Utilizing type I interferon expression in the identification of antiphospholipid syndrome subsets

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Article highlights:

- The type I interferon pathway may be a central player in APS pathogenesis and development
- Type I interferon signature have shown promising results in discriminating distinct APS phenotypes
- The translational application of the type I interferon signature in routine clinical practice, although fascinating, needs further investigation
- Heterogeneous methodological approaches in type I interferon signature quantification represent a major limitation for its translational applicability in APS setting
- The use of therapeutic agents directly or indirectly targeting interferon production and functions is promising, especially for those aPL-positive patients who presented higher IFN-I signature

Abstract

Introduction: Antiphospholipid Syndrome (APS) is a systemic autoimmune disease with a complex multifactorial pathogenesis, combining genetic background, traditional cardiovascular risk factors, disease-specific features such as the presence of antiphospholipid antibodies (aPL), and an imbalance of various immune system functions. Recent data support the role of interferons (IFN), especially type I IFN (IFN-I), in the onset and development of APS clinical manifestations, including thrombotic events and obstetric complications. *Areas covered:* In this review we aimed to discuss the growing body of evidences on the relevance of IFN-I pathways in APS, both from a basic mechanistic perspective, focusing on its possible use in disease/patients stratification. The IFN-I signature has shown promising, although preliminary, results in segregating aPL-positive subjects by aPL profile, association with other autoimmune conditions such as lupus, age at onset, and current treatment, among others. *Expert opinion:* To date, the scarce available data as well as methodological and technical heterogeneity among studies limit the comparability of the results, thus requiring further validation to translate these findings to routine clinical practice. Therefore, further research is required in pursuit of more nuanced patient profiling and the development of new immunomodulatory therapeutic strategies for APS beyond anti-coagulant and anti-platelet agents.

1. Introduction

The Antiphospholipid Syndrome (APS) is clinically defined by the occurrence of vascular thromboses, both venous and arterial, and by pregnancy morbidity events, such as recurrent unexplained early miscarriages, foetal deaths and late obstetrical complications in patients persistently positive for antiphospholipid antibodies (aPL), including lupus anticoagulant (LA), anti-cardiolipin (aCL), and anti-β_2-glycoprotein I (aβ_2GPI) antibodies [1]. Commonly, affected patients manifest exclusively one of the two forms, either thrombotic or obstetric APS, and only a minority of subjects have characteristics of both, suggesting the existence of pathogenic mechanisms that are only partially overlapping [2].

While aPL positivity is necessary to confirm a diagnosis of APS, their mere presence seems not to be sufficient to cause the full APS pathophysiology, and the hypothesis of a second trigger, therefore called "second hit", is usually suggested [3]. Furthermore, since its first description, the clinical spectrum of APS has been expanded, including a wide range of pathologic features, the so-called "extra criteria manifestations", such as cardiac valve disease, cognitive impairment and haematological abnormalities, which cannot be explained solely by the hypercoagulable state typical of the disease [4]. In parallel, new autoantibody specificities and pathogenic pathways have been identified and their role in APS pathogenesis is emerging with variable degrees of relevance to APS clinical scenarios [5–11]. Overall, these advances reflect the complex multifactorial nature of APS pathogenesis and support the difficulties encountered by physicians in managing this systemic condition [12].

Interferons (IFNs) are a large family of proteins with autocrine and paracrine actions. IFNs are mostly secreted by infected cells in response to viral infections but they also play numerous roles in modulating innate and adaptive responses, including in cancer protection and autoimmunity. Based on their receptors, IFNs have been categorized into three main distinct groups: IFN type I (including IFN-α and IFN-β among others), II, and III. These groups are further divided into subtypes based on their structural and antigenic characteristics [13].

Type I IFN (IFN-I) exert several crucial functions, such as balancing the innate immune response and favouring immunologic memory and antigen-specific T and B cells

responses. Nowadays, IFN-I is also considered a key player in the initiation, progression and treatment response of a wide number of immune-rheumatic diseases, such as systemic lupus erythematosus (SLE), Sjögren'syndrome, systemic sclerosis, rheumatoid arthritis (RA) and myositis [14].

In this review, we aimed to summarise the available evidence regarding the pathogenic pathways and mechanisms associated with IFN-I activation in APS, as well as potential clinical and therapeutic implications.

2. Interferon activation and signature in APS patients: pathogenic pathways and mechanisms

2.1 Sources of type I interferons in APS

2.1.1 Cells producing type I interferons

Plasmacytoid dendritic cells (pDCs) recognize viruses via toll like receptors (TLRs) and release IFN-I, thus modulating anti-viral responses [15,16]. pDCs are also known to be major producers of the specific IFN-I, interferon alpha (IFN-α) in many autoimmune diseases [17–22]. Beyond pDCs, fibroblasts [23], epithelial cells [24], endothelial cells [25], and neutrophils [26,27] can be primed to produce IFN-I. Which cells are the most important producers of IFN-I in APS remains to be determined.

2.1.2 TLR-triggered generation of type I interferon

In 2011, Prinz et al. [28] demonstrated increased expression of TLR7/8 by peripheral blood mononuclear cells (PBMCs) in the specific population of APS patients. In this study exposing pDCs to either total IgG fractions from patients or non-cofactor-dependent monoclonal aPL promoted IFN-α expression in a TLR7-dependent manner; this only occurred in the presence of traditional TLR7 ligands such as single-stranded RNA, suggesting that aPL sensitize pDCs to these ligands [28]. This ligand dependence is somewhat reminiscent of lupus, where IFN-I induction likely depends on the presence of both RNA and ribonucleoprotein-binding antibodies [29–33]. From a mechanistic standpoint, aPL appeared to prime pDCs to internalize RNA, while also promoting translocation of TLRs from the endoplasmic reticulum to the endosome [28]. All these events were downstream of endosomal NADPH activation and superoxide production, as inhibiting either blocked aPL-mediated effects [28]. Based on these findings, it has

been suggested that, irrespective of the concomitant presence of SLE, aPL are able to directly maximize the sensitization to TLR7. This could represent, therefore, an additional pathogenic factor in APS and in aPL- positive SLE patients. In the context of SLE, a large amount of data indicates that different TLRs, mainly TLR2-5 and TLR7-9, are involved in SLE susceptibility, onset, and progression [34]. Regarding the production of IFN-I in SLE patients, it is recognized that pDCs are induced via TLR9 stimuli to produce IFN-α, increasing the production of B cell activating factor (BAFF), which in turns activates B cells autoreactivity [35]. In addition, IFN-α levels are reported to correlate with TLR5, TLR7, and TLR9 mRNA in PBMCs of SLE patients [36].

It is worth mentioning that other TLRs, such as TLR2 and TLR4, which are not directly linked to IFN-I, are also involved in APS pathogenesis. They have been shown to have a central role in thrombosis, endothelial dysfunction, and inflammatory cytokine and chemokine production, with their activation mediated by the presence of aPL, especially aβ2GPI IgG and aβ2GPI IgG/β2GPI immune complexes [37].

2.1.3 Potential connections between β_2-glycoprotein I and type I interferon

β_2GPI, a circulating phospholipid-binding protein with roles in innate immunity and apoptotic cell clearance, is the most studied antigen for aPL [38–41]. Using a lupus mouse model that relies on duplication of TLR7, Giannakopoulos et al. [42] found impaired apoptotic cell clearance when β_2GPI was knocked out. The knockout mice also demonstrated higher IFN-I signatures, higher autoantibody titers, and an exaggerated lupus phenotype as compared with the β_2GPI-expressing mice [42]. The Authors speculated on a TLR7 inhibitory function of β_2GPI in this model, which might be disrupted by aPL [42]. To this end, several studies have found a correlation between the presence of aβ_2GPI antibodies and the IFN-I signature in primary APS patients [43–45]. One study showed that while IFN-I levels in patients with primary APS, SLE only, and SLE with APS were similar, and aβ2GPI positivity was correlated with higher IFN-I score in primary APS patients. This suggests there may be different and independent pathways that drive disease pathogenicity [46].

An area for potential future research is the fact that basal levels of IFN-I appear to be maintained by commensal bacteria [47–50]. Interestingly, a recent study hinted that the commensal bacterium *Roseburia intestinalis*, which has homologous sequences to

β_2GPI, may be a nidus of autoimmunity in APS patients [51]. The extent to which the microbiome may be contributing to aPL and/or IFN-I production in APS is an area we expect to see investigated in the coming years.

2.1.4 MicroRNA and type I interferon

MicroRNAs are short non-coding RNA molecules that bind to the 3' end of untranslated mRNAs and reduce their translation, thereby regulating gene expression [52]. There is ample evidence that specific microRNAs destabilize and thereby suppress IFN-pathway transcripts in different cell types; not surprisingly, dysregulation of these microRNAs have been associated with autoimmune diseases [52–59]. In a recent study, global pDC microRNA expression was found to be downregulated in APS patients (both primary and associated with SLE), where it tracked with the IFN-I signature [60]. Pathway analysis of the downregulated microRNA target genes demonstrated "pDC activation" as a regulated pathway, thereby hinting at interplay between pDCs, microRNAs, and IFN-I [60]. Interestingly, related studies have found APS patient microRNAs to be associated with accelerated atherosclerosis and oxidative stress, as well as the prothrombotic phenotype inherent to many APS patients [61,62].

