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Type I interferon gene expression in antiphospholipid syndrome: Pathogenetic, clinical and therapeutic implications

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ABSTRACT

Type I Interferon gene expression has been shown to play an important role in the pathogenesis of several systemic autoimmune disorders, paving the way for its potential use as a surrogate marker or a therapeutic tool. While the concept of type I interferon signature and its correlation with clinical phenotypes and disease activity, along with anti-interferon targeted therapy have been vastly investigated in patients with systemic lupus erythematosus, there is a paucity of data concerning antiphospholipid syndrome patients. In this review, we summarize the current knowledge on the pathogenetic and clinical implications of type I interferon expression in antiphospholipid syndrome and discuss the therapeutic possibility of targeting molecules along the interferon signaling pathway. A number of recent studies have shown a type I interferon gene expression induction in patients with primary antiphospholipid syndrome via the plasmacytoid dendritic cell pathway, toll like receptors (TLRs) such as TLR7 and TLR9, anti-beta2glycoprotein I antibody-mediated neutrophil activation and neutrophil extracellular traps (NETs) release in a TLR4-dependent fashion, and a subsequent B cell and plasmablast activation. An association between type I interferon expression and several demographic, clinical and laboratory characteristics including age, gender, pregnancy complications such as eclampsia, anti-beta2glycoprotein I antibodies, and a negative correlation with hydroxychloroquine and/or statin use, has been shown. Correlation of high interferon scores to worse outcomes in prospective studies could direct the initiation for a prompt treatment in high-risk populations. Potential therapeutic approaches targeting type I interferon production and signaling pathway components might include anti-interferon or interferon receptor monoclonal antibodies, or an interferon based therapeutic vaccine as was indicated from previous systemic lupus erythematosus studies, TLR inhibitors including hydroxychloroquine and *anti*-TLR antibodies, plasmacytoid dendritic cell inhibition, adenosine-receptor agonists, and plasmablast targeting treatments. Well-designed studies are needed to further assess the immunomodulatory potential of the above targets for therapeutic intervention in patients with primary antiphospholipid syndrome.

1. Introduction

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by arterial and venous thrombosis along with pregnancy-related complications in the presence of persistent medium-high titers of anticardiolipin (aCL) and/or anti-beta 2 glycoprotein (*anti*-β_2GPI) antibodies and/or the lupus anticoagulant (LA), named antiphospholipid antibodies (aPL) [1]. APS can present with several distinct clinical phenotypes including classic APS manifestations with recurrent thrombotic events and/or pregnancy morbidity or the non-criteria APS manifestations including thrombocytopenia, heart valve lesions, APS-related nephropathy, cognitive dysfunction, and others [2–5]. Furthermore, patients with single, double or triple aPL positivity but no clinically apparent disease as well as individuals with APS-related manifestations but no measurable aPL have been described. Since the presence of aPL itself, even at medium-high titers, is not always accompanied by APS-related manifestations nor does it accurately predict the impending appearance of an overt clinical syndrome, the hypothesis of a "two-hit model" has been proposed.

Pathogenetic mechanisms of APS include a combination of

Abbreviations: aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; PAPS, primary antiphospholipid syndrome; SLE, systemic lupus erythematosus; SLE/APS, systemic lupus erythematosus-associated antiphospholipid syndrome; IFN, interferon; IFN-I, interferon type I; IFNAR, type I interferon receptor; HCQ, hydroxychloroquine

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coagulation activation and fibrinolysis impairment via aPL-mediated functional alteration of protein C, annexin V, complement components, platelets, serum proteases, tissue factor and toll-like receptors, as well as interactions between aPL and several other molecules directed against heparin, thromboplastin, thrombomodulin, kininogen, platelet-activating factor, lipoproteins and factors VII, VIIa and XII of the coagulation cascade [6,7]. Following interaction with aPL, monocyte and endothelial cell activation leads to an upregulation of tissue factor, *E*-selectin, pro-inflammatory cytokines such as IL-6 and IL-8, and adhesion molecules, alterations promoting intravascular thrombosis and inflammation [8]. Furthermore, aPL-mediated disruption of normal platelet functionality is achieved through a multitude of distinct mechanisms including an increase in receptors and molecules promoting platelet aggregation such as glycoprotein IIb-IIIa, thromboxane A2 and platelet factor-4 combined with a decrease in inhibitory molecules like annexin V [9]. A role for complement components has been suggested in the pathophysiology of APS, based on the observations that C5a amplifies tissue factor activity in neutrophils, models of C3 or C6 deficient mice are resistant to aPL-mediated thrombosis, an effect also reproduced by C5 inhibition and the fact that eculizumab, a humanized monoclonal antibody hindering the activity of C5b-C9 terminal complex has been successfully used in the settings of catastrophic APS and APS-related thrombotic microangiopathy [10]. Another pathway through which innate immune response is thought to contribute in APS pathogenesis is the release of neutrophil extracellular traps (NETs), a mechanism that is overly activated by neutrophil interaction with *anti*-β_2GPI antibodies promoting thrombus formation [11]. The complex interplay between underlying genetic mechanisms and infectious or other triggers inducing the production of aPL, along with the systemic action of the latter, remain largely unexplored, impeding in turn the development of a causative treatment.

2. Type I interferon signature in primary antiphospholipid syndrome – pathogenetic and clinical implications

An increased expression of type I interferon (IFN)-responding genes, the so-called IFN signature, has been implicated in the pathogenetic pathways of a multitude of systemic autoimmune diseases, including systemic lupus erythematosus (SLE), Sjogren's syndrome, rheumatoid arthritis, myositis and scleroderma affecting both the innate and acquired arms of the immune system [12–14]. Cytosolic nucleic acid sensing-pattern recognition receptors such as toll-like and the cytosolic retinoic acid-inducible gene 1 (RIG-I)- receptors induce type I IFN-stimulated gene expression mainly by plasmacytoid dendritic cells (pDCs) and to a lesser extent by macrophages, neutrophils and epithelial cells or fibroblasts [15] via activation of Janus kinase (JAK) and signal transducer and activator of transcription (STAT)1 and STAT2 [16].

While it is well known that SLE is associated with type I IFN signature, the type I IFN gene expression in primary APS (PAPS), independent of correlation with SLE, and its clinical and therapeutic implications, remain under investigation. The methodology and the results of published studies examining type I IFN expression in PAPS are summarized in Table 1.

In a genome-wide array study performed by Bernales et al. [17], among a total of 93 genes differentially expressed between patients with PAPS and healthy controls ($p < 0.01$), both type I and II IFN-inducible families exhibited upregulated expression in subjects with PAPS. IFN-related regulators of the coagulation cascade such as the glycoprotein 1B alpha polypeptide and the phospholipid scramblase 1 (PLSCR1) were also overexpressed. Despite the overlapping immune response gene expression patterns among patients with SLE and PAPS, differences in PLSCR1, tumor necrosis factor ligand superfamily member 13, CD14 and TLR-8 discern the patient cohorts, underlining the similar yet distinct pathophysiologic substrates of two disease entities.

Gene profiling of monocytes by Perez-Sanchez et al. revealed that differential expression patterns between patients with PAPS, SLE-associated APS (SLE/APS) and SLE substantiated the pro-inflammatory and pro-atherosclerotic phenotypes having previously proven to be shared by these patients, with the PAPS group exerting amplified expression of mitochondrial metabolism genes, specifically those related to oxidative stress reduction pathways [18]. The finding of altered mitochondrial gene expression leading to increased oxidative stress was regarded by the authors to be part of the pathogenetic substrate for thrombosis in APS. This hypothesis was supported by the fact that accelerated thrombosis was described as a potentially reversible process following administration of a central component of the mitochondrial respiratory chain named coenzyme Q [19]. Type I IFN scores among the three groups revealed that only SLE and SLE/APS had upregulated gene expression when compared to controls. This finding could be attributed to the fact that following the first gene-wide analysis, the threshold set for further analysis and quantification excluded from the study any gene with less than twofold differential expression vs controls.

