an additive effect in synovial fibroblasts [97]. Given the fact that IL-1 β and TNF- α may induce GBP expression in these cells and concomitantly play a pathogenic role in psoriasis and PsA, a similar combinatorial assessment in psoriasis skin fibroblast may yield insightful results.

GBPs in IBD

Crohn's disease (CD) and ulcerative colitis (UC), collectively known as IBD, are characterized by the infiltration of leukocytes, ulceration, oedema, and inflammation of the intestine [98, 99]. IBD is associated with loss of function in the intestinal epithelial barrier, leading to increased bacterial translocation. However, the exact aetiology of IBD is still unknown. In both CD and UC, IFN- γ is one of the upregulated cytokines [100]. IFN-induced GBP1 has been the most extensively characterized member of the GBP family that was found to be elevated in IBD and colorectal carcinoma patients [45, 46]. Importantly, the expression of GBP1 colocalized with the tight junction protein coxsackievirus and adenovirus receptor in the intestinal epithelial cells from colonic mucosa of individuals with IBD, suggesting its

importance in maintaining the integrity of epithelial barrier formation and intercellular interactions. Furthermore, the knockdown of GBP1 by siRNA caused a significant impairment of transepithelial electrical resistance and exposed epithelial cells to an increased rate of apoptosis induced by pro-inflammatory cytokines, which resulted in damaged intestinal barrier function. Thus, the expression of GBP1 in the colonic mucosal tissue affected with IBD is thought to be an early event to protect the intestinal barrier function in response to mucosal inflammation [46]. Further studies are needed to understand the mechanism of GBP1 induction in acute or chronic inflammation so that its use as a potential target gene for IBD therapies and other pathologic conditions with mucosal inflammation can be evaluated.

Therapeutic opportunities and future directions

IFNs and the ISGs play important roles in host immune defence mechanisms and in an increasing list of autoimmune diseases. Currently, the use of therapies directly targeting the IFNs are quite controversial and may be a double-edged sword. This is largely attributed to the redundant role played by type I–III IFNs in various immunological conditions. For example, type I IFNs are associated with SLE [101], SS [102], and may also be important in other autoimmune diseases such as RA and type 1 diabetes mellitus [103]. Interestingly though, IFN- β from the type I IFN class is highly expressed in the synovial membrane of RA and postulated to elicit anti-inflammatory actions [104, 105]. Therapeutic approaches targeting type I IFNs have shown some promise. A recent randomized, double-blind, placebo-controlled clinical trial testing the efficacy of BIIB059, a humanized mAb that binds to blood DC antigen 2 receptor expressed specifically by plasmacytoid DCs, in SLE patients with active cutaneous disease showed a favourable safety profile and sustained clinical benefit as evident from decreased MxA in the skin lesions [106]. In another recent phase 3 clinical trial, monthly i.v. administration of anifrolumab (300 mg), a human monoclonal antibody to type I IFN receptor subunit 1, showed a higher response than placebo at week 52 in active SLE patients [107]. These findings open the door to targeting the type I IFN pathway in SLE. Similarly, a combination inhibitory approach of targeting TYK2 and JAK1 has shown some preclinical promise in imiquimod-induced psoriasis-like dermatitis by inhibiting the IL-23/IL-17 axis [108]. Banking on the experimental success of signalling inhibitors, the search for small-molecule inhibitors of the type I IFN signalling pathway has gained significant attention in drug discovery [109, 110].