2.1.5 Neutrophil extracellular traps and type I interferon

Neutrophil extracellular traps (NETs) are extracellular webs of DNA and microbicidal proteins that have the potential to cause tissue damage, trigger endothelial activation, and serve as a source of autoantigens [63–65]. As compared with healthy neutrophils, APS neutrophils release NETs in an exaggerated fashion [64,66], while administration of aPL to mice triggers large thrombi enriched in NETs [67]. APS patients also appear to have increased circulating numbers of so-called low-density granulocytes (LDGs)[68], which have a lower threshold to release NETs as compared with normal-density neutrophils. In lupus, it is well established that NETs activate pDCs via TLRs and thereby induce production of IFN-I, which in turn primes neutrophils to release more NETs [64,69,70]. Interestingly, recent data showed that while augmented NET release and an increase in LDG numbers were observed in those SLE patients who display higher IFN-I signatures, LDG numbers did not associate with IFN-I signatures in APS [71]. These findings, although preliminary, further strengthen the idea of APS and SLE as distinct pathological entities, with different pathogenic pathways and

underlying mechanisms. Nevertheless, whether NETs are a driver of IFN-I production in APS setting is still an area deserving of future research [72].

An interesting study related to this topic reported that SLE patients with a single nucleotide polymorphism (SNP) in the neutrophil cytosolic factor 1 gene, which encodes a subunit of NADPH oxidase 2, not only have higher IFN-I activity (as compared to SLE patients with the normal genotype), but are also more likely to have high levels of circulating aPL and to actually develop APS [73]. While overall NET production in patients with the implicated SNP was apparently impaired due to reduced production of cellular reactive oxygen species (ROS), neutrophil expression of mitochondrial ROS was enhanced [73], with mitochondrial ROS being known to trigger NETs that are particular interferogenic [74]. Another study profiling gene expression of primary APS neutrophils as compared with control neutrophils found IFIT1 (an IFN-I regulated gene) as the most upregulated gene [75], hinting at a potential bidirectional relationship between neutrophils and IFN-I in the context of APS. Many questions still remain including the extent to which IFN-I may regulate neutrophils in APS.

2.2 Downstream effects of type I interferon in APS

2.2.1 Vascular damage and type I interferon

IFN-I are a well-known driver of the endothelial dysfunction and premature atherosclerosis inherent to SLE [76,77]. Accelerated endothelial damage and atherosclerosis have also been recognized in many APS patients [78–81]. In a cohort of patients with primary APS, Grenn et al. [43] evaluated the association between the IFN-I signature and endothelial progenitor cell (EPC) dysfunction, which have been linked together in other autoimmune diseases [82–86]. They found that both classic EPCs and circulating angiogenic cells (CACs, which are myeloid-derived and have proangiogenic properties) from APS patients, failed to differentiate into mature endothelial cells [43]. This defect did not correlate with the presence of aPL, but rather with levels of IFN-I. Furthermore, blockade of the IFN-I receptor *in vitro* rescued the differentiation into healthy endothelial cells [43]. In pursuit of other mechanisms that could lead to vascular damage in APS, a different group found IFN-I expression to correlate with both increased tissue factor expression and increased intermediate and non-classical monocytes, the latter having been associated with cardiovascular disease in SLE and RA [44].

2.2.2 Type I interferon and adaptive immunity

As a bridge between innate and adaptive immunity, IFN-I activate antigen-presenting cells (APCs), increase the expression of CD86 and MHC class I and II molecules on APCs, and enable B cell differentiation [87–89]. Specific to humoral immunity, IFN-I upregulate BAFF on DCs [90] and efficiently trigger BAFF mobilization from intracellular stores to the cell surface of lupus monocytes [91]. B cells and pDCs from SLE patients demonstrate increased BAFF expression, which can be decreased by anti-IFN-I agents [91–93]. In APS, clinical studies have found a correlation between BAFF levels and higher adjusted global APS scores [94]. Higher levels of both BAFF and IFN-α have also been appreciated in APS patients with adverse pregnancy outcomes, albeit without a clear correlation between the two cytokines [95]. Whether disrupting IFN-I activity in APS may also negate BAFF expression is an area deserving of future research [96], including disease stratification to guide treatment-decision making.

When focusing on pregnancy complications in the context of APS, a very limited number of studies have observed an association between obstetric events, mainly preeclampsia and higher IFN-I levels [97], which is in line with previous data from SLE patients suggesting a role for IFN-α in inducing angiogenic imbalance [98]. Further studies are needed to elucidate the possible role of IFN-I in pregnancy morbidity in APS patients.

As discussed above, ligands of TLR7 trigger IFN-I production by pDCs, with subsequent differentiation of B cells into antibody-producing plasmablasts [99]. Recently, a study linked a specific TLR7 SNP to higher IFN-I production from APS PBMCs; the patients also demonstrated increased plasmablast numbers along with decreased memory and regulatory B cell populations [100]. When studied *ex vivo*, it appeared that CD20-negative B cells (a population that includes plasmablasts) were the most important producers of aPL [100]. These results are notable given that similar derangements in B cell populations have been found in SLE, where circulating plasmablasts are predictive biomarkers in at least a subset of patients [100–102].

3. IFN signature and APS: from different laboratory techniques to diagnostic and therapeutic implications

Due to the rapidly growing body of evidence supporting a role for IFN-I in the immunopathogenesis of APS, and other systemic diseases, there has been a parallel interest in capturing the activation of the IFN-I pathway from a translational perspective. The assessment of the IFN-I pathway activation through the so-called 'IFN signature' has been hypothesized to provide an added value in diagnosis, patient stratification and treatment decisions in APS. However, despite several efforts, some obstacles still exist before considering the IFN signature as a biomarker to be used in the routine clinical setting. To date, the cross-sectional nature of the majority of the in vivo studies limits clinical validation. Moreover, the heterogeneity of assays used to measure the IFN signature and IFN activation described in the literature represents a further challenge to be addressed. In the following section, a summary of the approaches to capture the IFN signature in APS together with their potential clinical relevance are discussed.

A major challenge to assess the IFN activation is the great diversity of IFN ligands and their relatively short half-lives, which hinder our ability to capture IFN activation at the protein level. Although recent developments such as ultrasensitive single molecule assays (Simoa) [20] or dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA)[103] seem to partially overcome these issues, there is limited evidence on their actual added value. Besides, their use in the setting of APS needs to be explored. Consequently, most of the available studies focused on the assessment of the cellular response to IFN-I, that is, the assessment of IFN-inducible gene expression, usually in whole blood or sorted immune cell populations. These approaches have been conducted by qPCR classical methods or by large-scale microarrays. Pre-analytical, technical-related, and methodological limitations can be also observed for this approach, the choice of the candidate IFN-inducible genes and the calculation of the score or signature being the most evident ones. With a similar rationale, the quantification of IFN-induced gene encoded proteins in serum has been also explored in APS. Additionally, the use of cellular assays to measure IFN activity using different cell substrates has been also proposed. Finally, the identification of epigenetic fingerprints to the IFN signature has added another layer of complexity to this scenario. In sum, a considerable number of distinct approaches have been described to measure the IFN signature in APS, although they cover a notable variety of biological processes and readouts. Consequently, there is a relatively solid evidence about the presence of the IFN-I pathway in APS, but its

clinical implication needs to be considered with caution. Although grouped under a common umbrella, these methods likely provide a different, and probably complementary, information for the clinical setting.

Microarray studies have revealed that a number of IFN-related genes are differentially expressed in APS as compared with healthy controls and/or SLE patients, either in whole blood or in sorted cell subsets (monocytes or neutrophils)[43,44,75,104,105]. Actually, IFN-related genes were ranked among the most upregulated genes [43,104]. Although a significant overlap was observed with SLE and SLE-associated with APS, current evidence suggests that differential, APS-specific components can be found in the IFN signature, which reinforces differential pathogenic substrates among these conditions [104], as previously discussed. Interestingly, this specific imprint was related to mitochondria biogenesis and function and oxidative stress, hence underlining the links between IFN and mitochondrial activity in APS. Nevertheless, although interesting from a basic perspective, in order to relate individual genes to complex pathogenic pathways, the added value of microarray studies for clinical and therapeutic decisions is uncertain.

Most of the studies analysing the IFN signature in APS are based on gene expression analyses by qPCR methods. However, a large variation in the number of genes assessed (from 3 to 41 genes) and a lack of consistent methods to select the target genes (candidate genes vs unsupervised methods) and score calculations (averages, normalizations, z-scores, etc.) make difficult to compare the results obtained across studies [44,106–109]. In general terms, an IFN signature can be distinguished in APS patients, and both quantitative and qualitative differences seem to emerge compared to SLE or secondary APS patients. In fact, an IFN signature can be observed in about a half of the APS patients (38-49%)[44,106], whereas a higher activation could be found in SLE (70-90%)[44,106,110]. Additionally, not all genes exhibited similar alterations in APS compared to SLE or secondary APS patients, thus reinforcing their potential disease-specific involvement. Interestingly, the genes more consistently analysed were those ubiquitously found to be elevated in systemic diseases, such as MX1, IFI44, IFI44L or IFIT1. Although the increase in the IFN signature in APS is clear, there is limited evidence on their actual value as a diagnostic biomarker, and whether it should be considered a patient subset feature rather than a disease feature is still under debate. Since the former will be more relevant for patient stratification whereas the latter will be

for disease diagnosis, this represents a point of major interest for the clinical setting. In patients with established disease, some studies have highlighted an association between the IFN signature and diverse disease outcomes, such as antibody status (aβ_2GPI positivity and triple positivity)[43,106], obstetric events [108] or age at onset [108], but replication studies are needed.