Grenn et al. assessed endothelial progenitor cell (EPC) functional status and IFN I gene activity in 42 patients with PAPS and 63 healthy controls [20]. While there was only a trend of cell population reduction for classic EPCs in PAPS, a functional assay including both classic EPCs and myeloid-derived progenitor cells named circulating angiogenic cells (CAC), revealed that the combination of EPCs/CACs failed to differentiate into mature endothelial cells in a statistically significant rate for those patients when compared to the control group. The addition of APS serum to control EPC/CAC cell cultures indicated that endothelial maturation dysfunction was caused by a serum-related factor. APS sera IgG were excluded as a confounding factor for EPC/CAC hindered differentiation, since IgG-purified APS sera added to cell cultures reproduced the effect caused by non-purified PAPS sera. Furthermore, type I IFN augmented gene expression in PAPS was confirmed from serum samples of an independent cohort of 26 patients, while dysfunctional endothelial cells were shown to bear an IFN signature. The hypothesis that IFN type I gene induction could be the causative factor for the EPC/CACs dysfunction was further solidified by the fact that IFN type I receptor blockade rescued the progenitor cellular differentiation. The results of this study suggest that impaired EPC maturation in the setting of PAPS could be partially attributed to type I IFN gene expression, the higher level of which in peripheral blood mononuclear cells (PBMCs) was, in turn, correlated with IgG *anti*-β_2GPI presence. A possible mechanism for type I IFN altered patterns in PAPS discussed in this study was toll-like receptor (TLR)-7/8 overexpression. SLE-related autoantibodies but no concurrent clinical SLE features were present in a portion of the study population.

In a study performed by van den Hoogen et al. comparing IFN expression patterns in SLE, SLE/APS and PAPS, upregulated type I IFN expression was present in 77%, 71% and 46% of patients, respectively and in a statistically significant manner when compared to age-matched healthy controls [21]. Type I IFN signature was not related to aPL antibodies or type of thrombotic events. In a subsequent analysis, a trend between monocyte tissue factor expression and amplified IFN gene expression was present among all groups but without reaching statistical significance ($p = 0.10$, 0.055 and 0.67 for SLE, SLE/APS and PAPS, respectively). Intermediate ($CD14++$, $CD16^+$) and non-classical ($CD14^+$, $CD16^+$) monocyte subgroups with a phenotype related to accelerated cardiovascular disease development were positively correlated with IFN signature ($r = 0.47$, $p < 0.001$ and $r = 0.39$, $p = 0.006$, respectively). This correlation is in accordance to the established link between vascular dysfunction and IFN. Although a trend of interconnection between lupus autoantibodies and higher IFN scores was observed, 45% of individuals without *anti*-dsDNA positivity showed similar gene expression amplification. Furthermore, the use of hydroxychloroquine and/or statins attenuated IFN signature.

Transcriptome mapping in neutrophils from patients with PAPS and age-, sex-, and ethnicity-matched healthy controls performed by Knight et al. revealed a universal upregulation of several type I IFN-inducible

Table 1
Studies examining type I IFN signature in patients with PAPS.

Authors, year	Patient groups	Methods used for type I IFN gene expression	Findings
Bernales et al., 2008 [17]	6 w/PAPS, 5 w/SLE and 6 healthy controls (initial cohort) 7 w/PAPS, 12 w/SLE, 5 w/non-PAPS thrombosis and 17 healthy controls (verification cohort)	Microarray study and quantitative PCR for genes differentially expressed in PAPS and SLE (93, $p < 0.01$ and 88, $p < 0.01$, respectively) vs healthy controls	In the initial cohort: Upregulated expression of genes related to monocyte functionality, coagulation, nucleic acid metabolism, including the type I IFN-inducible genes LGALS3BP, WARS, OAS2, G1P2, GTPBP2, TNFSF13, PLSCR1, H1F0 and STAT1. In the verification cohort: Nine genes (NCF4, TLR8, CD14, CD163, PLSCR1, TNFSF13, STAT1, G1P2, OAS2), of which five are IFN- inducible, were overexpressed in PAPS vs controls ($P < 0.01$). Type I IFN signature related genes PLSCR1 and TNFSF13 and receptors CD14 and TLR8 differentially expressed between PAPS and SLE ($p < 0.05$).
Perez-Sanchez et al., 2015 [18]	42 w/PAPS, 56 w/SLE, 31 w/SLE/APS and 61 healthy controls	Microarray study and quantitative PCR w/ 555, 1224 and 518 genes being differentially expressed for SLE, SLE/APS and PAPS, respectively, vs controls	IFN signature (resulting from IFI27, IFI53, IFI44, IFI44L, IFI6, IFIT1, IFIT5, IFITM1, IFITM4P etc.) shared by SLE/APS and SLE groups.
Grenn et al., 2017 [20]	68 w/PAPS (26 from an independent cohort)	Microarray study and quantitative PCR for IFIT1, IFI44 and PRKR and IFN-α serum testing	Upregulation of IFN-α in PAPS serum and of type I IFN-inducible genes IFIT1, IFI44, PRKR vs controls ($p = 0.013$, $p = 0.025$ and $p < 0.001$, respectively) IFN-I-related hindering of EPC/CACs maturation, reversible by IFN-I blockade ($p < 0.001$). Relatively higher IFIT1 ($p = 0.021$), IFI44 ($p = 0.041$) and PRKR ($p = 0.42$) expression in PAPS w/IgG *anti*-β_2GPI positivity vs PAPS without *anti*-β_2GPI.
van den Hoogen et al., 2016 [21]	24 w/PAPS, 47 w/SLE, 28 w/SLE/APS and 24 healthy controls	Quantitative PCR for 11 IFN-I inducible genes	Type I IFN signature (defined as scores exceeding the 95th percentile of healthy controls) was present in 46% of patients with PAPS. Higher IFN-I scores negatively correlated with classical monocytes ($r = -0.47$, $p < 0.001$), positively correlated with intermediate ($r = 0.47$, $p < 0.001$) and non-classical monocytes ($r = 0.39$, $p = 0.006$). Lower IFN scores w/hydroxychloroquine and statin administration ($p < 0.05$).
Knight et al., 2017 [22]	9 w/PAPS and 9 healthy controls	Quantitative PCR for several IFN-regulated genes	Upregulated IFN-regulated genes in PAPS vs controls ($p = 4.0 \times 10^{-27}$) w/IFIT1, IFIT3, IFI6, MX1, HERC5 (8.5-, 5.2-, 4.9-, 4.8-, 4.8-fold increase vs controls, respectively).
van den Hoogen et al., 2018 [23]	10 w/PAPS, 20 w/SLE, 10 w/SLE/APS and 12 healthy controls	Quantitative PCR for IFN scoring and miRNA sequencing in plasmacytoid dendritic cells	Type I IFN levels and TLR-7 ligation inversely correlated w/ miRNA-361-5p, -128-3p and -181-2-3p expression (false discovery rate < 0.05).
Weeding et al., 2018 [24]	12 w/PAPS (10 included in the final analysis) and 12 healthy controls	DNA genome-wide methylation	Hypomethylation of IFN-regulated gene IFI44L promoter was able to differentiate SLE from PAPS (sensitivity: 93.3%, specificity: 80% for methylation fraction 0.329).
Ugolini-Lopes et al., 2019 [25]	53 w/PAPS, a group of nonimmune-mediated thrombophilia patients (positive controls) and 50 healthy controls	Quantitative PCR for 41 type I IFN-inducible genes w/IFN signature resulting from DNAJA1, IFIT5, IFI27, MX1, IFI6 and TYK2	Type I IFN signature in 49% of PAPS patients vs 14% for healthy and 17% for positive controls ($p < 0.0001$). Upregulated IFN-I expression correlated w/earlier-onset PAPS manifestations ($p = 0.023$) and obstetric events ($p = 0.032$). Lower IFN scores w/statin administration ($p = 0.026$).
Palli et al., 2019 [27]	55 w/PAPS, 48 w/SLE, 34 w/SLE/APS and 28 healthy controls	Quantitative PCR for MX1, IFIT1 and IFI44	Type I IFN in PAPS vs controls ($p = 0.028$). Upregulated IFN-I expression correlated positively w/female gender (b-coefficient = 0.49, $p = 0.034$) and moderately high *anti*-β_2GPI positivity (b-coefficient = 0.53, $p = 0.017$) and negatively w/age (b-coefficient = −0.02/year, $p = 0.027$) and hydroxychloroquine administration (b-coefficient = −0.51, $p = 0.027$).
Hisada et al., 2019 [29]	26 w/PAPS, 19 w/SLE/APS and 10 healthy controls	Quantitative PCR w/IFN signature resulting from LY6E, MX1, IFIT1 and IFIT3	Upregulated IFN-I expression correlated w/TLR-7 polymorphisms (SNP rs3853839, GG allele, $p = 0.023$).
van den Hoogen et al., 2018 [30]	43 (initial cohort) and 143 (repeat cohort) w/SLE, SLE/APS, PAPS and healthy controls	Serum levels of galectin-9, CXCL-10 and TNF-RII	Serum levels of galectin-9, CXCL-10 and TNF-RII were elevated among all patient groups ($p < 0.05$) and correlated positively with tissue factor expression and disease activity. Galectin-9 exhibited superior correlation with the IFN score ($r = 0.70$, $p < 0.001$) and was more potent in detecting type I IFN signature (area under the curve: 0.86) than the other proposed biomarkers.

