The importance of the type I interferon system in autoimmunity

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ABSTRACT

The type I interferon (IFN) system is our main defense against viral infections and consists of a large number of sensors of nucleic acid that can trigger the production of more than 15 different proteins with antiviral and immunostimulatory capacity. There are several observations suggesting an important role for this system in the etiopathogenesis of systemic lupus erythematosus (SLE) and other autoimmune diseases. Among these are the development of autoimmune diseases during IFN-α treatment, a prominent increase in the expression of type I IFN regulated genes (an IFN signature) in a number of rheumatic diseases, the existence of endogenous IFN inducers in SLE patients and a genetic association between autoimmune diseases and gene variants within the type I IFN signalling pathway. Collectively, these observations suggests that inhibition of the type I IFN system could be beneficial in SLE and possible also other autoimmune diseases. Many different therapeutic targets exist and several studies are in progress aiming to block or down-regulate the activated type I IFN system. A number of studies with monoclonal anti-IFN-α antibodies in SLE patients have been reported, and a small study investigating vaccination with an interferon-α-kinoid against IFN-α has been published. Trials targeting the type I IFN receptor are under way, and other possibilities include elimination of the endogenous IFN inducers and inhibition of key molecules in the type I IFN signalling pathway. Results so far show that it is possible to partially suppress the IFN signature, improve several biomarkers and ameliorate clinical manifestations by some of these new treatment strategies.

Type I IFN system

There are 3 different types of IFNs (I-III) and among these type I IFNs are the largest family, which can be divided into five classes (IFN-α, -β, -ω, -ε and -κ). The IFN-α subgroup can be further divided into 12 subtypes, encoded by 13 highly homologous genes clustered on chromosome 9. The type I IFN system is defined as the type I IFNs themselves and all inducers, cells and molecules involved in the pathways leading to the production and effects of type I IFN (1). The most potent producer of type I IFN is the plasmacytoid dendritic cell (pDC), which upon virus infection can synthesis 10^9 IFN molecules within 24 hours (2). Produced type I IFN proteins bind to the type I IFN receptor (IFNAR) and ligation of IFNAR initiates several signal transduction pathways leading to expression of up to two thousand FN stimulated genes (ISGs), see the Interferome database (http://www.interferome.org/). The gene profile induced by type I IFN is today known as the IFN signature and the ISGs regulate a number of cellular functions, besides inducing antiviral proteins. Type II and type III IFNs have a low degree of homology with type I IFN and signal through their own receptors. Despite this, there is a large overlap between the IFN regulated genes (IRGs) induced by the different IFNs, which might be explained by the crosstalk between the shared components in the JAK/STAT signalling pathways. Notably, no specific type III IFN regulated genes have been reported so far, and due to the shared IRGs induced by the different IFNs it is sometimes impossible to determine the type of IFN responsible for an observed IRG profile.

Type I IFN genes are strictly regulated and in healthy individuals almost no constitutive IFN-α production can be detected. The type I IFN production is classically induced by viruses, bacteria or microbial nucleic acids when sensed by the pattern recognition receptors (PRRs), which can be localised in the cytosol or in the endosome. There is growing number of identified PRRs,

as exemplified by the Toll like receptors (TLRs), retinoic acid inducible gene 1 (RIG-I) like receptors (RLRs) and nucleotide oligomerisation domain (NOD)-like receptors (NLRs) (reviewed in (3)). The pDC expresses TLR7 and TLR9 in their endosomal membranes and can therefore become activated by pathogens that invade the pDC through receptor-mediated endocytosis. The first step in the activation chain involves myeloid differentiation factor 88 (MyD88), which finally leads to transcription of type I IFN genes and release of type I IFNs. Nucleated cells express IFNAR and via activated kinases recruit and phosphorylate the transcription factors Stat1 and Stat2, which finally associate with interferon regulatory factor 9 (IRF-9). The Stat1/Stat2/IRF-9 complex translocates to the nucleus and binds to IFN-stimulated response elements (ISRE) and induces the IFN signature.