Of note, a recent study from Flessa et al. have moved beyond IFN-I and tried to explore the significance of a new member of the IFN family, the type III IFNs (IFNλ, particularly IFNλ1) which is thought to play an additional role in the pathogenesis of several autoimmune conditions, such as APS [111]. Interestingly, lower IFNλ1 gene expression in PBMCs showed an independent association with obstetric APS and pregnancy complications. This data might suggest an impaired function of IFNλ1 in modulating neutrophil activation and NETs formation leading to foetal damage in the affected patients. Moreover, the study reported higher levels of IFN-I score/IFNλ1 ratio in those primary APS patients who presented with triple aPL positivity and aβ_2GPI [111]. Overall, these evidences seem to confirm both the pivotal role of IFNs in APS pathogenesis and its link with autoantibodies production.

Finally, IFN signature by qPCR has been reported to be influenced by pharmacological agents, such as hydroxychloroquine (HCQ) [44,106] and statins [44].

On the one hand, these findings may suggest that the IFN signature could be a targetable mechanism in APS, as proposed in other conditions. On the other hand, these results highlight that treatments can be a significant confounding factor for the IFN signature, hence requiring a dedicated analysis, especially since studies addressing the very early stage of the disease in treatment-naïve cohorts are lacking.

Detection of IFN signature in APS at the protein level, including IFNα or IFN-induced proteins detection [43,107], as well as functional assays using cell-reporter lines have also been used in APS [43]. Although some studies showed promising results, the findings are far from providing a clear added value for the clinical setting. Finally, a recent genome-wide methylation study has characterized several differentially methylated sites between APS and controls [112]. Among them, the hypomethylation of the IFI44L locus was found to discriminate between APS and SLE.

4. Conclusions

In conclusion, available data suggest that aPL have the ability to potentiate TLR activity, which results in IFN-I production by pDCs and possibly other cell types. This process is likely to be modulated by microRNAs and NETs, with hints that bidirectional relationships may be at play. Regarding pathogenesis, elevated levels of IFN-I may contribute to endothelial damage and increased in plasmablast numbers, the latter likely to be an important source of aPL. In Table 1 and Figure 1 the main mechanisms involved in IFN-I production and its effects are presented, along with main therapeutic immunomodulatory approaches and possible future treatment targets in the APS context.

While IFN-I represent a fascinating and promising tool in APS clinical setting, both for diagnostic, clinical management, and prognostic purposes, the high heterogeneity in pre-analytical and methodological approaches along with the relatively low number of available studies (and lack of long-term trials), have limited researchers to reach solid conclusions negatively impacting the translation of these findings into routine clinical practice. Nevertheless, current literature has pointed out the existence of a distinct IFN-I signature when comparing APS patients with healthy controls and SLE with or without APS, along with its correlation with clinical and disease-specific serological features, thus supporting its potential role in identifying different subsets of the disease.

5. Expert opinion

5.1 *How can type I interferon expression help us in identifying APS subsets?*

To date, IFN-I have been extensively proven to be relevant in SLE setting, for diagnosis, patient characterization and disease activity assessment [24,113,114]. Subsequently, fewer studies have evaluated the ability of the IFN-I signature and activation to identify specific subgroups of aPL-positive subjects in larger SLE cohorts with promising results [107]. In fact, Iwamoto et al. reported an independent correlation between aPL positivity and IFNα activation in SLE African-American patients. Moreover, this correlation was not observed in either Hispanic-American or European-American subjects, supporting the link between IFN pathways not only with disease-specific features, such as autoantibody production, but also with genetic background, which is well known to play a central role in APS pathogenesis [12,115]. Interestingly,

a recent attempt by Idborg et al. to characterize a cohort of SLE patients from a molecular perspective based on their autoantibody profile, have identified two distinct main subgroups, one associated with the presence of SSA/SSB positivity and the other characterized by LA positivity [116]. Higher degrees of IFN-I activation were found in the SSA/SSB+ subgroup as compared with the aPL-positive patients where complement activation and systemic inflammation seemed to be more prominent mechanisms, with possible therapeutic implications [116]. Furthermore, recent experiences have specifically addressed the role of IFN-I in primary APS showing clear associations with specific clinical manifestations and autoantibody profiles [45,108]. Moreover, different degrees of global activation of the IFN-I signature along with distinct activation patterns of IFN-I-regulated gene networks have been appreciated among healthy controls, aPL-carriers, primary APS patients, and SLE patients with and without APS; furthermore, a positive association has been detected between IFN-I signatures and the presence of the anti-phosphatidylserine/prothrombin IgG isotype, an autoantibody specificity of emerging importance in APS [117,118]. Taken together, these limited data support the possible use of the IFN-I signature in APS characterization and disease subset identification, with potential implications for more effectively personalizing prognosis and therapeutic strategies. Moreover, the identification of disease mechanisms occurring at the earliest stages of the disease will broaden the understanding about disease-initiating pathogenic mechanisms, with potential relevance for disease prevention and/or early management.

5.2 *How can type I interferon expression play a role in guiding the therapeutic strategies in APS?*

Evaluating the potential associations between the IFN-I signature and the clinical, laboratory and treatment characteristics of patients with primary APS is crucial in pursuit of identifying potential new approaches directly or indirectly targeting IFN pathways by using both highly-specific anti-IFN antibodies or therapeutic approaches that can modulate IFN production and functions. In this context, the use of HCQ, which is largely employed in both thrombotic and obstetric APS as well as a prophylactic strategy in high-risk aPL-positive subjects, has been shown to be associated with lower degrees of IFN-I activation in patients with primary APS [45], in line with its ability to modulate NETs formation and TLR signalling [44]. Monoclonal anti-IFNα agents, such as sifalimumab and rontalizumab, have been developed and tested in SLE cohorts, with

heterogeneous results [119,120]. Other monoclonal antibodies with a potentially wider spectrum of anti-IFN-I functions, such as anifrolumab, have demonstrated more promising results [121].

Overall, given the likely role of the IFN-I signature in APS pathogenesis and progression, as well as the complex interplay between the various cellular functions and pathologic mechanisms regulated by IFN-I, the employment of therapeutic agents able to directly or indirectly target the IFN-I-associated pathways represents an interesting treatment option both in SLE and APS. Further prospective studies are however needed in order to elucidate clinical effectiveness and safety profiles, especially in APS.

5.3 *How can type I interferon signature can be useful in APS patients stratification and profiling?*

Although the presence of the IFN-I signature in APS patients has been demonstrated by different and complementary approaches, a number of knowledge gaps still remain that prevent their implementation in routine clinical practice at present. First, the lack of consensus about how to best quantify the IFN-I signature is a major impediment. Standardized recommendations are therefore warranted. Furthermore, large, well-designed studies, with an adequate appraisal of confounding factors and long-term follow-up, are needed to delineate the added value of the IFN-I signature especially for disease activity assessment and individual risk stratification. In fact, the concept of an APS disease activity index represents an unmet need giving the lack of reliable biomarkers which directly correlate with the development of discrete thrombotic and obstetric complications as well as organ damage over time. On the other hand, numerous attempts have been made with the aim of identifying homogeneous groups of subjects at greater risk of developing clinical manifestations of the syndrome which however seem inadequate to cover the entire spectrum of the disease [122–124]. Scoring systems have been also developed and validated over the years combining disease specific features and traditional cardiovascular risk factors, which have a pivotal role in clot formation [125,126]. Two recent studies, in which a clustering-analysis approach was employed, confirmed the presence of relatively homogeneous subgroups of aPL-positive subjects sharing similar clinical and serological characteristics corresponding to known and defined nosological entities [127,128]. At the same time, both studies also demonstrated the extreme heterogeneity of APS manifestations and the existence of an interesting subgroup of aPL-positive patients showing intermediate

characteristics between SLE and primary APS, particularly with lower risk for developing thrombotic events and higher rates of systemic features such as anti-nuclear antibodies (ANA) positivity (97%) and cytopenia (mainly thrombocytopenia), supporting the idea of a *continuum* in APS clinical spectrum [128]. In line with these data, the employment of an approach based on patient profiling, rather than its mere categorization into discrete disease groups, should allow for a more real-life, precise, and personalized management of these patients. Interestingly, given its crucial role in anti-viral responses, IFN-I has been further studied in the context of SARS-CoV-2 infection. Based on the available data, the dysregulation of IFN functions has been associated with the most severe forms of the disease [129], through an exaggerated enhancement of pro-inflammatory factors that lead to tissue damage, a hypercoagulable state and autoimmune reactions, including the production of autoantibodies, especially in susceptible individuals [129–131]. These data would seem to both confirm the complexity of IFN pathways in disease pathogenesis and progression, while also emphasizing that we must move beyond a dichotomous perspective and rather employ more sophisticated approaches that will enable more insightful clinical application.

Available data suggest that the IFN-I signature may represent an additional tool not only to better define APS patients, but also to better characterized aPL-positive patients who do not fulfil traditional classification criteria, in particular aPL asymptomatic subjects, patients who are refractory to standard treatment and those patients who present with intermediate and "systemic" forms of APS who could benefit from alternative therapeutic approaches beyond anti-coagulant and anti-platelets agents (with anti-IFN-I agents being one consideration). In addition, a special focus should be made in the early stages of the disease, in order to assess the role of IFN-I signature in early diagnosis and to evaluate drivers of its fluctuations during follow-up.