IFN: interferon, IFN-I: interferon type I, APS: antiphospholipid syndrome, PAPS: primary antiphospholipid syndrome, SLE: systemic lupus erythematosus, SLE/APS: systemic lupus erythematosus-associated antiphospholipid syndrome.

genes, in conjunction with amplified expression of cell-cell interaction mediator molecules [22]. IFIT1 expression levels were increased 8.5 times in the cohort of PAPS vs controls, a pattern followed by several other genes with the most notable being IFIT3, IFI6, MX1 and HERC5 (5.2-, 4.9-, 4.8- and 4.8-fold increase vs controls, respectively). Members of the toll-like receptor and leucocyte immunoglobulin-like receptor families were also overexpressed in the patient group, adding to the pro-inflammatory phenotype of neutrophils in PAPS. Further investigation of surface molecules in circulating neutrophils indicated the prothrombotic role of P-selectin glycoprotein ligand-1 (PSGL-1), a factor involved in leucocyte transposition.

Based on the postulation of the pivotal role of dendritic cells as major sources of IFN expression in PAPS and SLE, van den Hoogen et al. examined the miRNA expression profile of pDCs in 40 patients (20 with

SLE, 10 with SLE/APS and 10 with PAPS) and 12 healthy controls [23]. Despite the uniformity of miRNA profiles among all patient cohorts, most of the noncoding molecules selected as candidate differential markers for disease were markedly under-expressed when compared to the control group. While the genes involved in miRNA production and transport were not differentially expressed between patients and controls, pDCs activation through administration of a TLR-7 agonist resulted in prompt miRNA downregulation. Stratification of patients into high- and low-IFN expression groups allowed for further correlation between miRNA levels and type I IFN signature. Namely, miRNA-361-5p, -128-3p and -181-2-3p levels were inversely related with IFN type I (IFN–I) scores ($r = -0.44$, -0.45 and -0.48, respectively, $p < 0.001$). The interplay between TLR-7-related pDC activity, miRNA levels fluctuation and IFN production provides insight on many possible disease-modifying and monitoring targets for individuals with both APS and SLE.

In a genome-wide DNA methylation analysis performed on extracted neutrophil DNA from patients with PAPS, SLE and healthy controls, Weeding et al. observed that none of the differentially methylated sites in PAPS were differentially methylated in SLE neutrophils, and that although there was demethylation of IFN signature in SLE this was not detected in PAPS [24]. Hypomethylation within a single probe in the interferon induced protein 44 like IFI44L promoter yielded a sensitivity of 93.3% and a specificity of 80% (for a methylation fraction of 0.329) for the differentiation of PAPS and SLE, while the differential methylation profile of PAPS compared to healthy controls was relatively modest ($p = 0.05$). Interestingly, an association was noted between differential methylation in PAPS and genetic regions regulating pregnancy such as ETS1 and EMP2, both implicated in trophoblast differentiation and migration that could contribute to pregnancy complications in APS.

Ugolini-Lopes et al. attempted to further elucidate the IFN expression patterns in PBMCs of patients with PAPS [25] excluding those with specific SLE autoantibodies. Gene analysis by real-time quantitative PCR was performed in patients with PAPS patients, age-matched controls, and nonimmune-mediated thrombophilia (NIMT) individuals treated with vitamin K antagonists. Upregulated type I IFN expression was detected in 49% of PAPS patients compared with 17% of NIMT individuals and 14% of healthy controls ($p < 0.0001$). Upregulated IFN expression was defined as a composite z-score of 6 genes (DNAJA1, IFI27, IFI6, IFIT5, MX1 and TYK2) with cutoff values defined by a ROC curve for optimal sensitivity and specificity, while a total of 11 genes were found to be markedly expressed in the PAPS cohort. Further subgroup analysis showed a correlation of altered IFN expression with younger age ($p = 0.023$) and preeclampsia ($p = 0.032$). The latter is also supported by previous studies reporting higher IFNα levels in SLE patients with preeclampsia, a complication attributed to relationship between IFN-α and angiogenic imbalance [26]. Additionally, IFN expression was related solely to statin use ($p = 0.026$), with hydroxychloroquine (HCQ) exerting a neutral effect, a finding attributed to the fact that the majority of patients were treated with HCQ.

In a study from our group [27], the expression patterns of three IFN-inducible genes (MX-1, IFIT-1 and IFI-44) were measured in 34 patients with SLE/APS, 48 with SLE and 55 with PAPS, with 35% of the latter showing low-titer positivity for ANA and no positivity for SLE-specific antibodies. In accordance with the results by van Hoogen et al. [21], SLE/APS and SLE patients had the highest IFN scores, with slight superiority for the SLE/APS group, while all groups exhibited higher gene expression than healthy controls. IFN-I signature in the PAPS group was correlated with *anti*-β_2GPI positivity while a concurrent trend with triple aPL positivity was observed, an interesting finding given that there are conflicting results among different studies as far as the association between aPL and IFN-I score [21,25]. The notion that *anti*-β_2GPI are functionally related to the IFN-I pathway is supported by the similar results by Grenn et al. [20] and the fact that these antibodies have been shown to induce IFN-α production in vitro [28]. Multivariate analysis yielded a positive correlation between IFN-I score and female gender (beta coefficients = 0.49, $p = 0.034$), along with a negative correlation with age and hydroxychloroquine treatment (beta coefficients = -0.02 per year, $p = 0.027$ and -0.51, $p = 0.027$, respectively).

Hisada et al. performed T- and B-cell subset analysis in conjunction with analysis of TLR-7 and type I IFN-regulated genes in PBMCs of 26 patients with PAPS, 19 with SLE/APS and 10 healthy controls [29]. Carriers of TLR-7 single nucleotide polymorphism (SNP) rs3853839 from the PAPS population presented significantly higher type I FN scores ($p = 0.023$) calculated based on the mRNA expression of LYE6, MX1, IFIT1 and IFIT3. The risk allele of TLR-7 SNP was also correlated with increased plasmablast ($CD3^-$, $CD19^+$, $CD20^-$, CD27++, CD38++) and decreased memory B-cell populations, supporting the hypothesis that TLR7 expression induced by aPL is associated with plasmablast differentiation via IFN signature.