IFN- γ has been at the centre of controversy in terms of its therapeutic use and purported benefits. For decades, IFN- γ has been considered as a pro-inflammatory cytokine [111]. Over time, some studies have also pointed to its anti-inflammatory effects [112]. The reason for this dichotomy could be based on the particular

pathogenic mechanisms or the preclinical models used in the studies, the precise timing of interventions that manipulate expression or action of IFN- γ , and the lack of understanding of the genes it stimulates that categorically play protective or damaging roles. Recent studies suggest that the pharmacologic inhibition of IFN- γ may be an underlying reason for opportunistic varicella zoster virus (VZV) infections in several rheumatic diseases, including RA, SLE and psoriasis [107, 113–115]. IFN- γ was found to be more potent than IFN- α in blocking VZV infections primarily through the IFN- γ –IRF1 axis [116]. Importantly, human embryonic lung fibroblasts treated with IFN- γ showed significantly less VZV-infected viral titre compared with IFN- α , which may be attributed to 224 uniquely upregulated and 103 specifically downregulated genes by IFN- γ in functional enrichment analyses of genes involved in IFN-signalling [116]. Of particular interest to us, will be to investigate further to examine whether any of the GBP family member(s) are involved in this process. Another finding supports this inhibitory activity of IFN- γ against VZV infections in a variety of cell lines and identifies the JAK/STAT1 signalling pathway as central to this phenomenon [117].

While there are growing concerns about VZV infection in patients on anti-rheumatic therapies [115], it will be important to understand the impact of these therapies on IFN- γ -activated genes, GBPs in particular, to determine their potential therapeutic value. Furthermore, this will allow the implementation of adjunct treatment approaches that can effectively exogenously deliver these proteins or bypass impact on endogenously expressed GBPs to combat such infections in rheumatic diseases.

Currently, there are no therapies centred on GBPs in autoimmune or rheumatic diseases. This presents an exciting opportunity, given the putative role of GBP5 as an endogenous regulator of inflammation and tissue destruction. Targeted delivery or overexpression of GBPs would maintain or even enhance anti-viral activity, in contrast with current antirheumatic therapies whose immunosuppressive effects can increase susceptibility to opportunistic infections. As discussed earlier, GBP2 and GBP5 together inhibit the proprotein convertase furin, which itself is an investigational target in rheumatic diseases. A primary challenge of furin inhibitors is the unintended inhibition of other proprotein convertases that recognize the same or similar polybasic cleavage sites [71]. Targeted delivery or overexpression of GBP2 and GBP5 could enhance natural inhibition of furin with optimal specificity.

Collectively, the studies summarized here uncover a unique opportunity to examine the role of GBPs beyond the shadow of IFN- γ , warranting further research to identify potential biomarkers or therapeutic targets from this large and diverse pool of genes.

Acknowledgements

The figure in this review article was created with BioRender.com.

Funding: This study was supported by the National Institutes of Health NIH [R01 AR072615 grant] (S.A.), a Rheumatology Research Foundation preceptorship award (M.H.) & (R.S.), and funds from Washington State University.

Disclosure statement: The authors have declared no conflicts of interest.