Besides the antiviral properties, type I IFN influences many key functions in the innate and adaptive immunity (reviewed in (2, 4)), which best can be described as a general activation of immune cells. This is important for an efficient clearance of viruses and development of long-lasting immunity, but if not properly regulated can cause autoimmune reactions. Type I IFN induces DC maturation and activation with increased expression of MHC class I and II molecules, as well as costimulatory molecules. This facilities cross-presentation of exogenous antigens and detection of virus infected cells by cytotoxic T cells. Upregulation of chemokine receptors promote cell migration to sites of inflammation, which is demonstrated by a reduced number of pDCs in the peripheral blood of patients with an activated type I IFN system such as SLE patients. The development of T cells along the Th1 pathway is promoted and cytotoxic T cells are stimulated by type I IFNs. Furthermore, type I IFNs prolong the survival of activated T lymphocytes, stimulate the development of CD4^{+} and CD8+ memory T cells, increase the differentiation of Th17 cells and suppress Treg functions, which all can lead to an expansion of autoreactive T cells (5). Type I IFN increases

the production of B-lymphocyte stimulator (BLyS), lowers the threshold required for activation through the B cell receptor and stimulates B cell differentiation antibody production (6-7). Thus, the B cell compartment and the type I IFN system are closely integrated, which is of importance for the understanding of the pathogenesis of systemic lupus erythematosus (SLE). Type I IFNs have also effects outside the immune system, such as increased expression of certain autoantigens and impairment of endothelial function by induction of apoptosis. The effect on endothelial cells can contribute to the increased risk for cardiovascular disease, which has been noted in patients with an IFN signature (8).

The connection between the type I IFN system and autoimmunity

The first indications of a causative role of type I IFN in human autoimmune diseases was the observation of an increased occurrence of autoantibodies and autoimmune diseases during type I IFN treatment (9). We noted in a cohort of patients with malignant carcinoid tumours that 19% of patients receiving long-term treatment with IFN-α eventually manifested an autoimmune disease (10), including SLE. Presence of autoantibodies before IFN-α therapy considerably increased the risk for autoimmune disease. Later, it was also shown that administration of IFN-α to patients with systemic sclerosis caused a dramatic progression and worsening of the disease (11). The conclusions from these observations were that type I IFN can induce loss of tolerance and promote an established autoimmune process.

Given the fact that many patients with systemic autoimmune diseases have increased serum levels of type I IFN, a seminal observation was that immune complexes (ICs) containing nucleic acids have the capacity to activate pDC to type I IFN production (12). Further studies revealed that such interferogenic ICs are internalised via the FcγRIIa expressed on pDCs (13), reach the endosome and stimulate the relevant TLR with subsequent activation of transcription factors and IFN-α production

(14). The nucleic acid containing autoantigens in the interferogenic ICs can be generated from apoptotic or necrotic cells (15), which is relevant given the increased apoptosis and reduced clearance of apoptotic cells in lupus (16). Recent studies have shown that neutrophils undergoing so called NETosis also have the capacity to provide interferogenic autoantigens (17), demonstrating that several pathways can lead to pDC activation.

In 2003 several research groups observed that a majority of patients with SLE display an IFN signature, which is connected to a more severe clinical picture with nephritis or haematological manifestations (18-21). Paediatric lupus patients, who usually have a more severe disease compared to adult lupus patients, almost invariably display an IFN signature at early disease stages (18), suggesting that activation of the type I IFN system may be especially important in the initiation of the disease process. Later studies demonstrated that patients with several other rheumatic diseases show an IFN signature in both peripheral blood mononuclear cells (PBMC) and tissues from affected organs. Among these are patients with primary Sjögrens syndrome (22, 23) myositis, systemic sclerosis and a subgroup of patients with rheumatoid arthritis (24). Collectively, these observations suggest a central role of the type I IFN system in the development of autoimmune diseases (25).

