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EDITORIAL

Interferon signature in systemic autoimmune diseases: what does it mean?

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The first description of the involvement of interferons (IFN) in systemic autoimmune diseases (AID) dates back more than 40 years.[1] IFNs play a crucial role in numbers of immunological pathways involve in AID, such as induction of dendritic cell (mDC), MHC expression, cytokines like BAFF, IL-2, IL-7. The aim of this editorial is not to discuss all these mechanisms linking IFNs to autoimmunity but to discuss the origin and signification of what is called 'the IFN signature' and how measuring it. The diseases in which this signature plays a prominent role are systemic erythematosus lupus (SLE), Sjögren's syndrome (SS),[2 3] inflammatory myositis[4] and scleroderma.[5] The study of this IFN signature is still attracting the interest of research teams around the world, and for good reason: the advent of IFN-targeted therapies could revolutionise the outcome of patients with IFN-mediated diseases. However, behind this so-called IFN signature, there are still many grey areas: what type(s) of IFNs are we talking about? Who are the producers of IFNs? What is the origin of this IFN signature? What tools for assessing the IFN signature? Several techniques have been developed including IFN-inducible gene signatures; IFN-inducible proteins such as MxA, Galectin 9, Siglec1, IP-10; reporter system with WISH cells; dosage of the different subtypes of circulating IFNs. And finally, what are the consequences of this IFN signature in clinical practice, both in the classification of patients with the aim of achieving personalised medicine, and in the management of treatments, particularly at a time when therapies targeting IFNs are being developed?

The so-called signature corresponds to the evidence of an upregulation of transcripts induced by the different IFN subtypes: type I (IFN-α (of which there are 13 subtypes), β, ε, κ and ω), type II (IFN-γ) and type III (IFN-ʎ 1 to 4). However, the limitation of this transcriptomic definition was because until recently it did not allow differentiation between the three families of IFNs and only IFN-γ could be easily measured. The IFN signature is classically assessed by the level of expression of different mRNA induced by IFN. Several combinations of genes have been proposed to calculate IFN score. A five-gene signature (IFI44, IFI44L, IFIT1, IFIT3, MxA) has been proposed first.[6] A four-gene set (IFI27, IFI44, IFI44L and RSAD2) was applied for the analysis of the anti-IFNAR therapeutic antibody—anifrolumab—trials.[7] This score allows just a differentiation into IFN score high and low. Moreover, the transcriptomic overlap between the three types of IFNs, notably between type I and type III, is still a limit.[8] It is the reason why the development of new techniques for the determination of type I IFN such as the single molecule array (Simoa),[9] mesoscale diffusion technique and new methods of analysing the transcriptomic signature have changed the situation Importantly, it became apparent that within a single disease, different IFN profiles could coexist. In SLE, it was shown that the IFN signature was not limited to IFN-α, but involved the gradual activation of three distinct modules underpinned by various IFNs, including type II.[10] In SS, a multiomic study was able to define four clusters of patients.[11] Among them, three seem to be driven by IFNs: cluster C1 corresponds to the strongest IFN type I and type II signature, cluster C3 to an intermediate IFN signature associated with B-cell activation and finally cluster C4 would be mainly linked to IFN type II. More recently, it has been shown that during SS, in the blood, it is IFN-α that drives this IFN signature. The level of circulating IFN-α was also associated with the clinical and biological characteristics of SS patients and with the prognosis, with

an association between the baseline IFN-α level and the development of systemic complications within 5 years.[12] Lastly, comparing the serum dosage of type I and type II IFN with the IFN signature will allow knowing if the high IFN signature is due to a high level of IFN, to an increased activation of pattern recognition receptors, or to an excessive response of IFN-responsive genes to IFN.

The cellular origin of the different IFN subtypes has also been re-evaluated. Initially, the focus was on plasmacytoid dendritic cells (pDCs), which are 'professionals' IFN-α secretors. However, the role of resident cells, keratinocytes, renal tubular cells, salivary gland epithelial cells appears to be important both in the early and late phases of SLE and SS.[13 14] In SLE, it has been shown that the IFN signature in tubular cells is associated with proliferative histology and markers of fibrosis, and IFN signature in keratinocytes is associated with clinical non-response to therapy.[15] In addition, in SLE, the role of neutrophils, via NETosis, has also been demonstrated in inducing IFN signature.[16]