References

- Martens S, Howard J. The interferon-inducible GTPases. *Annu Rev Cell Dev Biol* 2006;22:559–89.
- Pilla-Moffett D, Barber MF, Taylor GA, Coers J. Interferon-inducible GTPases in host resistance, inflammation and disease. *J Mol Biol* 2016;428:3495–513.
- Meyer O. Interferons and autoimmune disorders. *Joint Bone Spine* 2009;76:464–73.
- Lee AJ, Ashkar AA. The dual nature of type I and type II interferons. *Front Immunol* 2018;9:2061.
- de Weerd NA, Nguyen T. The interferons and their receptors—distribution and regulation. *Immunol Cell Biol* 2012;90:483–91.
- Mora-Arias T, Amezcua-Guerra LM. Type III interferons (lambda interferons) in rheumatic autoimmune diseases. *Arch Immunol Ther Exp* 2020;68:1.
- Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol* 2005;5:375–86.
- Barrat FJ, Crow MK, Ivashkiv LB. Interferon target-gene expression and epigenomic signatures in health and disease. *Nat Immunol* 2019;20:1574–83.
- Psarras A, Emery P, Vital EM. Type I interferon-mediated autoimmune diseases: pathogenesis, diagnosis and targeted therapy. *Rheumatology* 2017;56:1662–75.
- Vestal DJ, Jeyaratnam JA. The guanylate-binding proteins: emerging insights into the biochemical properties and functions of this family of large interferon-induced guanosine triphosphatase. *J Interferon Cytokine Res* 2011;31:89–97.
- Haller O, Staeheli P, Kochs G. Interferon-induced Mx proteins in antiviral host defense. *Biochimie* 2007;89:812–8.
- Taylor GA, Feng CG, Sher A. Control of IFN-gamma-mediated host resistance to intracellular pathogens by immunity-related GTPases (p47 GTPases). *Microbes Infect* 2007;9:1644–51.
- Boehm U, Klamp T, Groot M, Howard JC. Cellular responses to interferon-gamma. *Annu Rev Immunol* 1997;15:749–95.
- Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. *Annu Rev Biochem* 1998;67:227–64.
- MacMicking JD. Immune control of phagosomal bacteria by p47 GTPases. *Curr Opin Microbiol* 2005;8:74–82.
- Taylor GA, Feng CG, Sher A. p47 GTPases: regulators of immunity to intracellular pathogens. *Nat Rev Immunol* 2004;4:100–9.