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References

[1] Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4:295–306.

[2] Meroni PL, Borghi MO, Grossi C, et al. Obstetric and vascular antiphospholipid syndrome: Same antibodies but different diseases? [Internet]. Nat. Rev. Rheumatol. Nature Publishing Group; 2018 [cited 2020 Dec 6]. p. 433–440. Available from: https://pubmed.ncbi.nlm.nih.gov/29891914/.

[3] Meroni PL, Borghi MO, Raschi E, et al. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol [Internet]. 2011 [cited 2018 Apr 23];7:330–339. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21556027. ***Exeptional overview on APS pathogenesis and manifestations**

[4] Sciascia S, Amigo M-C, Roccatello D, et al. Diagnosing antiphospholipid syndrome: “extra-criteria” manifestations and technical advances. Nat Rev Rheumatol. 2017 Sep;13(9):548-560. doi: 10.1038/nrrheum.2017.124. ***Complete overview on "extra-criteria" manifestations of APS**

[5] Radin M, Foddai SG, Cecchi I, et al. Antiphosphatidylserine/Prothrombin Antibodies: An Update on Their Association with Clinical Manifestations of Antiphospholipid Syndrome. Thromb Haemost [Internet]. 2020 [cited 2020 Dec 6];120:592–598. Available from: https://pubmed.ncbi.nlm.nih.gov/32185783/.

[6] Bertolaccini ML, Sanna G. The Clinical Relevance of Noncriteria Antiphospholipid Antibodies. Semin Thromb Hemost [Internet]. 2018 [cited 2020 Dec 6];44:453–457. Available from: https://pubmed.ncbi.nlm.nih.gov/28470650/.

[7] Shoenfeld Y, Gerli R, Doria A, et al. Accelerated atherosclerosis in autoimmune

rheumatic diseases. Circulation. 2005;112:3337–3347.

[8] Girardi G, Berman J, Redecha P, et al. Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. J Clin Invest [Internet]. 2003 [cited 2020 Dec 8];112:1644–1654. Available from: https://pubmed.ncbi.nlm.nih.gov/14660741/.

[9] Canaud G, Bienaimé F, Tabarin F, et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. N Engl J Med. 2014;371:303–312. ****Exeptional step forward in APS knowledge and treatment**

[10] Sciascia S, Cecchi I, Radin M, et al. IgG Anti-high-Density Lipoproteins Antibodies Discriminate Between Arterial and Venous Events in Thrombotic Antiphospholipid Syndrome Patients. Front Med [Internet]. 2019 [cited 2020 Dec 8];6. Available from: https://pubmed.ncbi.nlm.nih.gov/31612138/.

[11] Zuo Y, Yalavarthi S, Gockman K, et al. Anti–Neutrophil Extracellular Trap Antibodies and Impaired Neutrophil Extracellular Trap Degradation in Antiphospholipid Syndrome. Arthritis Rheumatol [Internet]. 2020 [cited 2020 Dec 8];72:2130–2135. Available from: https://pubmed.ncbi.nlm.nih.gov/32729667/.

[12] Barinotti A, Radin M, Cecchi I, et al. Genetic Factors in Antiphospholipid Syndrome: Preliminary Experience with Whole Exome Sequencing. Int J Mol Sci [Internet]. 2020 [cited 2021 Jan 1];21:9551. Available from: https://www.mdpi.com/1422-0067/21/24/9551.

[13] Zhang K. Overview of Interferon: Characteristics, signaling and anti-cancer effect. Arch Biotechnol Biomed. 2017;1:001-016.

[14] Muskardin TLW, Niewold TB. Type i interferon in rheumatic diseases [Internet]. Nat. Rev. Rheumatol. Nature Publishing Group; 2018 [cited 2020 Dec 11]. p. 214–228. Available from: https://pubmed.ncbi.nlm.nih.gov/29559718/.

[15] Banchereau J, Steinman RM. Dendritic cells and the control of immunity [Internet]. Nature. Nature; 1998 [cited 2020 Dec 11]. p. 245–252. Available from: https://pubmed.ncbi.nlm.nih.gov/9521319/.

[16] Steinman RM, Idoyaga J. Features of the dendritic cell lineage [Internet].

Immunol. Rev. Immunol Rev; 2010 [cited 2020 Dec 11]. p. 5–17. Available from: https://pubmed.ncbi.nlm.nih.gov/20193008/.

[17] Sisirak V, Ganguly D, Lewis KL, et al. Genetic evidence for the role of plasmacytoid dendritic cells in systemic lupus erythematosus. J Exp Med [Internet]. 2014 [cited 2020 Dec 11];211:1969–1976. Available from: /pmc/articles/PMC4172218/?report=abstract.

[18] Kioon MDA, Tripodo C, Fernandez D, et al. Plasmacytoid dendritic cells promote systemic sclerosis with a key role for TLR8. Sci Transl Med [Internet]. 2018 [cited 2020 Dec 11];10. Available from: https://pubmed.ncbi.nlm.nih.gov/29321259/.

[19] Rowland SL, Riggs JM, Gilfillan S, et al. Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model. J Exp Med [Internet]. 2014 [cited 2020 Dec 11];211:1977–1991. Available from: https://pubmed.ncbi.nlm.nih.gov/25180065/.

[20] Rodero MP, Decalf J, Bondet V, et al. Detection of interferon alpha protein reveals differential levels and cellular sources in disease. J Exp Med. 2017;214:1547–1555.

[21] Decker P. Neutrophils and interferon-α-producing cells: Who produces interferon in lupus? [Internet]. Arthritis Res. Ther. BioMed Central; 2011 [cited 2020 Dec 11]. p. 118. Available from: /pmc/articles/PMC3239333/?report=abstract.

[22] Eloranta ML, Alm G V., Rönnblom L. Disease mechanisms in rheumatology - Tools and pathways: Plasmacytoid dendritic cells and their role in autoimmune rheumatic diseases. Arthritis Rheum [Internet]. 2013 [cited 2020 Dec 11];65:853–863. Available from: https://pubmed.ncbi.nlm.nih.gov/23280551/.

[23] Satta N, Dunoyer-Geindre S, Reber G, et al. The role of TLR2 in the inflammatory activation of mouse fibroblasts by human antiphospholipid antibodies. Blood [Internet]. 2007 [cited 2020 Dec 11];109:1507–1514. Available from: https://pubmed.ncbi.nlm.nih.gov/17082324/.

[24] Crow MK. Type I Interferon in the Pathogenesis of Lupus. J Immunol [Internet]. 2014 [cited 2020 Dec 11];192:5459–5468. Available from: https://pubmed.ncbi.nlm.nih.gov/24907379/.

[25] Raschi E, Testoni C, Bosisio D, et al. Role of the MyD88 transduction signaling pathway in endothelial activation by antiphospholipid antibodies. Blood [Internet]. 2003 [cited 2020 Dec 11];101:3495–3500. Available from: https://pubmed.ncbi.nlm.nih.gov/12531807/.

[26] Denny MF, Yalavarthi S, Zhao W, et al. A Distinct Subset of Proinflammatory Neutrophils Isolated from Patients with Systemic Lupus Erythematosus Induces Vascular Damage and Synthesizes Type I IFNs. J Immunol [Internet]. 2010 [cited 2020 Dec 11];184:3284–3297. Available from: https://pubmed.ncbi.nlm.nih.gov/20164424/.

[27] Shirafuji N MS ,Ogura HTKKHOK, NSASTF. Granulocyte colony-stimulating factor stimulates human mature neutrophilic granulocytes to produce interferon-alpha - PubMed. Blood [Internet]. 1990 [cited 2020 Dec 11];75:17–19. Available from: https://pubmed.ncbi.nlm.nih.gov/1688496/.

[28] Prinz N, Clemens N, Strand D, et al. Antiphospholipid antibodies induce translocation of TLR7 and TLR8 to the endosome in human monocytes and plasmacytoid dendritic cells. Blood. 2011;118:2322–2332. ****Novel and crucial insight on APS pathogenesis**

[29] Vallin H, Blomberg S, Alm G V., et al. Patients with systemic lupus erythematosus (SLE) have a circulating inducer of interferon-alpha (IFN-α) production acting on leucocytes resembling immature dendritic cells. Clin Exp Immunol [Internet]. 1999 [cited 2020 Dec 11];115:196–202. Available from: https://pubmed.ncbi.nlm.nih.gov/9933442/.

[30] Barrat FJ, Meeker T, Gregorio J, et al. Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. J Exp Med [Internet]. 2005 [cited 2020 Dec 11];202:1131–1139. Available from: https://pubmed.ncbi.nlm.nih.gov/16230478/.

[31] Lövgren T, Eloranta ML, Båve U, et al. Induction of interferon-α production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. Arthritis Rheum [Internet]. 2004 [cited 2020 Dec 11];50:1861–1872. Available from: https://pubmed.ncbi.nlm.nih.gov/15188363/.

[32] Kelly KM, Zhuang H, Nacionales DC, et al. “Endogenous adjuvant” activity of the RNA components of lupus auto antigens Sm/RNP and Ro 60. Arthritis Rheum [Internet]. 2006 [cited 2020 Dec 11];54:1557–1567. Available from: https://pubmed.ncbi.nlm.nih.gov/16645989/.

[33] Means TK, Latz E, Hayashi F, et al. Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. J Clin Invest [Internet]. 2005 [cited 2020 Dec 11];115:407–417. Available from: https://pubmed.ncbi.nlm.nih.gov/15668740/.