Given the limitations in gene expression quantification and subsequent calculation of IFN-I signature in routine clinical practice, the use of IFN gene status biomarkers has been predicated. To this end, three biomarkers correlating with disease activity in patients with SLE, SLE/APS and PAPS ($n = 43$ at identification, $n = 148$ at replication step) were selected by van den Hoogen et al. [30]. Elevated levels of galectin-9, tumor necrosis factor receptor type II (TNF-RII) and C-X-C motif chemokine 10 (CXCL-10) reflected amplified IFN-I expression in all patient groups. Galectin-9 exhibited the most prevalent correlation with elevated IFN score for both SLE and APS ($r = 0.70$, $p < 0.001$ vs $r = 0.52$, $p < 0.001$ and $r = 0.46$, $p < 0.001$ for CXCL-10 and TNF-RII, respectively), supporting its role as a potent biomarker for indirect quantification of IFN-I signature and disease activity. Notably, treatment with leflunomide/hydroxychloroquine and disease activity attenuation with subsequent changes in markers of IFN activity in a randomized controlled trial of patients with primary Sjögren's syndrome was mirrored by a decline of galectin-9 levels, supporting their possible additional use as a biomarker of IFN expression and changes in disease activity in patients with other autoimmune diseases [31]. Despite the notable results, the aforementioned biomarker should be implemented in clinical practice with caution, since viral diseases can induce fluctuation of its levels, mimicking flares of autoimmune disease activity.

3. Type I interferon signature in primary antiphospholipid syndrome – therapeutic implications

As the pathogenetic mechanisms of APS are gradually being elucidated, involving inflammatory along with classic thrombotic mechanisms, several novel therapeutic approaches have emerged. HCQ, statins, abciximab, defibrotide, peptides, B-cell depletion and inhibition of mechanistic target of rapamycin (mTOR), or complement are some of the potential novel treatment options currently being investigated [32,33]. Given the recently recognized role of type I IFN gene expression in APS pathogenesis, its implementation in potential new treatments could include a direct targeting of IFN expression by using highly-specific *anti*-IFN antibodies or therapeutic approaches targeting other parts of the type I IFN production and signaling pathway (Fig. 1).

3.1. Hydroxychloroquine (HCQ)

HCQ, an alkalinizing agent with high affinity for lysosomal accumulation has a combination of anti-inflammatory, immunoregulatory and metabolic effects [11]. An additional antithrombotic role of HCQ has been demonstrated in experimental and clinical studies. In vitro and animal APS models showed that HCQ can reverse aPL mediated platelet activation, the binding of aPL-β2GPI complexes to phospholipid bilayers, and the activation of TLR3, TLR7, and TLR9 receptors [34–36]. In a previous study of SLE patients including aPL-stratified data, we showed that the duration of hydroxychloroquine played a protective role against thrombosis in both aPL-positive (HR per month 0.99,

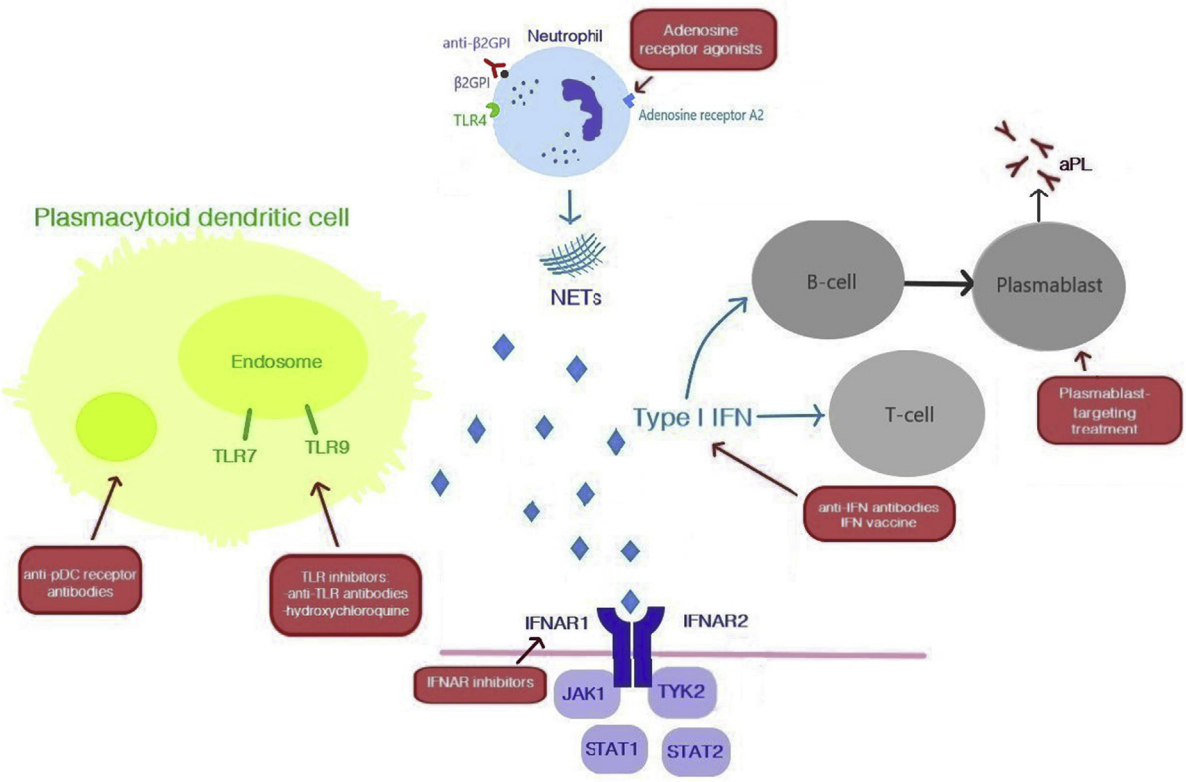

Fig. 1. Potential therapeutic approaches targeting type I IFN production and signaling pathway components in antiphospholipid syndrome.

P = 0.05) and aPL-negative patients (HR per month 0.98, P = 0.04) [37]. Other clinical studies have also shown its thromboprophylactic effect in asymptomatic aPL carriers [38,39] as well as in patients with PAPS and prior history of thrombosis [40] or obstetric complications [41].

HCQ has been shown to act as a TLR-7 and TLR-9 inhibitor by interfering with endosomal interaction of nucleic acids derived from apoptotic debris and immune complexes in SLE, hindering the downstream signaling leading to IFN-α production by pDCs [42]. Previous studies have also shown that HCQ use can reduce the levels of circulating Type I IFN [21,27]. Furthermore, owing to its alkalinizing properties, HCQ impairs lysosomal-induced autophagy, a process based on acidic degradation of cellular components, and subsequent antigenic presentation to antibody-producing cells. Concerning patients with APS, HCQ treatment has been associated with reduced IFN-I scores in patients with PAPS as indicated by the study of van den Hoogen et al. [21]. Consistent results were described in the study of Palli et al., where type I IFN expression was negatively correlated with HCQ use (b-coefficient = −0.51, p = 0.027), a phenomenon attributed to a combination of TLR inhibition and neutrophil swarming and extracellular trap formation impairment, with the latter being an important facilitator of thrombotic events occurrence [27]. Ugolini-Lopes et al. reported no correlation between HCQ administration and IFN-I signature, a result attributed to the fact that the majority of the study sample was receiving HCQ (58%), not allowing for satisfactory subgroup analysis [25]. Ongoing trials are currently investigating the role of HCQ for the secondary prevention of thrombotic and obstetric complications in PAPS [43].