- 17 Xu Y, He Z, Li Z *et al.* Irgm1 is required for the inflammatory function of M1 macrophage in early experimental autoimmune encephalomyelitis. *J Leukoc Biol* 2017;101:507–17.
- 18 Wang C, Wang C, Dong H *et al.* Immune-related GTPase Irgm1 exacerbates experimental auto-immune encephalomyelitis by promoting the disruption of blood–brain barrier and blood–cerebrospinal fluid barrier. *Mol Immunol* 2013;53:43–51.
- 19 Sorace JM, Johnson RJ, Howard DL, Drysdale BE. Identification of an endotoxin and IFN-inducible cDNA: possible identification of a novel protein family. *J Leukoc Biol* 1995;58:477–84.
- 20 Gilly M, Wall R. The *IRG-47* gene is IFN-gamma induced in B cells and encodes a protein with GTP-binding motifs. *J Immunol* 1992;148:3275–81.
- 21 MacMicking JD. IFN-inducible GTPases and immunity to intracellular pathogens. *Trends Immunol* 2004;25:601–9.
- 22 de Jong TD, Snoek T, Mantel E *et al.* Dynamics of the type I interferon response during immunosuppressive therapy in rheumatoid arthritis. *Front Immunol* 2019;10:902.
- 23 Lambers WM, de Leeuw K, Doornbos-van der Meer B *et al.* Interferon score is increased in incomplete systemic lupus erythematosus and correlates with myxovirus-resistance protein A in blood and skin. *Arthritis Res Ther* 2019;21:260.
- 24 Soponkanaporn S, Deakin CT, Schutz PW *et al.* Expression of myxovirus-resistance protein A: a possible marker of muscle disease activity and autoantibody specificities in juvenile dermatomyositis. *Neuropathol Appl Neurobiol* 2019;45:410–20.
- 25 Bodewes ILA, Huijser E, van Helden-Meeuwse CG *et al.* TBK1: a key regulator and potential treatment target for interferon positive Sjögren's syndrome, systemic lupus erythematosus and systemic sclerosis. *J Autoimmun* 2018;91:97–102.
- 26 Wahadat MJ, Bodewes ILA, Maria NI *et al.* Type I IFN signature in childhood-onset systemic lupus erythematosus: a conspiracy of DNA- and RNA-sensing receptors? *Arthritis Res Ther* 2018;20:4.
- 27 Castañeda-Delgado JE, Bastián-Hernandez Y, Macias-Segura N *et al.* Type I interferon gene response is increased in early and established rheumatoid arthritis and correlates with autoantibody production. *Front Immunol* 2017;8:285.
- 28 Lande R, Giacomini E, Serafini B *et al.* Characterization and recruitment of plasmacytoid dendritic cells in synovial fluid and tissue of patients with chronic inflammatory arthritis. *J Immunol* 2004;173:2815–24.
- 29 Raterman HG, Vosslander S, de Ridder S *et al.* The interferon type I signature towards prediction of non-response to rituximab in rheumatoid arthritis patients. *Arthritis Res Ther* 2012;14:R95.
- 30 Maria NI, Brkic Z, Waris M *et al.* MxA as a clinically applicable biomarker for identifying systemic interferon type I in primary Sjögren's syndrome. *Ann Rheum Dis* 2014;73:1052–9.
- 31 de Gannes GC, Ghoreishi M, Pope J *et al.* Psoriasis and pustular dermatitis triggered by TNF- α inhibitors in patients with rheumatologic conditions. *Arch Dermatol* 2007;143:223–31.
- 32 Klamp T, Boehm U, Schenk D, Pfeffer K, Howard JC. A giant GTPase, very large inducible GTPase-1, is inducible by IFNs. *J Immunol* 2003;171:1255–65.
- 33 Rhodes LD, Wallis S, Demlow SE. Genes associated with an effective host response by Chinook salmon to *Renibacterium salmoninarum*. *Dev Comp Immunol* 2009;33:176–86.
- 34 Tretina K, Park ES, Maminska A, MacMicking JD. Interferon-induced guanylate-binding proteins: guardians of host defense in health and disease. *J Exp Med* 2019;216:482–500.
- 35 Prakash B, Praefcke GJ, Renault L, Wittinghofer A, Herrmann C. Structure of human guanylate-binding protein 1 representing a unique class of GTP-binding proteins. *Nature* 2000;403:567–71.
- 36 Li LF, Yu J, Li Y *et al.* Guanylate-binding protein 1, an interferon-induced GTPase, exerts an antiviral activity against classical swine fever virus depending on its GTPase activity. *J Virol* 2016;90:4412–26.
- 37 Guenzi E, Töpolt K, Cornali E *et al.* The helical domain of GBP-1 mediates the inhibition of endothelial cell proliferation by inflammatory cytokines. *EMBO J* 2001;20:5568–77.
- 38 Krapp C, Hotter D, Gawanbacht A *et al.* Guanylate binding protein (GBP) 5 is an interferon-inducible inhibitor of HIV-1 infectivity. *Cell Host Microbe* 2016;19:504–14.
- 39 Lubeseder-Martellato C, Guenzi E, Jörg A *et al.* Guanylate-binding protein-1 expression is selectively induced by inflammatory cytokines and is an activation marker of endothelial cells during inflammatory diseases. *Am J Pathol* 2002;161:1749–59.
- 40 Hammon M, Herrmann M, Bleiziffer O *et al.* Role of guanylate binding protein-1 in vascular defects associated with chronic inflammatory diseases. *J Cell Mol Med* 2011;15:1582–92.
- 41 Zhang F, Wei K, Slowikowski K *et al.* Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat Immunol* 2019;20:928–42.
- 42 Bowcock AM, Shannon W, Du F *et al.* Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Hum Mol Genet* 2001;10:1793–805.
- 43 Naschberger E, Wenzel J, Kretz CC *et al.* Increased expression of guanylate binding protein-1 in lesional skin of patients with cutaneous lupus erythematosus. *Exp Dermatol* 2011;20:102–6.
- 44 Haep L, Britzen-Laurent N, Weber TG *et al.* Interferon gamma counteracts the angiogenic switch and induces vascular permeability in dextran sulfate sodium colitis in mice. *Inflamm Bowel Dis* 2015;21:2360–71.
- 45 Britzen-Laurent N, Lipnik K, Ocker M *et al.* GBP-1 acts as a tumor suppressor in colorectal cancer cells. *Carcinogenesis* 2013;34:153–62.