An obvious question is why the type I IFN system is not down-regulated in patients with autoimmune diseases, because type I IFN production is normally tightly controlled. There exist at least three possible reasons behind the unabated type I IFN production and IFN signature in SLE and other autoimmune diseases. The first cause has already been discussed, namely the existence of self-derived triggers of type I IFN production. The second cause is a genetic predisposition to increased type I IFN production and response in patients with autoimmune diseases (reviewed in (26)). A third cause is a lack of proper control of pDCs or the expression of IRGs. For instance, NK cells enhance the IFN-α response by

pDC stimulated with RNA containing ICS and monocytes have a strong inhibitory effect on the NK cells, but this inhibitory function by monocytes is diminished in SLE patients (27).

The many findings concerning the type I IFN system in SLE patients can be put together into an etiopathogenic model of SLE, which also includes other observations in this disease (Fig 1, modified from (28)). This model can also be partially applied to other systemic autoimmune rheumatic diseases. It is envisioned that an initial infection by a virus induces type I IFN production and release of cellular material from dying cells. The extracellular autoantigens from apoptotic and necrotic cells as well as NETs from granulocytes then trigger B cells to autoantibody production against RNA and DNA binding proteins in individuals prone to autoimmune reactions. ICs will be formed, which act as endogenous type I IFN inducers causing se a prolonged stimulation of type I IFN production by pDC. The excessive release of endogenous DNA/RNA in combination with impaired clearance of apoptotic cell material will facilitate the generation of more interferogenic IC. Several drugs and environmental factors can contribute to the generation of autoantigens. Produced type I IFN activates B cells and T cells, which together with NK cells enhance the type I IFN production as described above. This will result in chronic activation of the type I IFN system, which will drive an autoimmune process leading to chronic inflammation and tissue damage in a vicious circle manner.

Targeting type I IFN system in autoimmune diseases

The proposed model of the pathogenesis of SLE suggests a number of different therapeutic strategies aiming to down regulate an activated type I IFN system. Some of these are listed in Table I, and most probably the optimal therapeutic target depends on the mechanism(s) behind an increased type I IFN production, or response, in a specific subset of patients. Despite this, both high doses of glucocorticosteroids and hydroxychloroquine
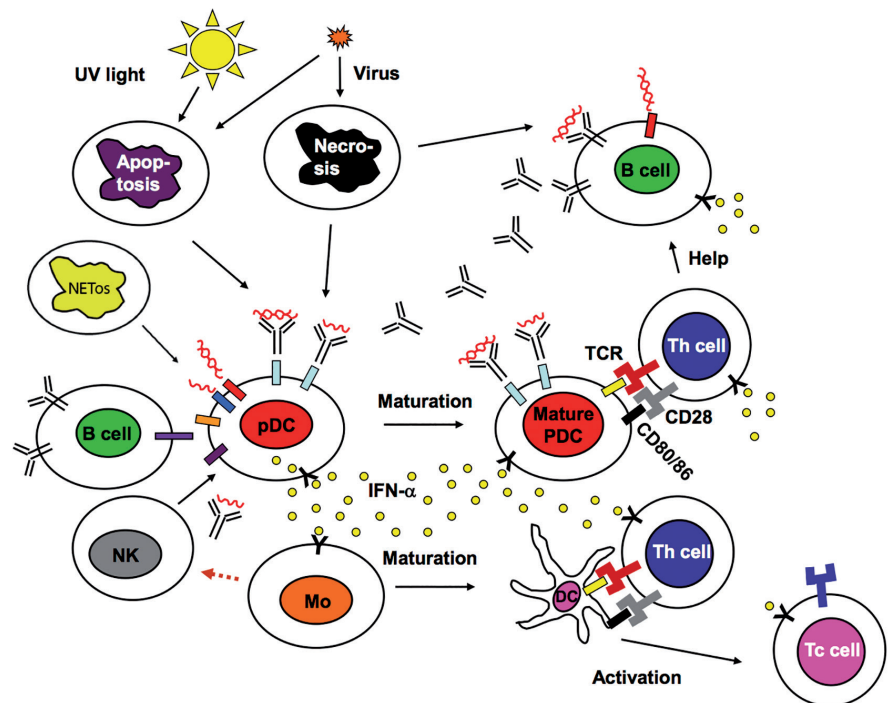
Fig. 1. An SLE model highlighting the role of the type I interferon (IFN) system in the etiopathogenesis of the disease. A viral infection induces IFN-α production and release of autoantigens from dying cells. Produced IFN-α act as an immune adjuvant and stimulates cells in both the innate and adaptive immune system. Autoantibodies are produced in response to the autoantigens and these antibodies together with nucleic acid containing antigens form immune complexes (ICs) with the capacity to stimulate plasmacytoid dendritic cells (pDCs) to IFN-α production. The IFN-α response is promoted by NK cells and B cells whereas monocytes (Mo) down-regulates the response. The latter function is deficient in SLE patients and together with the impaired clearance of interferogenic ICs this will result in an ongoing type I IFN production, which fuel the autoimmune process. The Figure is modified from ref. 28.