3.2. Anti-IFN treatments

As the evidence concerning the role of type I IFN signature in the pathogenesis of APS is continually increasing, targeting of type I IFN could be considered as a viable therapeutic option. In SLE, therapeutic approaches included direct IFN-α or IFN receptor targeting and a therapeutic vaccine composed of IFNα2b in combination with a carrier protein, named IFN-α-kinoid (IFN–K) [44].

Currently, three *anti*–IFN–α monoclonal antibodies have been tested in clinical trials of SLE patients. Rontalizumab, an IgG1 humanized monoclonal *anti*–IFN–α monoclonal antibody was tested in a phase I and phase II trials with limited clinical efficacy [45–47]. Sifalimumab, another *anti*–IFN–α monoclonal antibody tested in two small phase I trials induced an attenuation of type I IFN signature and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score [48–50]. Larger sample trials of both agents did not detect a larger difference with the control groups probably due to the involvement of a wider group of type I IFN family members or also of type II or III IFNs [46]. An IgG4 *anti*–IFN–α antibody named AGS-009 was tested in a phase I trial, inducing a dose-dependent decrease of IFN-α scores [51].

Anifrolumab (formerly MEDI-546), a fully human monoclonal antibody that binds to subunit 1 of type I interferon receptor (IFNAR1) acts in a wider spectrum of type I IFNs including IFN-α, IFN-β and IFN-ω, producing a dose-dependent reduction of IFN expression and clinical remission as defined by SRI response measurement in phase IIb trial, effects more prominent in patients with high baseline IFN-I levels. Although the TULIP 1 phase III trial did not reach its primary endpoint, the results of ongoing long-term extension phase III trials are pending [47].

In a different approach, active immunization with IFN α Kinoid (IFN–K), a therapeutic vaccine consisting of a carrier protein coupled with IFNα2b, was associated with higher *anti*-IFNα titers in IFN signature-positive SLE patients and with a significantly reduced expression of IFN-induced genes in a phase I/II dose-escalation study [52].

3.3. Therapeutic approaches targeting other type I IFN production and signaling pathway components

Given the pivotal role of pDCs in IFN expression in PAPS, antibodies

directly targeting cell type and/or components of the TLR-mediated innate inflammatory response could be of use in reducing IFN scores in this group of patients. Such an approach is supported by the fact that pDC-targeting therapies have emerged for autoimmune diseases and the interdependence between TLR signaling and IFN expression in PAPS described in several patient cohorts [21,23,28]. BDCA2 is a pDC-specific receptor whose binding results in hindering of IFN-response gene expression. BIIB059, a humanized monoclonal antibody that binds a pDC-specific receptor, the dendritic cell antigen 2, is currently being evaluated for the treatment of SLE [53].

The potential of *anti*-TLR-7 and *anti*-TLR-9 antibody treatment is also of high importance given the role of the two receptors as initiators in the cascade of pDC-induced IFN expression (Fig. 1). While TLR-7 and TLR-9 are localized intracellularly in lysosomal surface deeming their targeting difficult, researchers have suggested the concomitant existence of cell-surface receptor molecules, allowing for binding of receptor-specific antibodies [54]. Rather than direct inhibition of the receptors, impeding the action of accessory molecules facilitating their activation could lead to similar *in vivo* effects. An example of such molecules is miRNAs acting as epigenetic modifiers of mRNA transcripts modulating the TLR-mediated IFN production cascade. MiRNA-155 has been identified as an inflammatory response driver in rheumatoid arthritis patients, with two counteracting strands both promoting (miRNA-155-5p) and halting (miRNA-155-3p) type I IFN production from pDCs, regulated by the TLR family [55]. As it was shown by van den Hoogen et al., other miRNAS related to pDC activation are miRNA-361-5p, -128-3p and −181a-2-3p with an inverse relationship with IFN-scores, harboring immunomodulatory potential [23].

Considering that circulating nucleic acid molecules can activate IFN signaling mediated through TLR pattern recognition, the use of enzymes capable of mitigating these inflammatory signals could aid in attenuating IFN expression. In a multicenter phase 1b, randomized placebo-controlled study, RSLV-132, an RNase-Fc fusion protein, was tested in SLE patients but without any notable effect on IFN signature, a finding attributed to small sample size, variation among patients, and the short duration of treatment [56].

NETosis, a regulated form of neutrophil cell death characterized by the secretion of networks of extracellular fibers composed of DNA (NETs), is a major inducer of type I IFN production. Previous studies have shown the role of neutrophils and NETosis in APS suggesting that they might represent emerging therapeutic targets. Neutrophils display β2GPI on their surface, and can be triggered to release NETs in TLR4-dependent fashion by exposure to *anti*-β2GPI [11] (Fig. 1). It has been recently shown that adenosine A2A receptor agonism protects against NETosis in vitro and venous thrombosis in APS mice [57] and the effect of agents with adenosine-amplifying properties such as dipyridamole, dilazep and defibrotide should be further investigated in patients with APS.

In addition, a pathway associated with type I IFN upregulation is the recently observed TLR7-mediated plasmablast differentiation via IFN signature [29]. Given that plasmablasts represent a major source of aPL production, targeting plasmablasts might represent a novel therapeutic approach in APS.

Well-designed studies are needed to further assess the immunomodulatory potential and clinical outcomes of the aforementioned treatment targets.

4. Conclusions

Several studies have demonstrated a type I IFN signature in patients with APS and its association with several demographic, clinical and laboratory characteristics of patients including age, gender, eclampsia, *anti*-β2GPI antibodies as well as a negative relationship with HCQ and/or statin use. The upregulated expression of type I IFN-inducible genes in patients with SLE and PAPS is considered to be an important driver of both clinical and subclinical disease, deeming the blockade of related pathways a prime therapeutic target. While several IFN targeting therapies have been described in SLE, there is lack of data about their safety and efficacy in patients with PAPS, underlining the need for well-designed randomized controlled trials. Another interesting prospect concerning the implementation of type I IFN gene expression in management strategies of patients with APS would be as an index of treatment response or disease severity marker. Correlation of high IFN scores to a more aggressive disease course could allow for a prompt treatment in high-risk patients, facilitating thrombosis prevention. Lastly, shortcomings such as the difficulty of implementing type I IFN signature in routine clinical practice could be overcome by the use of easily measured biomarkers that can accurately reflect aberrant IFN-I gene expression.

Authorship

All authors have contributed to the conception and design, drafting of the article and the final approval of the version to be submitted.

Conflicts of interest

The authors have no competing interests to declare.

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Declarations of interest

None.

Appendix A. Supplementary data

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References

[1] S. Miyakis, M.D. Lockshin, T. Atsumi, D.W. Branch, R.L. Brey, R. Cervera, R.H.W.M. Derksen, P.G. De Groot, T. Koike, P.L. Meroni, G. Reber, Y. Shoenfeld, A. Tincani, P.G. Vlachoyiannopoulos, S.A. Krilis, International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS), J. Thromb. Haemost. 4 (2006) 295–306, https://doi.org/10.1111/j.1538-7836.2006.01753.x.

[2] M.M. Abreu, A. Danowski, D.G. Wahl, M.-C. Amigo, M. Tektonidou, M.S. Pacheco, N. Fleming, V. Domingues, S. Sciascia, J.O. Lyra, M. Petri, M. Khamashta, R.A. Levy, The relevance of "non-criteria" clinical manifestations of antiphospholipid syndrome: 14th international congress on antiphospholipid antibodies technical task force report on antiphospholipid syndrome clinical features, Autoimmun. Rev. 14 (2015) 401–414, https://doi.org/10.1016/j.autrev.2015.01.002.