- 46 Schnoor M, Betanzos A, Weber DA, Parkos CA. Guanylate-binding protein-1 is expressed at tight junctions of intestinal epithelial cells in response to interferon- γ and regulates barrier function through effects on apoptosis. *Mucosal Immunol* 2009;2: 33–42.
- 47 Feng J, Cao Z, Wang L *et al.* Inducible GBP5 mediates the antiviral response via interferon-related pathways during influenza A virus infection. *J Innate Immun* 2017; 9:419–35.
- 48 Zou Z, Meng Z, Ma C *et al.* Guanylate-binding protein 1 inhibits nuclear delivery of Kaposi's Sarcoma-associated herpesvirus virions by disrupting formation of actin filament. *J Virol* 2017;91:e00632–17.
- 49 Balasubramanian S, Fan M, Messmer-Blust AF *et al.* The interferon- γ -induced GTPase, mGBP-2, inhibits tumor necrosis factor α (TNF- α) induction of matrix metalloproteinase-9 (MMP-9) by inhibiting NF- κ B and Rac protein. *J Biol Chem* 2011;286: 20054–64.
- 50 Wang J, Min H, Hu B, Xue X, Liu Y. Guanylate-binding protein-2 inhibits colorectal cancer cell growth and increases the sensitivity to paclitaxel of paclitaxel-resistant colorectal cancer cells by interfering Wnt signaling. *J Cell Biochem* 2020;121:1250–9.
- 51 Theofilopoulos AN, Koundouris S, Kono DH, Lawson BR. The role of IFN-gamma in systemic lupus erythematosus: a challenge to the Th1/Th2 paradigm in autoimmunity. *Arthritis Res* 2001;3:136–41.
- 52 Smolen JS, Aletaha D. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. *Nat Rev Rheumatol* 2015;11:276–89.
- 53 Yang L, Soonpaa MH, Adler ED *et al.* Human cardiovascular progenitor cells develop from a KDR⁺ embryonic-stem-cell-derived population. *Nature* 2008; 453:524–8.
- 54 Vasa M, Fichtlscherer S, Aicher A *et al.* Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;89:E1–7.
- 55 Szekanecz Z, Besenyei T, Szentpétery A, Koch AE. Angiogenesis and vasculogenesis in rheumatoid arthritis. *Curr Opin Rheumatol* 2010;22:299–306.
- 56 Silverman MD, Haas CS, Rad AM, Arbab AS, Koch AE. The role of vascular cell adhesion molecule 1/ very late activation antigen 4 in endothelial progenitor cell recruitment to rheumatoid arthritis synovium. *Arthritis Rheum* 2007;56:1817–26.
- 57 Grisar J, Aletaha D, Steiner CW *et al.* Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. *Circulation* 2005;111: 204–11.
- 58 Lee TH, Wisniewski HG, Vilcek J. A novel secretory tumor necrosis factor-inducible protein (TSG-6) is a member of the family of hyaluronate binding proteins, closely related to the adhesion receptor CD44. *J Cell Biol* 1992;116:545–57.
- 59 Milner CM, Higman VA, Day AJ. TSG-6: a pluripotent inflammatory mediator? *Biochem Soc Trans* 2006;34: 446–50.
- 60 Day AJ, Milner CM. TSG-6: a multifunctional protein with anti-inflammatory and tissue-protective properties. *Matrix Biol* 2019;78–9:60–83.
- 61 Milner CM, Day AJ. TSG-6: a multifunctional protein associated with inflammation. *J Cell Sci* 2003;116: 1863–73.
- 62 Mindrescu C, Thorbecke GJ, Klein MJ, Vilček J, Wisniewski H-G. Amelioration of collagen-induced arthritis in DBA/1J mice by recombinant TSG-6, a tumor necrosis factor/interleukin-1-inducible protein. *Arthritis Rheum* 2000;43:2668–77.
- 63 Mindrescu C, Dias AA, Olszewski RJ *et al.* Reduced susceptibility to collagen-induced arthritis in DBA/1J mice expressing the TSG-6 transgene. *Arthritis Rheum* 2002;46:2453–64.
- 64 Glant TT, Kamath RV, Bárdos T *et al.* Cartilage-specific constitutive expression of TSG-6 protein (product of tumor necrosis factor alpha-stimulated gene 6) provides a chondroprotective, but not antiinflammatory, effect in antigen-induced arthritis. *Arthritis Rheum* 2002;46: 2207–18.
- 65 Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 2014;32:513–45.
- 66 Elshabrawy HA, Chen Z, Volin MV *et al.* The pathogenic role of angiogenesis in rheumatoid arthritis. *Angiogenesis* 2015;18:433–48.
- 67 Koch AE. The role of angiogenesis in rheumatoid arthritis: recent developments. *Ann Rheum Dis* 2000; 59(Suppl 1):i65–71.
- 68 Paleolog EM. Angiogenesis in rheumatoid arthritis. *Arthritis Res* 2002;4:S81–90.
- 69 Guenzi E, Topolt K, Lubeseder-Martellato C *et al.* The guanylate binding protein-1 GTPase controls the invasive and angiogenic capability of endothelial cells through inhibition of MMP-1 expression. *EMBO J* 2003; 22:3772–82.
- 70 Braun E, Hotter D, Koepke L *et al.* Guanylate-binding proteins 2 and 5 exert broad antiviral activity by inhibiting furin-mediated processing of viral envelope proteins. *Cell Rep* 2019;27:2092–104.e10.
- 71 Braun E, Sauter D. Furin-mediated protein processing in infectious diseases and cancer. *Clin Transl Immunol* 2019;8:e1073.
- 72 Couture F, Kwiatkowska A, Dory YL, Day R. Therapeutic uses of furin and its inhibitors: a patent review. *Expert Opin Ther Pat* 2015;25:379–96.
- 73 Sato H, Kinoshita T, Takino T, Nakayama K, Seiki M. Activation of a recombinant membrane type 1-matrix metalloproteinase (MT1-MMP) by furin and its interaction with tissue inhibitor of metalloproteinases (TIMP)-2. *FEBS Lett* 1996;393:101–4.
- 74 Srour N, Lebel A, McMahon S *et al.* TACE/ADAM-17 maturation and activation of sheddase activity require proprotein convertase activity. *FEBS Lett* 2003;554: 275–83.
- 75 Rose BJ, Kooyman DL. A tale of two joints: the role of matrix metalloproteases in cartilage biology. *Dis Markers* 2016;2016:4895050.