[34] Wu YW, Tang W, Zuo JP. Toll-like receptors: Potential targets for lupus treatment [Internet]. Acta Pharmacol. Sin. Nature Publishing Group; 2015 [cited 2021 Feb 23]. p. 1395–1407. Available from: /pmc/articles/PMC4816237/.

[35] Lau CM, Broughton C, Tabor AS, et al. RNA-associated autoantigens activate B cells by combined B cell antigen receptor/Toll-like receptor 7 engagement. J Exp Med [Internet]. 2005 [cited 2021 Feb 23];202:1171–1177. Available from: https://pubmed.ncbi.nlm.nih.gov/16260486/.

[36] Komatsuda A, Wakui H, Iwamoto K, et al. Up-regulated expression of Toll-like receptors mRNAs in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. Clin Exp Immunol [Internet]. 2008 [cited 2021 Feb 23];152:482–487. Available from: /pmc/articles/PMC2453201/.

[37] Wang M, Kong X, Xie Y, et al. Role of TLR-4 in anti-β2-glycoprotein I-induced activation of peritoneal macrophages and vascular endothelial cells in mice. Mol Med Rep. 2019;49:4353–4363.

[38] De Groot PG, Urbanus RT. Antiphospholipid Syndrome-Not a Noninflammatory Disease. Semin Thromb Hemost [Internet]. 2015 [cited 2020 Dec 11];41:607–614. Available from: https://pubmed.ncbi.nlm.nih.gov/26276935/.

[39] Balasubramanian K, Maiti SN, Schroit AJ. Recruitment of beta-2-glycoprotein 1 to cell surfaces in extrinsic and intrinsic apoptosis. Apoptosis [Internet]. 2005 [cited 2020 Dec 11];10:439–446. Available from: https://pubmed.ncbi.nlm.nih.gov/15843904/.

[40] Maiti SN, Balasubramanian K, Ramoth JA, et al. β-2-glycoprotein 1-dependent macrophage uptake of apoptotic cells: Binding to lipoprotein receptor-related

protein receptor family members. J Biol Chem [Internet]. 2008 [cited 2020 Dec 11];283:3761–3766. Available from: https://pubmed.ncbi.nlm.nih.gov/18073216/.

[41] Rovere P, Manfredi AA, Vallinoto C, et al. Dendritic cells preferentially internalize apoptotic cells opsonized by anti-β2-glycoprotein I antibodies. J Autoimmun [Internet]. Academic Press; 1998 [cited 2020 Dec 11]. p. 403–411. Available from: https://pubmed.ncbi.nlm.nih.gov/9802923/.

[42] Giannakopoulos B, Mirarabshahi P, Qi M, et al. Deletion of the antiphospholipid syndrome autoantigen β2- glycoprotein i potentiates the lupus autoimmune phenotype in a toll-like receptor 7-mediated murine model. Arthritis Rheumatol [Internet]. 2014 [cited 2020 Dec 11];66:2270–2280. Available from: https://pubmed.ncbi.nlm.nih.gov/24692206/.

[43] Grenn RC, Yalavarthi S, Gandhi AA, et al. Endothelial progenitor dysfunction associates with a type I interferon signature in primary antiphospholipid syndrome. Ann Rheum Dis [Internet]. 2017 [cited 2019 Nov 18];76:450–457. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27432357.

[44] van den Hoogen LL, Fritsch-Stork RDE, Versnel MA, et al. Monocyte type I interferon signature in antiphospholipid syndrome is related to proinflammatory monocyte subsets, hydroxychloroquine and statin use. Ann Rheum Dis [Internet]. 2016 [cited 2019 Nov 18];75:e81–e81. Available from: http://ard.bmj.com/lookup/doi/10.1136/annrheumdis-2016-210485.

[45] Palli E, Kravvariti E, Tektonidou MG. Type I Interferon Signature in Primary Antiphospholipid Syndrome : Clinical and Laboratory Associations. 2019;10:1–7.

[46] Palli E, Kravvariti E, Tektonidou MG. Type I interferon signature in primary antiphospholipid syndrome: Clinical and laboratory associations. Front Immunol [Internet]. 2019 [cited 2020 Dec 11];10. Available from: https://pubmed.ncbi.nlm.nih.gov/30930907/.

[47] Nir U MLCBMI. Priming affects the transcription rate of human interferon-beta 1 gene - PubMed. J Biol Chem [Internet]. 1985 [cited 2020 Dec 11];260:14242–14247. Available from: https://pubmed.ncbi.nlm.nih.gov/2865261/.

[48] Severa M, Remoli ME, Giacomini E, et al. Sensitization to TLR7 Agonist in IFN-β-Preactivated Dendritic Cells. J Immunol [Internet]. 2007 [cited 2020 Dec 11];178:6208–6216. Available from: https://pubmed.ncbi.nlm.nih.gov/17475848/.

[49] Weiss G, Maaetoft-Udsen K, Stifter SA, et al. MyD88 Drives the IFN-β Response to Lactobacillus acidophilus in Dendritic Cells through a Mechanism Involving IRF1, IRF3, and IRF7 . J Immunol [Internet]. 2012 [cited 2020 Dec 11];189:2860–2868. Available from: https://pubmed.ncbi.nlm.nih.gov/22896628/.

[50] Abt MC, Osborne LC, Monticelli LA, et al. Commensal Bacteria Calibrate the Activation Threshold of Innate Antiviral Immunity. Immunity [Internet]. 2012 [cited 2020 Dec 11];37:158–170. Available from: https://pubmed.ncbi.nlm.nih.gov/22705104/.

[51] Ruff WE, Dehner C, Kim WJ, et al. Pathogenic Autoreactive T and B Cells Cross-React with Mimotopes Expressed by a Common Human Gut Commensal to Trigger Autoimmunity. Cell Host Microbe [Internet]. 2019 [cited 2020 Dec 11];26:100–113.e8. Available from: https://pubmed.ncbi.nlm.nih.gov/31227334/.

[52] Chen JQ, Papp G, Szodoray P, et al. The role of microRNAs in the pathogenesis of autoimmune diseases [Internet]. Autoimmun. Rev. Elsevier B.V.; 2016 [cited 2020 Dec 11]. p. 1171–1180. Available from: https://pubmed.ncbi.nlm.nih.gov/27639156/.

[53] Carlsen AL, Schetter AJ, Nielsen CT, et al. Circulating microRNA expression profiles associated with systemic lupus erythematosus. Arthritis Rheum. 2013;65:1324–1334.

[54] Zhou H, Huang X, Cui H, et al. MiR-155 and its star-form partner miR-155* cooperatively regulate type I interferon production by human plasmacytoid dendritic cells. Blood [Internet]. 2010 [cited 2020 Dec 11];116:5885–5894. Available from: https://pubmed.ncbi.nlm.nih.gov/20852130/.

[55] Rossato M, Affandi AJ, Thordardottir S, et al. Association of MicroRNA-618 Expression With Altered Frequency and Activation of Plasmacytoid Dendritic Cells in Patients With Systemic Sclerosis. Arthritis Rheumatol [Internet]. 2017

[cited 2020 Dec 11];69:1891–1902. Available from: https://pubmed.ncbi.nlm.nih.gov/28556560/.

[56] Gracias DT, Stelekati E, Hope JL, et al. The microRNA miR-155 controls CD8+ T cell responses by regulating interferon signaling. Nat Immunol [Internet]. 2013 [cited 2020 Dec 11];14:593–602. Available from: https://pubmed.ncbi.nlm.nih.gov/23603793/.

[57] David M. Interferons and microRNAs. J Interf Cytokine Res [Internet]. 2010 [cited 2020 Dec 11];30:825–828. Available from: https://pubmed.ncbi.nlm.nih.gov/20939680/.

[58] Hillen MR, Chouri E, Wang M, et al. Dysregulated miRNome of plasmacytoid dendritic cells from patients with Sjögren's syndrome is associated with processes at the centre of their function. Rheumatol (United Kingdom) [Internet]. 2019 [cited 2020 Dec 11];58:2305–2314. Available from: https://pubmed.ncbi.nlm.nih.gov/31131409/.

[59] Yan S, Yim LY, Tam RCY, et al. MicroRNA-155 mediates augmented CD40 expression in bone marrow derived plasmacytoid dendritic cells in symptomatic lupus-prone NZB/W F1 mice. Int J Mol Sci [Internet]. 2016 [cited 2020 Dec 11];17. Available from: https://pubmed.ncbi.nlm.nih.gov/27509492/.

[60] Van Den Hoogen LL, Rossato M, Lopes AP, et al. MicroRNA downregulation in plasmacytoid dendritic cells in interferon-positive systemic lupus erythematosus and antiphospholipid syndrome. Rheumatol (United Kingdom) [Internet]. 2018 [cited 2020 Dec 11];57:1669–1674. Available from: https://pubmed.ncbi.nlm.nih.gov/29873766/.

[61] Teruel R, Pérez-Sánchez C, Corral J, et al. Identification of miRNAs as potential modulators of tissue factor expression in patients with systemic lupus erythematosus and antiphospholipid syndrome. J Thromb Haemost [Internet]. 2011 [cited 2020 Dec 11];9:1985–1992. Available from: https://pubmed.ncbi.nlm.nih.gov/21794077/.