[3] C. Kampolis, M. Tektonidou, I. Moyssakis, G.E. Tzelepis, H. Moutsopoulos, P.G. Vlachoyiannopoulos, Evolution of cardiac dysfunction in patients with antiphospholipid antibodies and/or antiphospholipid syndrome: a 10-year follow-up study, Semin. Arthritis Rheum. 43 (2014) 558–565, https://doi.org/10.1016/j.semarthrit.2013.07.016.

[4] M.G. Tektonidou, F. Sotsiou, L. Nakopoulou, P.G. Vlachoyiannopoulos, H.M. Moutsopoulos, Antiphospholipid syndrome nephropathy in patients with systemic lupus erythematosus and antiphospholipid antibodies: prevalence, clinical associations, and long-term outcome, Arthritis Rheum. 50 (2004) 2569–2579, https://doi.org/10.1002/art.20433.

[5] M.G. Tektonidou, N. Varsou, G. Kotoulas, A. Antoniou, H.M. Moutsopoulos, Cognitive deficits in patients with antiphospholipid syndrome, Arch. Intern. Med. 166 (2006) 2278, https://doi.org/10.1001/archinte.166.20.2278.

[6] B. Giannakopoulos, S.A. Krilis, The pathogenesis of the antiphospholipid syndrome, N. Engl. J. Med. 368 (2013) 1033–1044, https://doi.org/10.1056/NEJMra1112830.

[7] C.B. Chighizola, E. Raschi, M.O. Borghi, P.L. Meroni, Update on the pathogenesis and treatment of the antiphospholipid syndrome, Curr. Opin. Rheumatol. 27 (2015) 476–482, https://doi.org/10.1097/BOR.0000000000000200.

[8] M.D. Patsouras, P. Karagianni, P. Kogionou, P.G. Vlachoyiannopoulos, Differential CpG methylation of the promoter of interleukin 8 and the first intron of tissue factor in Antiphospholipid syndrome, J. Autoimmun. (2019), https://doi.org/10.1016/J.JAUT.2019.05.001.

[9] R.T. Urbanus, R.H.W.M. Derksen, P.G. de Groot, Platelets and the antiphospholipid syndrome, Lupus 17 (2008) 888–894, https://doi.org/10.1177/0961203308096344.

[10] S. Chaturvedi, R.A. Brodsky, K.R. McCrae, Complement in the pathophysiology of the antiphospholipid syndrome, Front. Immunol. 10 (2019) 449, https://doi.org/10.3389/fimmu.2019.00449.

[11] S. Yalavarthi, et al., Release of neutrophil extracellular traps by neutrophils stimulated with antiphospholipid antibodies: a newly identified mechanism of thrombosis in the antiphospholipid syndrome, Arthritis Rheum. 67 (2015) 2990–3003.

[12] J.J. Hooks, H.M. Moutsopoulos, S.A. Geis, N.I. Stahl, J.L. Decker, A.L. Notkins, Immune interferon in the circulation of patients with autoimmune disease, N. Engl. J. Med. 301 (1979) 5–8, https://doi.org/10.1056/NEJM197907053010102.

[13] M.K. Crow, Type I interferon in the pathogenesis of lupus, J. Immunol. 192 (2014) 5459–5468, https://doi.org/10.4049/jimmunol.1002795.

[14] A. Nezos, F. Gravani, A. Tassidou, E.K. Kapsogeorgou, M. Voulgarelis, M. Koutsilieris, M.K. Crow, C.P. Mavragani, Type I and II interferon signatures in Sjogren's syndrome pathogenesis: contributions in distinct clinical phenotypes and Sjogren's related lymphomagenesis, J. Autoimmun. 63 (2015) 47–58, https://doi.org/10.1016/j.jaut.2015.07.002.

[15] A. Szabo, E. Rajnavolgyi, Collaboration of Toll-like and RIG-I-like receptors in human dendritic cells: tRIGgering antiviral innate immune responses, Afr. J. Clin. Exp. Immunol. 2 (2013) 195–207.

[16] B.D. Bell, M. Kitajima, R.P. Larson, T.A. Stoklasek, K. Dang, K. Sakamoto, K.-U. Wagner, D.H. Kaplan, B. Reizis, L. Hennighausen, S.F. Ziegler, The transcription factor STAT5 is critical in dendritic cells for the development of TH2 but not TH1 responses, Nat. Immunol. 14 (2013) 364–371, https://doi.org/10.1038/ni.2541.

[17] I. Bernales, A. Fullaondo, M.J. Marín-Vidalled, E. Ucar, V. Martínez-Taboada, M. López-Hoyos, A.M. Zubiaga, Innate immune response gene expression profiles characterize primary antiphospholipid syndrome, Genes Immun. 9 (2008) 38–46, https://doi.org/10.1038/sj.gene.6364443.

[18] C. Perez-Sanchez, N. Barbarroja, S. Messineo, P. Ruiz-Limon, A. Rodriguez-Ariza, Y. Jimenez-Gomez, M.A. Khamashta, E. Collantes-Estevez, M.J. Cuadrado, M.A. Aguirre, C. Lopez-Pedrera, 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. 74 (2015) 1441–1449, https://doi.org/10.1136/ANNRHEUMDIS-2013-204600.

[19] C. Perez-Sanchez, P. Ruiz-Limon, M.A. Aguirre, M.L. Bertolaccini, M.A. Khamashta, A. Rodriguez-Ariza, P. Segui, E. Collantes-Estevez, N. Barbarroja, H. Khraiwesh, J.A. Gonzalez-Reyes, J.M. Villalba, F. Velasco, M.J. Cuadrado, C. Lopez-Pedrera, Mitochondrial dysfunction in antiphospholipid syndrome: implications in the pathogenesis of the disease and effects of coenzyme Q10 treatment, Blood 119 (2012) 5859–5870, https://doi.org/10.1182/blood-2011-12-400986.

[20] R.C. Grenn, S. Yalavarthi, A.A. Gandhi, N.M. Kazzaz, C. Núñez-Álvarez, D. Hernández-Ramírez, A.R. Cabral, W.J. McCune, P.L. Bockenstedt, J.S. Knight, Endothelial progenitor dysfunction associates with a type I interferon signature in primary antiphospholipid syndrome, Ann. Rheum. Dis. 76 (2017) 450–457, https://doi.org/10.1136/annrheumdis-2016-209442. 5.

[21] L.L. van den Hoogen, R.D.E. Fritsch-Stork, M.A. Versnel, R.H.W. Derksen, J.A.G. van Roon, T.R.D. Radstake, Monocyte type I interferon signature in antiphospholipid syndrome is related to proinflammatory monocyte subsets, hydroxychloroquine and statin use, Ann. Rheum. Dis. 75 (2016), https://doi.org/10.1136/annrheumdis-2016-21048 e81–e81.

[22] J.S. Knight, H. Meng, P. Coit, S. Yalavarthi, G. Sule, A.A. Gandhi, R.C. Grenn, L.F. Mazza, R.A. Ali, P. Renauer, J.D. Wren, P.L. Bockenstedt, H. Wang, D.T. Eitzman, A.H. Sawalha, Activated signature of antiphospholipid syndrome neutrophils reveals potential therapeutic target, JCI Insight 2 (2017), https://doi.org/10.1172/jci.insight.93897.

[23] L.L. van den Hoogen, M. Rossato, A.P. Lopes, A. Pandit, C.P.J. Bekker, R.D.E. Fritsch-Stork, J.A.G. van Roon, T.R.D.J. Radstake, microRNA down-regulation in plasmacytoid dendritic cells in interferon-positive systemic lupus erythematosus and antiphospholipid syndrome, Rheumatology 57 (2018) 1669–1674, https://doi.org/10.1093/rheumatology/key159.