- 76 Ma Z, Qin H, Benveniste EN. Transcriptional suppression of matrix metalloproteinase-9 gene expression by IFN- γ and IFN- β : critical role of STAT-1 α . *J Immunol* 2001;167:5150–9.
- 77 Nguyen J, Knapnougel P, Lesavre P, Bauvois B. Inhibition of matrix metalloproteinase-9 by interferons and TGF- β 1 through distinct signalings accounts for reduced monocyte invasiveness. *FEBS Lett* 2005;579: 5487–93.
- 78 Pasoto SG, Adriano de Oliveira Martins V, Bonfa E. Sjögren's syndrome and systemic lupus erythematosus: links and risks. *Open Access Rheumatol* 2019;11: 33–45.
- 79 Hu S, Gao K, Pollard R *et al.* Preclinical validation of salivary biomarkers for primary Sjögren's syndrome. *Arthritis Care Res* 2010;62:1633–8.
- 80 Hjelmervik TO, Petersen K, Jonassen I, Jonsson R, Bolstad AI. Gene expression profiling of minor salivary glands clearly distinguishes primary Sjögren's syndrome patients from healthy control subjects. *Arthritis Rheum* 2005;52:1534–44.
- 81 Katsiogiannis S, Tenta R, Skopouli FN. Autoimmune epithelitis (Sjögren's syndrome); the impact of metabolic status of glandular epithelial cells on auto-immunogenicity. *J Autoimmun* 2019;104:102335.
- 82 Konno T, Takano K, Kaneko Y *et al.* Guanylate binding protein-1-mediated epithelial barrier in human salivary gland duct epithelium. *Exp Cell Res* 2018;371: 31–41.
- 83 Fisch D, Bando H, Clough B *et al.* Human GBP1 is a microbe-specific gatekeeper of macrophage apoptosis and pyroptosis. *EMBO J* 2019;38:e100926.
- 84 Shenoy AR, Wellington DA, Kumar P *et al.* GBP5 promotes NLRP3 inflammasome assembly and immunity in mammals. *Science* 2012;336:481–5.
- 85 Pérez P, Goicovich E, Alliende C *et al.* Differential expression of matrix metalloproteinases in labial salivary glands of patients with primary Sjögren's syndrome. *Arthritis Rheum* 2000;43:2807–17.
- 86 Maciejczyk M, Pietrzykowska A, Zalewska A, Knaś M, Daniszewska I. The significance of matrix metalloproteinases in oral diseases. *Adv Clin Exp Med* 2016;25:383–90.
- 87 Ito K, Funayama S, Hitomi Y *et al.* Proteome analysis of gelatin-bound salivary proteins in patients with primary Sjögren's Syndrome: identification of matrix metalloproteinase-9. *Clin Chim Acta* 2009;403:269–71.
- 88 Sirobhusanam S, Parsa N, Reed TJ *et al.* *Staphylococcus aureus* colonization is increased on lupus skin lesions and is promoted by IFN-mediated barrier disruption. *J Invest Dermatol* 2020;140: 1066–74.e4.
- 89 Shi H, Gudjonsson JE, Kahlenberg JM. Treatment of cutaneous lupus erythematosus: current approaches and future strategies. *Curr Opin Rheumatol* 2020;32: 208–14.
- 90 Sarkar MK, Hile GA, Tsoi LC *et al.* Photosensitivity and type I IFN responses in cutaneous lupus are driven by epidermal-derived interferon kappa. *Ann Rheum Dis* 2018;77:1653–64.