[62] Pérez-Sánchez C, Aguirre MA, Ruiz-Limón P, et al. "Atherothrombosis-Associated microRNAs in Antiphospholipid syndrome and Systemic Lupus Erythematosus patients." Sci Rep [Internet]. 2016 [cited 2020 Dec 11];6.

Available from: https://pubmed.ncbi.nlm.nih.gov/27502756/.

[63] Grayson PC, Schauer C, Herrmann M, et al. Review: Neutrophils as Invigorated Targets in Rheumatic Diseases [Internet]. Arthritis Rheumatol. John Wiley and Sons Inc.; 2016 [cited 2020 Dec 11]. p. 2071–2082. Available from: https://pubmed.ncbi.nlm.nih.gov/27159737/.

[64] Lande R, Ganguly D, Facchinetti V, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. Sci Transl Med [Internet]. 2011 [cited 2020 Dec 11];3. Available from: https://pubmed.ncbi.nlm.nih.gov/21389263/.

[65] Carmona-Rivera C, Zhao W, Yalavarthi S, et al. Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2. Ann Rheum Dis [Internet]. 2015 [cited 2020 Dec 11];74:1417–1424. Available from: https://pubmed.ncbi.nlm.nih.gov/24570026/.

[66] Yalavarthi S, Gould TJ, Rao AN, et al. Release of neutrophil extracellular traps by neutrophils stimulated with antiphospholipid antibodies: A newly identified mechanism of thrombosis in the antiphospholipid syndrome. Arthritis Rheumatol [Internet]. 2015 [cited 2020 Dec 11];67:2990–3003. Available from: https://pubmed.ncbi.nlm.nih.gov/26097119/.

[67] Meng H, Yalavarthi S, Kanthi Y, et al. In Vivo Role of Neutrophil Extracellular Traps in Antiphospholipid Antibody–Mediated Venous Thrombosis. Arthritis Rheumatol [Internet]. 2017 [cited 2020 Dec 11];69:655–667. Available from: https://pubmed.ncbi.nlm.nih.gov/27696751/.

[68] Van Den Hoogen LL, Fritsch-Stork RDE, Van Roon JAG, et al. Low-Density Granulocytes Are Increased in Antiphospholipid Syndrome and Are Associated with Anti-β2-Glycoprotein i Antibodies: Comment on the Article by Yalavarthi et al [Internet]. Arthritis Rheumatol. John Wiley and Sons Inc.; 2016 [cited 2020 Dec 11]. p. 1320–1321. Available from: https://pubmed.ncbi.nlm.nih.gov/26749538/.

[69] Villanueva E, Yalavarthi S, Berthier CC, et al. Netting Neutrophils Induce Endothelial Damage, Infiltrate Tissues, and Expose Immunostimulatory

Molecules in Systemic Lupus Erythematosus. J Immunol [Internet]. 2011 [cited 2020 Dec 11];187:538–552. Available from: https://pubmed.ncbi.nlm.nih.gov/21613614/.

[70] Garcia-Romo GS, Caielli S, Vega B, et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med [Internet]. 2011 [cited 2020 Dec 12];3. Available from: https://pubmed.ncbi.nlm.nih.gov/21389264/.

[71] Van Den Hoogen LL, Van Der Linden M, Meyaard L, et al. Neutrophil extracellular traps and low-density granulocytes are associated with the interferon signature in systemic lupus erythematosus, but not in antiphospholipid syndrome. Ann Rheum Dis. 2020;79:1–2.

[72] van der Linden M, van den Hoogen LL, Westerlaken GHA, et al. Neutrophil extracellular trap release is associated with antinuclear antibodies in systemic lupus erythematosus and anti-phospholipid syndrome. Rheumatol (United Kingdom) [Internet]. 2018 [cited 2020 Dec 11];57:1228–1234. Available from: https://pubmed.ncbi.nlm.nih.gov/29608758/.

[73] Linge P, Arve S, Olsson LM, et al. NCF1-339 polymorphism is associated with altered formation of neutrophil extracellular traps, high serum interferon activity and antiphospholipid syndrome in systemic lupus erythematosus. Ann Rheum Dis [Internet]. 2019 [cited 2020 Dec 11];79. Available from: https://pubmed.ncbi.nlm.nih.gov/31704719/.

[74] Lood C, Blanco LP, Purmalek MM, et al. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. Nat Med [Internet]. 2016 [cited 2020 Dec 11];22:146–153. Available from: https://pubmed.ncbi.nlm.nih.gov/26779811/.

[75] Knight JS, Meng H, Coit P, et al. Activated signature of antiphospholipid syndrome neutrophils reveals potential therapeutic target. JCI Insight. 2017;2.

[76] Denny MF, Thacker S, Mehta H, et al. Interferon-α promotes abnormal vasculogenesis in lupus: A potential pathway for premature atherosclerosis. Blood [Internet]. 2007 [cited 2020 Dec 11];110:2907–2915. Available from: https://pubmed.ncbi.nlm.nih.gov/17638846/.

[77] Tydén H, Lood C, Gullstrand B, et al. Endothelial dysfunction is associated with activation of the type i interferon system and platelets in patients with systemic lupus erythematosus. RMD Open [Internet]. 2017 [cited 2020 Dec 11];3. Available from: https://pubmed.ncbi.nlm.nih.gov/29119007/.

[78] Padjas A, Płazak W, Celińska-Lowenhoff M, et al. Myocardial ischaemia, coronary atherosclerosis and pulmonary pressure elevation in antiphospholipid syndrome patients. Adv Clin Exp Med [Internet]. 2016 [cited 2020 Dec 11];25:1199–1205. Available from: https://pubmed.ncbi.nlm.nih.gov/28028974/.

[79] Amaya-Amaya J, Rojas-Villarraga A, Anaya JM. Cardiovascular disease in the antiphospholipid syndrome. Lupus [Internet]. SAGE Publications Ltd; 2014 [cited 2020 Dec 11]. p. 1288–1291. Available from: https://pubmed.ncbi.nlm.nih.gov/25228729/.

[80] Der H, Kerekes G, Veres K, et al. Impaired endothelial function and increased carotid intima-media thickness in association with elevated von Willebrand antigen level in primary antiphospholipid syndrome. Lupus [Internet]. 2007 [cited 2020 Dec 11];16:497–503. Available from: https://pubmed.ncbi.nlm.nih.gov/17670848/.

[81] Štalc M, Poredoš P, Peternel P, et al. Endothelial function is impaired in patients with primary antiphospholipid syndrome. Thromb Res [Internet]. 2006 [cited 2020 Dec 11];118:455–461. Available from: https://pubmed.ncbi.nlm.nih.gov/16274735/.

[82] Kaplan MJ, Salmon JE. How does interferon-α insult the vasculature? Let me count the ways [Internet]. Arthritis Rheum. Arthritis Rheum; 2011 [cited 2020 Dec 11]. p. 334–336. Available from: https://pubmed.ncbi.nlm.nih.gov/21279989/.

[83] Somers EC, Zhao W, Lewis EE, et al. Type I interferons are associated with subclinical markers of cardiovascular disease in a cohort of systemic lupus erythematosus patients. PLoS One [Internet]. 2012 [cited 2020 Dec 11];7. Available from: https://pubmed.ncbi.nlm.nih.gov/22606325/.

[84] Lee PY, Li Y, Richards HB, et al. Type I interferon as a novel risk factor for endothelial progenitor cell depletion and endothelial dysfunction in systemic

lupus erythematosus. Arthritis Rheum. 2007;56:3759–3769.

[85] Rodríguez-Carrio J, De Paz B, López P, et al. IFNα serum levels are associated with endothelial progenitor cells imbalance and disease features in rheumatoid arthritis patients. PLoS One. 2014;9.

[86] Ekholm L, Michelle Kahlenberg J, Helmers SB, et al. Dysfunction of endothelial progenitor cells is associated with the type I IFN pathway in patients with polymyositis and dermatomyositis. Rheumatol (United Kingdom) [Internet]. 2016 [cited 2020 Dec 11];55:1987–1992. Available from: https://pubmed.ncbi.nlm.nih.gov/27498356/.

[87] Hervas-Stubbs S, Perez-Gracia JL, Rouzaut A, et al. Direct effects of type I interferons on cells of the immune system [Internet]. Clin. Cancer Res. Clin Cancer Res; 2011 [cited 2020 Dec 11]. p. 2619–2627. Available from: https://pubmed.ncbi.nlm.nih.gov/21372217/.

[88] Le Bon A, Schiavoni G, D'Agostino G, et al. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity [Internet]. 2001 [cited 2020 Dec 11];14:461–470. Available from: https://pubmed.ncbi.nlm.nih.gov/11336691/.

[89] Padovan Elisabetta pagnoli GCSFMHM. IFN-alpha2a induces IP-10/CXCL10 and MIG/CXCL9 production in monocyte-derived dendritic cells and enhances their capacity to attract and stimulate CD8+ effector T cells - PubMed. J Leukoc Biol [Internet]. 2002 [cited 2020 Dec 11];71:669–676. Available from: https://pubmed.ncbi.nlm.nih.gov/11927654/.

[90] Litinskiy MB, Nardelli B, Hilbert DM, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol [Internet]. 2002 [cited 2020 Dec 11];3:822–829. Available from: https://pubmed.ncbi.nlm.nih.gov/12154359/.

[91] López P, Scheel-Toellner D, Rodríguez-Carrio J, et al. Interferon-α-induced B-lymphocyte stimulator expression and mobilization in healthy and systemic lupus erthymatosus monocytes. Rheumatol (United Kingdom) [Internet]. 2014 [cited 2020 Dec 11];53:2249–2258. Available from: https://pubmed.ncbi.nlm.nih.gov/24942493/.