[24] E. Weeding, P. Coit, S. Yalavarthi, M.J. Kaplan, J.S. Knight, A.H. Sawalha, Genome-wide DNA methylation analysis in primary antiphospholipid syndrome neutrophils, Clin. Immunol. 196 (2018) 110–116, https://doi.org/10.1016/j.clim.2018.11.011.

[25] M.R. Ugolini-Lopes, G.T. Torrezan, A.P.R. Gândara, E.H.R. Olivieri, I.S. Nascimento, E. Okazaki, E. Bonfá, D.M. Carraro, D.C.O. de Andrade, Enhanced type I interferon gene signature in primary antiphospholipid syndrome: association with earlier disease onset and preeclampsia, Autoimmun. Rev. 18 (2019) 393–398, https://doi.org/10.1016/J.AUTREV.2018.11.004.

[26] D. Andrade, M. Kim, L.P. Blanco, S.A. Karumanchi, G.C. Koo, P. Redecha, K. Kirou, A.M. Alvarez, M.J. Mulla, M.K. Crow, V.M. Abrahams, M.J. Kaplan, J.E. Salmon, Interferon-α and angiogenic dysregulation in pregnant lupus patients who develop preeclampsia, Arthritis Rheum. 67 (2015) 977–987, https://doi.org/10.1002/art.39029.

[27] E. Palli, E. Kravvariti, M.G. Tektonidou, Type I interferon signature in primary antiphospholipid syndrome: clinical and laboratory associations, Front. Immunol. 10 (2019) 487, https://doi.org/10.3389/fimmu.2019.00487.

[28] N. Prinz, N. Clemens, D. Strand, I. Putz, M. Lorenz, A. Daiber, P. Stein, A. Degreif, M. Radsak, H. Schild, S. Bauer, P. von Landenberg, K.J. Lackner, Antiphospholipid antibodies induce translocation of TLR7 and TLR8 to the endosome in human monocytes and plasmacytoid dendritic cells, Blood 118 (2011) 2322–2332, https://doi.org/10.1182/blood-2011-01-330639.

[29] R. Hisada, M. Kato, E. Sugawara, M. Kanda, Y. Fujieda, K. Oku, T. Bohgaki, O. Amengual, T. Horita, S. Yasuda, T. Atsumi, Circulating plasmablasts contribute to antiphospholipid antibody production, associated with type I interferon upregulation, J. Thromb. Haemost. (2019), https://doi.org/10.1111/jth.14427 jth.14427.

[30] L.L. van den Hoogen, J.A.G. van Roon, J.S. Mertens, J. Wienke, A.P. Lopes, W. de Jager, M. Rossato, A. Pandit, C.G.K. Wichers, F. van Wijk, R.D.E. Fritsch-Stork, T.R.D.J. Radstake, Galectin-9 is an easy to measure biomarker for the interferon signature in systemic lupus erythematosus and antiphospholipid syndrome, Ann. Rheum. Dis. 77 (2018) 1810–1814, https://doi.org/10.1136/annrheumdis-2018-213497.

[31] T.R.D.J. Radstake, E.H.M. van der Heijden, F.M. Moret, M.R. Hillen, A.P. Lopes, T. Rosenberg, N. Janssen, A.A. Kruize, J.A.G. van Roon, Clinical efficacy of leflunomide/hydroxychloroquine combination therapy in patients with primary Sjogren's syndrome: results of a placebo-controlled double-blind randomized clinical trial [abstract], Arthritis Rheum. 70 (suppl 10) (2018).

[32] D. Andrade, M. Tektonidou, Emerging therapies in antiphospholipid syndrome, Curr. Rheumatol. Rep. 18 (2016) 22, https://doi.org/10.1007/s11926-016-0566-z.

[33] M.G. Tektonidou, L. Andreoli, M. Limper, Z. Amoura, R. Cervera, N. Costedoat-Chalumeau, M.J. Cuadrado, T. Dörner, R. Ferrer-Oliveras, K. Hambly, M.A. Khamashta, J. King, F. Marchiori, P.L. Meroni, M. Mosca, V. Pengo, L. Raio, G. Ruiz-Irastorza, Y. Shoenfeld, L. Stojanovich, E. Svenungsson, D. Wahl, A. Tincani, M.M. Ward, EULAR recommendations for the management of antiphospholipid syndrome in adults, Ann. Rheum. Dis. (2019), https://doi.org/10.1136/ANNRHEUMDIS-2019-215213.

[34] J.H. Rand, X.-X. Wu, A.S. Quinn, P.P. Chen, J.J. Hathcock, D.J. Taatjes, Hydroxychloroquine directly reduces the binding of antiphospholipid antibody- 2-glycoprotein I complexes to phospholipid bilayers, Blood 112 (2008) 1687–1695, https://doi.org/10.1182/blood-2008-03-144204.

[35] J.H. Rand, X.-X. Wu, A.S. Quinn, A.W. Ashton, P.P. Chen, J.J. Hathcock, H.A.M. Andree, D.J. Taatjes, Hydroxychloroquine protects the annexin A5 anticoagulant shield from disruption by antiphospholipid antibodies: evidence for a novel effect for an old antimalarial drug, Blood 115 (2010) 2292–2299, https://doi.org/10.1182/blood-2009-04-213520.

[36] A. Kužnik, M. Benčina, U. Švajger, M. Jeras, B. Rozman, R. Jerala, Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines, J. Immunol. 186 (2011) 4794–4804, https://doi.org/10.4049/jimmunol.1000702.

[37] M.G. Tektonidou, K. Laskari, D.B. Panagiotakos, H.M. Moutsopoulos, Risk factors for thrombosis and primary thrombosis prevention in patients with systemic lupus erythematosus with or without antiphospholipid antibodies, Arthritis Care Res. 61 (2008) 29–36, https://doi.org/10.1002/art.24232.

[38] D. Erkan, Y. Yazici, M.G. Peterson, L. Sammaritano, M.D. Lockshin, A cross-sectional study of clinical thrombotic risk factors and preventive treatments in antiphospholipid syndrome, Rheumatology 41 (2002) 924–929, https://doi.org/10.1093/rheumatology/41.8.924.

[39] D. Erkan, M. Lockshin, APS ACTION members, APS ACTION – AntiPhospholipid syndrome alliance for clinical trials and InternatiOnal networking, Lupus 21 (2012) 695–698, https://doi.org/10.1177/0961203312437810.

[40] A. Schmidt-Tanguy, J. Voswinkel, D. Henrion, J. Subra, L. Loufrani, V. Rohmer, N. Ifrah, C. Belizna, Anti-thrombotic effects of hydroxychloroquine in primary antiphospholipid syndrome patients, J. Thromb. Haemost. 11 (2013) 1927–1929, https://doi.org/10.1111/jth.12363.

[41] A. Mekinian, M.G. Lazzaroni, A. Kuzenko, J. Alijotas-Reig, A. Ruffatti, P. Levy, V. Canti, K. Bremme, H. Bezanahary, T. Bertero, R. Dhote, F. Maurier, L. Andreoli, A. Benbara, A. Tigazin, L. Carbillon, P. Nicaise-Roland, A. Tincani, O. Fain, SNFMI and the European Forum on Antiphospholipid Antibodies, the efficacy of hydroxychloroquine for obstetrical outcome in anti-phospholipid syndrome: data from a European multicenter retrospective study, Autoimmun. Rev. 14 (2015) 498–502, https://doi.org/10.1016/j.autrev.2015.01.012.

[42] K. Sacre, L.A. Criswell, J.M. McCune, Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus erythematosus, Arthritis Res. Ther. 14 (2012) R155, https://doi.org/10.1186/ar3895.