- 91 De Groof A, Ducreux J, Aleva F *et al.* STAT3 phosphorylation mediates the stimulatory effects of interferon alpha on B cell differentiation and activation in SLE. *Rheumatology* 2020;59:668–77.
- 92 Mylonas A, Conrad C. Psoriasis: classical vs. paradoxical. The Yin-Yang of TNF and type I interferon. *Front Immunol* 2018;9:2746.
- 93 Sakkas LI, Zafiriou E, Bogdanos DP. Mini review: new treatments in psoriatic arthritis. Focus on the IL-23/17 axis. *Front Pharmacol* 2019;10:872.
- 94 Jeon C, Sekhon S, Yan D *et al.* Monoclonal antibodies inhibiting IL-12, -23, and -17 for the treatment of psoriasis. *Hum Vaccin Immunother* 2017;13:2247–59.
- 95 Lowes MA, Suárez-Fariñas M, Krueger JG. Immunology of psoriasis. *Annu Rev Immunol* 2014;32:227–55.
- 96 Marquis JF, Kapoustina O, Langlais D *et al.* Interferon regulatory factor 8 regulates pathways for antigen presentation in myeloid cells and during tuberculosis. *PLoS Genet* 2011;7:e1002097.
- 97 Noack M, Beringer A, Miossec P. Additive or synergistic interactions between IL-17A or IL-17F and TNF or IL-1 β depend on the cell type. *Front Immunol* 2019;10:1726.
- 98 Cromer WE, Mathis JM, Granger DN, Chaitanya GV, Alexander JS. Role of the endothelium in inflammatory bowel diseases. *World J Gastroenterol* 2011;17:578–93.
- 99 Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. *World J Gastroenterol* 2014;20:91–9.
- 100 Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 2011;140:1756–67.
- 101 Crow MK. Interferon-alpha: a therapeutic target in systemic lupus erythematosus. *Rheum Dis Clin North Am* 2010;36:173–86. x.
- 102 Mavragani CP, Niewold TB, Moutsopoulos NM *et al.* Augmented interferon- α pathway activation in patients with Sjögren's syndrome treated with etanercept. *Arthritis Rheum* 2007;56:3995–4004.
- 103 Wong FS, Wen L. IFN- α can both protect against and promote the development of type 1 diabetes. *Ann N Y Acad Sci* 2008;1150:187–9.
- 104 Adriaansen J, Kuhlman RR, van Holten J *et al.* Intraarticular interferon- β gene therapy ameliorates adjuvant arthritis in rats. *Hum Gene Ther* 2006;17: 985–96.
- 105 Treschow AP, Teige I, Nandakumar KS, Holmdahl R, Issazadeh-Navikas S. Stromal cells and osteoclasts are responsible for exacerbated collagen-induced arthritis in interferon- β -deficient mice. *Arthritis Rheum* 2005;52: 3739–48.
- 106 Furie R, Werth VP, Merola JF *et al.* Monoclonal antibody targeting BDCA2 ameliorates skin lesions in systemic lupus erythematosus. *J Clin Invest* 2019;129:1359–71.
- 107 Morand EF, Furie R, Tanaka Y *et al.* Trial of anifrolumab in active systemic lupus erythematosus. *N Engl J Med* 2020;382:211–21.
- 108 Works MG, Yin F, Yin CC *et al.* Inhibition of TYK2 and JAK1 ameliorates imiquimod-induced psoriasis-like