Lastly, a key question is what the origin of this IFN signature is. Genetic, epigenetic and environmental factors may be involved. The role of genetic factors and in particular single nucleotide polymorphisms located on IFN pathways is well established.[17 19] In SLE, more than 100 genetic risk loci have been identified. Half of them are related to IFN pathways.[20] Interestingly, type I and type II are involved. SNP within interferon regulatory factor (IRF) 5 locus has been shown to be associated with SLE and SS.[20 21] Among these SNPs a 5-bp CGGGG indel in the promoter of *IRF5* is associated with a high level of IRF5 mRNA, mainly after viral infection, which in turn correlate with higher IFN signature.[22] SNPs in *STAT4* loci are also associated with both SLE and SS.[13 23] Interestingly, STAT4 is a classical transcription factor of type 2 IFN but an association between STAT4α mRNA level and PKR, IFITM1 and MX1 mRNA levels that are type 1 IFN-induced genes has been described.[24] Epigenetic factors also play a role. Interestingly, epigenetic control seems to override genetic susceptibility. This has been demonstrated with methylation notably in SS where methylation alterations in B cells are more frequent in some specific pathways including IRF genes.[25] The most common pathological situation where type I IFN is increased is viral infections. A viral origin of systemic AID has been looked for decades. But even if some associations with a higher immune response to some viruses (especially Epstein-Barr virus[26]) have been observed in SLE and SS, no causal virus was found. If an exogenous viral infection cannot be found as the cause of this IFN signature, it might be useful to consider the possible role of endogenous viruses. Human endogenous retroviruses (HERVs) represent almost 10% of our genome.[27] They are exposed to epigenetic regulation and have been shown to be differentially expressed in patients with AID including multiple sclerosis or SLE. Differentially expressed HERVs are in close proximity with IFN-induced genes and could modulate their expression.[28]

Besides these viral endogenous sequences, a number of long non-coding RNAs (lncRNAs) are present in our genome, more or less expressed and having a regulatory role by specific interactions with DNA, RNA and proteins.[29] In this issue of RMD, Joachims *et al* focus on the role of long lncRNAs as regulators of the IFN response in SS patients. They assessed blood transcriptome of SS patients looking at RNA-seq and comparing patients with and without anti-SSA/Ro60 autoantibodies. They confirmed that the IFN signature is a characteristic feature of anti-SSA/Ro60+ patients. They assessed transcripts associated with IFN signature and found that 16 lncRNAs were strongly correlated with the IFN signature. Interestingly, through functional experiments, they showed that four antisense lncRNAs (NRIR, OAS123-AS1, MX1-AS1 and GBP5-AS1) were rapidly induced in response to IFN and that their increased expression acted as a massive upregulation signal to induce the expression of mRNAs. This study therefore demonstrates the potential role of these lncRNAs as targets and regulators of the IFN response, which may therefore participate in the amplification of this response during systemic AID.

Thus, our understanding of the origin and the consequences of the IFN signature is progressing. The question arises as to what this means in clinical practice. The development of therapies that target IFN (JAK inhibitors, anifrolumab) is an important step for the management of patients suffering from systemic AID. It was during the studies evaluating anifrolumab in SLE that the use of IFN signature monitoring in patients was developed. By pooling data from the TULIP trials into a post hoc study, it was shown that anifrolumab was more effective in the subgroup of SLE patients with high IFN signature.[30] Of note, the presence of serological biomarkers (anti-DNA, low C3, low C4) also allowed to discriminate patients who could benefit from anifrolumab treatment. In practice, it will therefore be interesting to compare the interest of monitoring the IFN signature (transcriptomic signature including 4 or 5 genes or inducible proteins) or the dosage of serum IFN itself by sensitive techniques (SIMOA or meso scale diffusion) versus the use of classical biomarkers more easily available in clinical practice. In addition, it will be important to assess the reproducibility of calculation of the IFN score. Actually, the IFN score calculated at different laboratories may be hardly comparable due to the distinct sets of IFN-stimulated genes assessed and different controls. The value of the IFN signature for stratification of patients treated with tsDMARDs is also being studied. JAK inhibitors may inhibit both type I by inhibiting JAK1 and TYK2 downstream IFNAR and also Type II IFN by inhibiting JAK1 and JAK2 downstream IFN gamma receptor. It was confirmed that they may decrease the IFN signature.[31] However, the IFN

signature at baseline has never been shown to predict clinical response to JAK inhibitors until recently. In a study presented at the 2022 ACR meeting focused on SS patients, it was shown that IFN signature at baseline correlated with response to filgotinib (JAK inhibitor) and to tirabrutinib (BTK inhibitor).[32] Finally, the evaluation of the IFN signature as a predictor of response to treatment could be also of interest in AID in which IFN is not the main driver. This is the case in rheumatoid arthritis (RA) in which an IFN signature is observed in only 20% of patients.[33] A recent study showed that in patients with early RA, an IFN signature, linked to IFN-α, was associated with a lower probability of response to csDMARD treatment at 6 months.[33] This was independent of conventional markers of disease activity. It was suggested that IFNs could drive disease persistence epigenetic reprograming of autoimmune lymphocytes.

In conclusion, the understanding of the IFN signature in systemic AID and its usefulness in clinical practice continues to progress. Nevertheless, there are still challenges to overcome for understanding the origin of this IFN signature in both blood and tissue and the role of non-coding RNA sequences, deciphering the specificity of this signature regarding type I, type II or type III IFN. It will be needed to compare the IFN signature to the dosages of IFN themselves to determine what is the most relevant and the most convenient in daily practice and to explore the usefulness of the IFN signature compared with the usual biomarkers, notably autoantibodies for stratifying patients and optimising therapeutic strategies. Clarifying these points will help optimise the management of patients with numbers of systemic AIDs in which IFN play a role.

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