[92] Yao Y, Richman L, Higgs BW, et al. Neutralization of interferon-α/β-inducible genes and downstream effect in a phase I trial of an anti-interferon-α monoclonal antibody in systemic lupus erythematosus. Arthritis Rheum [Internet]. 2009 [cited 2020 Dec 11];60:1785–1796. Available from: https://pubmed.ncbi.nlm.nih.gov/19479852/.

[93] Jacob N, Guo S, Mathian A, et al. B Cell and BAFF Dependence of IFN-α–Exaggerated Disease in Systemic Lupus Erythematosus-Prone NZM 2328 Mice. J Immunol [Internet]. 2011 [cited 2020 Dec 11];186:4984–4993. Available from: https://pubmed.ncbi.nlm.nih.gov/21383240/.

[94] van den Hoogen LL, Palla G, Bekker CPJ, et al. Increased B-cell activating factor (BAFF)/B-lymphocyte stimulator (BLyS) in primary antiphospholipid syndrome is associated with higher adjusted global antiphospholipid syndrome scores. RMD Open. 2018;4:e000693.

[95] Li XY, Duan HJ, Liu XY, et al. Change of serum B-cell activating factor level in patients with positive antiphospholipid antibodies and previous adverse pregnancy outcomes and its significance. Chin Med J (Engl) [Internet]. 2020 [cited 2020 Dec 11];133:2287–2294. Available from: https://pubmed.ncbi.nlm.nih.gov/32842014/.

[96] Roth DA, Thompson A, Tang Y, et al. Elevated BLyS levels in patients with systemic lupus erythematosus: Associated factors and responses to belimumab. Lupus [Internet]. 2016 [cited 2020 Dec 11];25:346–354. Available from: https://pubmed.ncbi.nlm.nih.gov/26385220/.

[97] Ugolini-Lopes MR, Torrezan GT, Gândara APR, et al. Enhanced type I interferon gene signature in primary antiphospholipid syndrome: Association with earlier disease onset and preeclampsia. Autoimmun. Rev. Elsevier B.V.; 2019. p. 393–398.

[98] Andrade D, Kim M, Blanco LP, et al. Interferon-α and angiogenic dysregulation in pregnant lupus patients who develop preeclampsia. Arthritis Rheumatol [Internet]. 2015 [cited 2021 Feb 24];67:977–987. Available from: https://pubmed.ncbi.nlm.nih.gov/25603823/.

[99] Gujer C, Sundling C, Seder RA, et al. Human and rhesus plasmacytoid dendritic

cell and B-cell responses to Toll-like receptor stimulation. Immunology [Internet]. 2011 [cited 2020 Dec 11];134:257–269. Available from: https://pubmed.ncbi.nlm.nih.gov/21977996/.

[100] Hisada RYO, Kato M, Sugawara ERI, et al. Circulating plasmablasts contribute to antiphospholipid antibody production, associated with type I interferon upregulation. J Thromb Haemost [Internet]. 2019 [cited 2020 Dec 11];17:1134–1143. Available from: https://pubmed.ncbi.nlm.nih.gov/30864219/.

[101] Jacobi AM, Mei H, Hoyer BF, et al. HLA-DRhigh/CD27high plasmablasts indicate active disease in patients with systemic lupus erythematosus. Ann Rheum Dis [Internet]. 2010 [cited 2020 Dec 11];69:305–308. Available from: https://pubmed.ncbi.nlm.nih.gov/19196727/.

[102] Rodríguez-Bayona B, Ramos-Amaya A, Pérez-Venegas JJ, et al. Decreased frequency and activated phenotype of blood CD27 IgD IgM B lymphocytes is a permanent abnormality in systemic lupus erythematosus patients. Arthritis Res Ther [Internet]. 2010 [cited 2020 Dec 11];12. Available from: https://pubmed.ncbi.nlm.nih.gov/20525218/.

[103] Båve U, Nordmark G, Lövgren T, et al. Activation of the type I interferon system in primary Sjögren's syndrome: A possible etiopathogenic mechanism. Arthritis Rheum. 2005;52:1185–1195.

[104] Perez-Sanchez C, Barbarroja N, Messineo S, et al. Gene profiling reveals specific molecular pathways in the pathogenesis of atherosclerosis and cardiovascular disease in antiphospholipid syndrome, systemic lupus erythematosus and antiphospholipid syndrome with lupus. Ann Rheum Dis. 2015;74:1441–1449.

[105] Bernales I, Fullaondo A, Marín-Vidalled MJ, et al. Innate immune response gene expression profiles characterize primary antiphospholipid syndrome. Genes Immun [Internet]. 2008 [cited 2019 Nov 18];9:38–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17960154.

[106] Palli E, Kravvariti E, Tektonidou MG. Type I Interferon Signature in Primary Antiphospholipid Syndrome: Clinical and Laboratory Associations. Front Immunol [Internet]. 2019 [cited 2019 Nov 20];10:487. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30930907.

[107] van den Hoogen LL, van Roon JAG, Mertens JS, et al. Galectin-9 is an easy to measure biomarker for the interferon signature in systemic lupus erythematosus and antiphospholipid syndrome. Ann Rheum Dis. 2018;77:1810–1814.

[108] Ugolini-Lopes MR, Torrezan GT, Gândara APR, et al. Enhanced type I interferon gene signature in primary antiphospholipid syndrome: Association with earlier disease onset and preeclampsia. Autoimmun Rev. 2019;18:393–398.

[109] Hisada R, Kato M, Sugawara E, et al. Circulating plasmablasts contribute to antiphospholipid antibody production, associated with type I interferon upregulation. J Thromb Haemost. 2019;17:1134–1143.

[110] Baechler EC, Batliwalla FM, Karypis G, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci. 2003;100:2610–2615.

[111] Flessa CM, Vlachiotis S, Nezos A, et al. Independent association of low IFNλ1 gene expression and type I IFN score/IFNλ1 ratio with obstetric manifestations and triple antiphospholipid antibody positivity in primary antiphospholipid syndrome. Clin Immunol [Internet]. 2019 [cited 2021 Jan 1];209. Available from: https://pubmed.ncbi.nlm.nih.gov/31639447/.

[112] Weeding E, Coit P, Yalavarthi S, et al. Genome-wide DNA methylation analysis in primary antiphospholipid syndrome neutrophils. Clin Immunol. 2018;196:110–116.

[113] Mathian A, Mouries-Martin S, Dorgham K, et al. Monitoring Disease Activity in Systemic Lupus Erythematosus With Single-Molecule Array Digital Enzyme-Linked Immunosorbent Assay Quantification of Serum Interferon-α. Arthritis Rheumatol [Internet]. 2019 [cited 2020 Dec 11];71:756–765. Available from: https://pubmed.ncbi.nlm.nih.gov/30507062/.

[114] Connelly KL, Kandane-Rathnayake R, Huq M, et al. Longitudinal association of type 1 interferon-induced chemokines with disease activity in systemic lupus erythematosus. Sci Rep [Internet]. 2018 [cited 2020 Dec 11];8. Available from: https://pubmed.ncbi.nlm.nih.gov/29459655/.

[115] Iwamoto T, Dorschner J, Jolly M, et al. Associations between type I interferon and antiphospholipid antibody status differ between ancestral backgrounds.

Lupus Sci Med. 2018;5:3–6.

[116] Idborg H, Zandian A, Sandberg AS, et al. Two subgroups in systemic lupus erythematosus with features of antiphospholipid or Sjögren's syndrome differ in molecular signatures and treatment perspectives. Arthritis Res Ther. 2019;21:1–11.

[117] Cecchi I, Radin M, Rubini E, et al. TYPE I INTERFERON EXPRESSION IDENTIFIES DIFFERENT SUBSETS OF ANTIPHOSPHOLIPID SYNDROME. Available from: http://ard.bmj.com/.

[118] Sciascia S, Sanna G, Murru V, et al. Anti-prothrombin (aPT) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies and the risk of thrombosis in the antiphospholipid syndrome. A systematic review. Thromb Haemost [Internet]. 2014 [cited 2018 May 20];111:354–364. Available from: http://www.thieme-connect.de/DOI/DOI?10.1160/TH13-06-0509.

[119] Kalunian KC, Merrill JT, Maciuca R, et al. A phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon-α) in patients with systemic lupus erythematosus (ROSE). Ann Rheum Dis [Internet]. 2015 [cited 2021 Jan 2];75:196–202. Available from: https://pubmed.ncbi.nlm.nih.gov/26038091/.

[120] Khamashta M, Merrill JT, Werth VP, et al. Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: A randomised, double-blind, placebo-controlled study. Ann Rheum Dis [Internet]. 2016 [cited 2021 Jan 2];75:1909–1916. Available from: https://pubmed.ncbi.nlm.nih.gov/27009916/.

[121] Felten R, Scher F, Sagez F, et al. Spotlight on anifrolumab and its potential for the treatment of moderate-to-severe systemic lupus erythematosus: Evidence to date [Internet]. Drug Des. Devel. Ther. Dove Medical Press Ltd.; 2019 [cited 2021 Jan 2]. p. 1535–1543. Available from: https://pubmed.ncbi.nlm.nih.gov/31190735/.