- dermatitis by inhibiting IL-22 and the IL-23/IL-17 axis. *J Immunol* 2014;193:3278–87.
- 109 Yuliantie E, Dai X, Yang D, Crack PJ, Wang MW. High-throughput screening for small molecule inhibitors of the type-I interferon signaling pathway. *Acta Pharm Sin B* 2018;8:889–99.
- 110 Kundu-Raychaudhuri S, Chen YJ, Wulff H, Raychaudhuri SP. Kv1.3 in psoriatic disease: PAP-1, a small molecule inhibitor of Kv1.3 is effective in the SCID mouse psoriasis – xenograft model. *J Autoimmun* 2014;55:63–72.
- 111 Kelchtermans H, Billiau A, Matthys P. How interferon- γ keeps autoimmune diseases in check. *Trends Immunol* 2008;29:479–86.
- 112 Mühl H, Pfeilschifter J. Anti-inflammatory properties of pro-inflammatory interferon- γ . *Int Immunopharmacol* 2003;3:1247–55.
- 113 Kato M. New insights into IFN- γ in rheumatoid arthritis: role in the era of JAK inhibitors. *Immunol Med* 2020;43:72–8.
- 114 Sun F, Chen Y, Wu W *et al.* Varicella zoster virus infections increase the risk of disease flares in patients with SLE: a matched cohort study. *Lupus Sci Med* 2019;6:e000339.
- 115 Stöllberger C, Finsterer J. Varicella zoster virus meningitis under ustekinumab because of plaque psoriasis. *J Dermatol* 2017;44:703–5.
- 116 Sen N, Sung P, Panda A, Arvin AM. Distinctive roles for type I and type II interferons and interferon regulatory factors in the host cell defense against Varicella-Zoster virus. *J Virol* 2018;92:e01151–18.
- 117 Shakya AK, O’Callaghan DJ, Kim SK. Interferon gamma inhibits Varicella-Zoster virus replication in a cell line-dependent manner. *J Virol* 2019;93:e00257–19.