Table I. Possible targets and strategies for treating the activated type I IFN system in SLE.

Target	Possible drugs, candidate molecules or strategies
IFN inducers	Nucleases, B cell modulation (autoAb reduction)
Receptors (FcγRIIa, TLR)	Hydroxychloroquine, inhibitory ODN
Type I IFN signalling pathway	IRF5 inhibitor
Genes/mRNA	siRNA, miRNA 146
Type I IFN	anti-IFN mAbs, vaccine
IFNAR	anti-IFNAR mAbs
IFN signalling	Kinase inhibitors
IFN-inducible genes	Histone deacetylase inhibitors
pDC	anti-BDCA-2, proteasome inhibitors
Cell interaction	mAbs targeting CD31, LFA-1

IFN: interferon; Ab: antibody; FcγRIIa: Fcγ receptor IIa; TLR: toll-like receptor; ODN: oligodeoxynucleotide; IRF5: interferon regulatory factor 5; siRNA: small interfering RNA; miRNA: micro RNA; IFNAR: type I IFN receptor; pDC: plasmacytoid dendritic cell; BDCA-2: Blood dendritic cell antigen 2; CD31: cluster of differentiation 31 or platelet endothelial cell adhesion molecule (PECAM-1); LFA-1: lymphocyte function-associated antigen 1.

down-regulate the IFN signature and is widely used in SLE patients. Today, a number of more specific inhibitors of the type I IFN system exist. The most obvious target is type I IFN itself and several monoclonal antibodies neutralising IFN-α have been tested in clinical trials, resulting in a partial decrease of the IFN signature and disease activity

(29-30). This treatment does not affect other type I IFNs and, a more complete inhibition of the type I IFN family can be achieved by targeting IFNAR. Recently, the first results from treatment of SLE with an anti-IFNAR were presented and a significant improvement in disease activity was noted, but at the cost of an increased frequency of viral

infections (31). New therapeutic strategies are to degrade the stimulatory TLR ligands in interferogenic ICs by nucleases or to use inhibitory oligodeoxynucleotides to block TLR activation (32). A broader therapeutic approach is to target the pDCs themselves with monoclonal antibodies against BDCA-2 (33), or to use proteasome inhibitors which efficiently suppress production of IFN-α by TLR-activated PDCs (34). Molecules of importance for the interaction between pDC and B cells (35) or NK cells (36) are other possible targets. A more specific treatment that has been suggested is administration of reverse transcriptase inhibitors for SLE patients with chronic stimulation of DNA sensors by retroelement cDNA (37). The challenge for the future is to modulate the type I IFN system in SLE without interfering with the antiviral defense, and realise that different therapeutic targets may be appropriate in different disease subsets.

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