[122] Cattini MG, Bison E, Pontara E, et al. Tetra positive thrombotic antiphospholipid syndrome: Major contribution of anti-phosphatidyl-serine/prothrombin antibodies to lupus anticoagulant activity. J Thromb Haemost. 2020;18:1124–1132.

[123] De Laat B, De Groot PG. Autoantibodies directed against domain i of beta2-

glycoprotein i. Curr Rheumatol Rep [Internet]. 2011 [cited 2020 Dec 8];13:70–76. Available from: https://pubmed.ncbi.nlm.nih.gov/21046294/.

[124] Galli M, Luciani D, Bertolini G, et al. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. Blood [Internet]. 2003 [cited 2018 Apr 23];101:1827–1832. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2002-02-0441.

[125] Sciascia S, Sanna G, Murru V, et al. GAPSS: The global anti-phospholipid syndrome score. Rheumatol (United Kingdom) [Internet]. 2013 [cited 2020 Sep 30];52:1397–1403. Available from: https://pubmed.ncbi.nlm.nih.gov/23315788/.

[126] Otomo K, Atsumi T, Amengual O, et al. Efficacy of the antiphospholipid score for the diagnosis of antiphospholipid syndrome and its predictive value for thrombotic events. Arthritis Rheum [Internet]. 2012 [cited 2020 Dec 8];64:504–512. Available from: https://pubmed.ncbi.nlm.nih.gov/21953404/.

[127] Zuily S, Clerc-Urmès I, Bauman C, et al. Cluster analysis for the identification of clinical phenotypes among antiphospholipid antibody-positive patients from the APS ACTION Registry. Lupus [Internet]. 2020 [cited 2020 Dec 8]; Available from: https://pubmed.ncbi.nlm.nih.gov/32703117/.

[128] Sciascia S, Radin M, Cecchi I, et al. Identifying phenotypes of patients with antiphospholipid antibodies: results from a cluster analysis in a large cohort of patients. Rheumatology [Internet]. 2019 [cited 2020 Dec 8]; Available from: https://pubmed.ncbi.nlm.nih.gov/31840749/.

[129] Lopez LA, Sang PC, Tian Y, et al. Dysregulated Interferon Response Underlying Severe COVID-19 [Internet]. Viruses. NLM (Medline); 2020 [cited 2021 Feb 21]. Available from: https://pubmed.ncbi.nlm.nih.gov/33322160/.

[130] Zhang Q, Liu Z, Moncada-Velez M, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science (80-) [Internet]. 2020 [cited 2021 Feb 21];370. Available from: https://pubmed.ncbi.nlm.nih.gov/32972995/.

[131] Merrill JT, Erkan D, Winakur J, et al. Emerging evidence of a COVID-19 thrombotic syndrome has treatment implications [Internet]. Nat. Rev. Rheumatol. Nature Research; 2020 [cited 2021 Feb 21]. p. 581–589. Available from:

Factors potentially associated with increased IFN-I in APS			
Factor	**Possible mechanism**	**Study design**	**References**

https://pubmed.ncbi.nlm.nih.gov/32733003/.

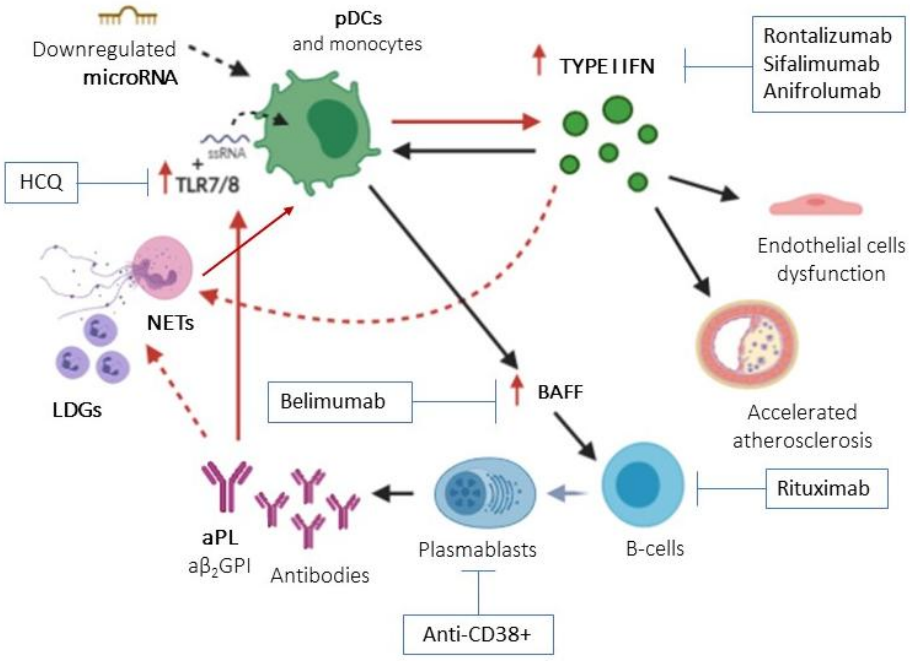
Table 1. Influence and regulation of type I interferon in antiphospholpid syndrome

aPL	• Associated with IFN-I signature in clinical studies • Increase IFN-I transcript expression by pDCs • Prime pDCs to internalize RNA • Promote translocation of TLRs from endoplasmic reticulum to endosome • Sensitize cells to TLR ligands • Possibly inhibit the anti-inflammatory function of β_2GPI • Microbiome (*Roseburia intestinalis*) due to the presence of homologous sequences to β_2GPI can play a role in IFN-I production	Human *in vivo*, *ex vivo*, *in vitro* and animal models [27,37-40], PAPS [38], SLE, SAPS and PAPS [39,40]	[28,42–45]
TLRs	• Increased expression in APS • pDCs produce IFN-I in a TLR7-dependent pathway • TLR7 ligands needed to produce IFN-I • Interferogenic TLR7 SNP associated with increased plasmablast numbers	Human *in vivo*, *ex vivo*, *in vitro* and animal models, PAPS, SAPS [92]	[28,100]
pDCs	• Quintessential producers of IFN-I	Human *in vivo*, *ex vivo*, *in vitro* and animal models [16-19]	[17–22]
Potential downstream effects of IFN-I in APS			
Endothelial injury	• IFN-I linked with EPC dysfunction • Increased pro-atherosclerotic monocyte subsets	Human *in vivo*, *ex vivo* assays, PAPS, SAPS and SLE patients [38, 39]	[43,44]
Increased BAFF	• Potential correlation between IFN-I and BAFF in APS patients	*In vivo* assays, aPL positive PAPS, SAPS and SLE patients [88, 89]	[94,95]
Potential bidirectional effects			
NETs	• APS patients have increased number of LDGs, which produce more NETs • NETs are a source of antigens that can stimulate TLRs on pDCs; as discussed above, TLRs and pDCs can enhance IFN-I production • APS NETs have enhanced expression of mitochondrial ROS, which are more interferogenic than cytosolic ROS • The IFN-I regulated gene group was the most significantly enriched in APS neutrophils • In SLE, IFN-I primes neutrophils to form NETs	Human *in vivo*, *ex vivo*, *in vitro* and animal models, PAPS, SAPS and SLE patients [64,66,68,70,73–75]	[64,66,68,70,73–75]
Plasmablasts	• A SNP causing increased TLR7 and IFN-I production was associated with increased plasmablast numbers • CD20-negative B cells (includes plasmablast population) produce aPL, which can then increase IFN-I expression as discussed above	Human *in vivo* and *ex vivo* assays, PAPS and SAPS patirents	[100]
Unclear association			
microRNA regulation	• Downregulated microRNA expression and IFN-I signature linked in APS	Human *in vivo* and *ex vivo* assays, PAPS, SAPS and SLE patients	[60]

1

aPL means antiphospholipid antibodies; APS, antiphospholipid syndrome;β_2GPI, $\beta_2$glycoprotein I; BAFF, B cell activating factor; EPC, endothelial progenitor cell; IFN-I, type I interferon; NETs, neutrophil extracellular traps;LDGs, low-density granulocytes; pDCs, plasmacytoid dendritic cells;SLE, systemic lupus erythematosus; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; TLRs, toll like receptors; PAPS, primary APS patients; SAPS, secondary APS patients.

Figure 1. Main sources and effects of type I interferon in antiphospholipid syndrome.



pDCs are recognized as the main source of IFN-I. Although not completely elucidated, it is known that a number of complex processes causes an increased production of IFN-I in APS setting, leading to endothelial dysfunction, accelerated atherosclerosis, and higher levels of BAFF which are responsible for an increased production of autoantibodies. APL, especially aβ2GPI, are thought to induce IFN-I production through the hyperactivation of TLR. Moreover, IFN-I production seems to be modulated by microRNA, which global expression have been reported to be downregulated in pDCs of APS patients, and by NETs. It is still debated whether the increase in NETs formation and LGDs number are directly responsible for higher IFN-I

levels, as it has been reported in SLE pathogenesis. This figure also shows the main treatment agents with immunomodulant properties employed in APS, along with possible future therapeutic targets including those with direct anti-IFN-I effects.

pDCs means plasmacytoid dendritic cells; IFN-I, type I interferon; BAFF, B-cell activating factor; aPL, antiphospholipid antibodies; aβ2GPI, anti-β2-glycoprotein I antibodies; LDGs, low-density granulocytes; NETs, neutrophil extracellular traps; TLR, toll like receptor; HCQ, hydroxychloroquine; APS, antiphospholipid syndrome.

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