[43] C. Belizna, F. Pregnolato, S. Abad, J. Alijotas-Reig, H. Amital, Z. Amoura, L. Andreoli, E. Andres, A. Aouba, S. Apras Bilgen, L. Arnaud, B. Bienvenu, V. Bitsadze, P. Blanco, M. Blank, M.O. Borghi, A. Caligaro, E. Candrea, V. Canti, L. Chiche, J.M. Chretien, J.W. Cohen Tervaert, L. Damian, T. Delross, E. Dernis, K. Devreese, A. Djokovic, E. Esteve-Valverde, M. Favaro, C. Fassot, R. Ferrer-Oliveras, A. Godon, M. Hamidou, M. Hasan, D. Henrion, B. Imbert, P.Y. Jeandel, P. Jeannin, P. Jego, N. Jourde-Chiche, J. Khizroeva, O. Lambotte, C. Landron, J.O. Latino, E. Lazaro, K. de Leeuw, T. Le Gallou, L. Kiliç, M. Limper, L. Loufrani, R. Lubin, N. Magy-Bertrand, G. Mahe, A. Makatsariya, T. Martin, C. Muchardt, G. Nagy, L. Omarjee, P. Van Paasen, G. Pernod, F. Perrinet, G. Pïres Rosa, M.A. Pistorius, A. Ruffatti, F. Said, P. Saulnier, D. Sene, L. Sentilhes, O. Shovman, J. Sibilia, C. Sinescu, N. Stanisavljevic, L. Stojanovich, L.S. Tam, A. Tincani, F. Tollis, S. Udry, M.N. Ungeheuer, M. Versini, R. Cervera, P.L. Meroni, HIBISCUS: hydroxychloroquine for the secondary prevention of thrombotic and obstetrical events in primary antiphospholipid syndrome, Autoimmun. Rev. 17 (2018) 1153–1168, https://doi.org/10.1016/j.autrev.2018.05.012.

[44] M.K. Crow, L. Ronnblom, Type I interferons in host defence and inflammatory diseases, Lupus Sci. Med. 6 (2019) e000336, , https://doi.org/10.1136/lupus-2019-000336.

[45] J.M. McBride, J. Jiang, A.R. Abbas, A. Morimoto, J. Li, R. Maciuca, M. Townsend, D.J. Wallace, W.P. Kennedy, J. Drappa, Safety and pharmacodynamics of rontalizumab in patients with systemic lupus erythematosus: results of a phase I, placebo-controlled, double-blind, dose-escalation study, Arthritis Rheum. 64 (2012) 3666–3676, https://doi.org/10.1002/art.34632.

[46] K.C. Kalunian, J.T. Merrill, R. Maciuca, J.M. McBride, M.J. Townsend, X. Wei, J.C. Davis, W.P. Kennedy, A Phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon-α) in patients with systemic lupus erythematosus (ROSE), Ann. Rheum. Dis. 75 (2016) 196–202, https://doi.org/10.1136/annrheumdis-2014-206090.

[47] R. Felten, F. Scher, F. Sagez, F. Chasset, L. Arnaud, Spotlight on anifrolumab and its potential for the treatment of moderate-to-severe systemic lupus erythematosus: evidence to date, Drug Des. Dev. Ther. 13 (2019) 1535–1543, https://doi.org/10.2147/DDDT.S170969.

[48] J.T. Merrill, D.J. Wallace, M. Petri, K.A. Kirou, Y. Yao, W.I. White, G. Robbie, R. Levin, S.M. Berney, V. Chindalore, N. Olsen, L. Richman, C. Le, B. Jallal, B. White, Lupus Interferon Skin Activity (LISA) Study Investigators, Safety profile and clinical activity of sifalimumab, a fully human anti-interferon monoclonal antibody, in systemic lupus erythematosus: a phase I, multicentre, double-blind randomised study, Ann. Rheum. Dis. 70 (2011) 1905–1913, https://doi.org/10.1136/ard.2010.144485.

[49] M. Khamashta, J.T. Merrill, V.P. Werth, R. Furie, K. Kalunian, G.G. Illei, J. Drappa, L. Wang, W. Greth, CD1067 study investigators, Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study, Ann. Rheum. Dis. 75 (2016) 1909–1916, https://doi.org/10.1136/annrheumdis-2015-208562.

[50] T. Takeuchi, Y. Tanaka, R. Matsumura, K. Saito, M. Yoshimura, K. Amano, T. Atsumi, E. Suematsu, N. Hayashi, L. Wang, R. Tummala, Safety and tolerability of sifalimumab, an anti-interferon-α monoclonal antibody, in Japanese patients with systemic lupus erythematosus: a multicenter, phase 2, open-label study, Mod. Rheumatol. (2019) 1–8, https://doi.org/10.1080/14397595.2019.1583832.

[51] I. Tcherepanova, M. Curtis, M. Sale, F. Miesowicz, C. Nicolette, SAT0193 Results of a randomized placebo controlled phase ia study of AGS-009, a humanized anti-interferon-α monoclonal antibody in subjects with systemic lupus erythematosus, Ann. Rheum. Dis. 71 (2013), https://doi.org/10.1136/annrheumdis-2012-eular.3140 536.3-537.

[52] B.R. Lauwerys, E. Hachulla, F. Spertini, E. Lazaro, C. Jorgensen, X. Mariette, E. Haelterman, G. Grouard-Vogel, B. Fanget, O. Dhellin, P. Vandepapelière, F.A. Houssiau, Down-regulation of interferon signature in systemic lupus erythematosus patients by active immunization with interferon α-kinoid, Arthritis Rheum. 65 (2013) 447–456, https://doi.org/10.1002/art.37785.

[53] A. Pellerin, K. Otero, J.M. Czerkowicz, H.M. Kerns, R.I. Shapiro, A.M. Ranger, K.L. Otipoby, F.R. Taylor, T.O. Cameron, J.L. Viney, D. Rabah, Anti-BDCA2 monoclonal antibody inhibits plasmacytoid dendritic cell activation through Fc-dependent and Fc-independent mechanisms, EMBO Mol. Med. 7 (2015) 464–476, https://doi.org/10.15252/emmm.201404719.

[54] A. Kanno, N. Tanimura, M. Ishizaki, K. Ohko, Y. Motoi, M. Onji, R. Fukui, T. Shimozato, K. Yamamoto, T. Shibata, S. Sano, A. Sugahara-Tobinai, T. Takai, U. Ohto, T. Shimizu, S. Saitoh, K. Miyake, Targeting cell surface TLR7 for therapeutic intervention in autoimmune diseases, Nat. Commun. 6 (2015) 6119, https://doi.org/10.1038/ncomms7119.

[55] S. Alivernini, E. Gremese, C. McSharry, B. Tolusso, G. Ferraccioli, I.B. McInnes, M. Kurowska-Stolarska, MicroRNA-155—at the critical interface of innate and adaptive immunity in arthritis, Front. Immunol. 8 (2018) 1932, https://doi.org/10.3389/fimmu.2017.01932.

[56] D.J. Burge, J. Eisenman, K. Byrnes-Blake, P. Smolak, K. Lau, S.B. Cohen, A.J. Kivitz, R. Levin, R.W. Martin, Y. Sherrer, J.A. Posada, Safety, pharmacokinetics, and pharmacodynamics of RSLV-132, an RNase-Fc fusion protein in systemic lupus erythematosus: a randomized, double-blind, placebo-controlled study, Lupus 26 (2017) 825–834, https://doi.org/10.1177/0961203316678675.

[57] R.A. Ali, A.A. Gandhi, H. Meng, S. Yalavarthi, A.P. Vreede, S.K. Estes, O.R. Palmer, P.L. Bockenstedt, D.J. Pinsky, J.M. Grve, J.A. Diaz, Y. Kanthi, J.S. Knight, Adenosine receptor agonism protects against NETosis and thrombosis in antiphospholipid syndrome, Nat. Commun. 10 (2019) 1916, https://doi.org/10.1038/s41467-019-09801-x.