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Interferon-alpha: a therapeutic target in systemic lupus erythematosus

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Summary

The long history of elevated IFN α in association with disease activity in patients with SLE has taken on high significance in the past decade with accumulating data strongly supporting broad activation of the type I IFN pathway in cells of lupus patients, association of IFN pathway activation with significant clinical manifestations of SLE, and increased disease activity based on validated measures. In addition, a convincing association of IFN pathway activation with the presence of autoantibodies specific for RNA-binding proteins has contributed to delineation of an important role for TLR activation by RNA-containing immune complexes in amplifying innate immune system activation and IFN pathway activation. While the primary triggers of SLE and the IFN pathway remain undefined, rapid progress in lupus genetics is helping to define lupus – associated genetic variants with a functional relationship to IFN production or response in lupus patients. Together, the explosion of data and understanding related to the IFN pathway in SLE have readied the lupus community for translation of those insights to improved patient care. Patience will be needed to allow the required collection of clinical data and biologic specimens across multiple clinical centers that will support the required testing of IFN activity, IFN-inducible gene expression or target chemokine gene products as candidate biomarkers. Meanwhile, promising clinical trials are moving forward to test the safety and efficacy of monoclonal antibody inhibitors of IFN α . Other therapeutic approaches to target the IFN pathway may follow close behind.

Keywords

Systemic lupus erythematosus; interferon-alpha;; innate immune response

Introduction

A role for type I interferon (IFN), predominantly IFN α , in the pathogenesis of SLE was first suggested based on the observation that serum from patients with active SLE disease had augmented capacity to inhibit the death of virus-infected cells.¹ Those data, published in the late 1970's, found an association between IFN activity and the standard serologic indicators of activity, anti-DNA antibody titer and low complement levels. Recent re-examination of the IFN system using newer technology has heightened attention to this important immune system

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mediator.²⁻⁶ Along with clinical observations demonstrating occasional induction of lupus-like autoantibodies and clinical disease in patients receiving recombinant IFN α for treatment of hepatitis C infection or malignancy,⁷ microarray gene expression analysis showing broad activation of IFN-inducible genes in blood cells of lupus patients has suggested that IFN α may be a central player in systemic autoimmune disease.²⁻⁴ As with any association of an immune system product with a clinical syndrome, it is important to address whether IFN α plays a pathogenic role in the disease, is an innocent bystander, or is possibly playing a protective role. A growing body of data relating IFN pathway activation to studies of genetic associations, clinical characterization of patients, murine models and results from therapeutic interventions support an important, and possibly central, role for this cytokine family in SLE.^{5,6} Together, the emerging data support the validity of IFN α as a therapeutic target in patients with SLE.

Type I IFN in immune responses

The family of type I IFNs comprises the protein products of multiple related genes encoded on the short arm of human chromosome 9.8 IFN β may be the prototype member of the family, but IFN α has the largest number of isoforms. Thirteen IFN α genes have evolved over time, presumably selected to effectively combat infection by viruses. The type I IFNs are rapidly induced by infection with DNA and RNA viruses, either through intracellular nucleic acid receptors or after engagement of a Toll-like receptor (TLR) that recognizes nucleic acids, such as TLR3 for double-stranded RNA, TLR7 or 8 for single-stranded RNA, or TLR9 for demethylated CpG-rich DNA.⁹ IFN α can be synthesized by many cells but plasmacytoid dendritic cells (pDCs) represent the cell type most capable of high level IFN α production.¹⁰

The broad expression of the type I IFN receptor (IFNAR) on many cell types contributes to the diverse cellular responses induced by this cytokine family. While T cells may be the conductors of the adaptive immune response orchestra, IFN α is an innate immune system product that orchestrates the immune system's initial response to viral infection prior to the activation of T cells. Among the immune system functions implemented by IFN α are differentiation of monocytes into dendritic-like cells capable of effective antigen presentation, induction of natural killer and NK T cells, promotion of production of IFN γ , support for B cell differentiation into class-switched antibody producing cells and in some cases induction of apoptosis, resulting in release of cell debris including potentially stimulatory self antigens.^{6, 11} As the presenting clinical features of SLE can sometimes resemble features of some virus infections, it is interesting to recognize that many of the immunologic alterations that are characteristic of SLE are similar to the immunologic effects of virus-induced IFN.

Insights from gene expression studies in patients with SLE

The advances in technology that permitted assessment of a broad spectrum of gene products in a population of cells emerged in the late 1990's and were used to demonstrate associations between the characteristic pattern of mRNA transcripts in monoclonal populations of cancer cells and disease prognosis.¹² It was initially presumed that clinically relevant insights from microarray gene expression studies in complex diseases, including the systemic autoimmune diseases, would not emerge from this experimental approach due to the variability in representation of diverse cell types among individuals. In fact, microarray studies of peripheral blood mononuclear cells (PBMC) from lupus patients were highly informative. Several laboratories analyzed large data sets and detected a pattern of gene expression rich in transcripts induced by IFNs.²⁻⁴ Among the overexpressed mRNAs were some that are well known as targets of type I IFN, such as *MX1*, the *OAS* family, *IFIT1* and others. However the broad pattern of increased expression of IFN-regulated genes included many induced by both type I IFNs and type II IFN (IFN γ). To determine the relative roles of type I and type II IFN in the "IFN signature", additional studies using more quantitative real-time polymerase chain

reaction (PCR) focused more narrowly on genes preferentially regulated by either type I or type II IFN.¹³ Those data clearly demonstrated the predominant picture of increased levels of type I IFN induced genes in lupus PBMC. Moreover, the level of expression of those gene products across a population of lupus patients showed a high level of statistically significant correlation of each type I IFN-induced transcript with the others. This pattern strongly suggested that type I IFN present *in vivo* in many lupus patients was driving a broad gene expression program, very similar to what has been seen in patients who have received either recombinant IFN α or IFN β for hepatitis C or multiple sclerosis.^{14,15} Some lupus patients also demonstrated increased expression of genes preferentially regulated by IFN γ , such as CXCL9 (monokine induced by gamma interferon; MIG), but they were less frequent than those who demonstrated activation of the type I IFN-induced genes.¹³

As noted, the type I IFN family includes multiple IFN α isoforms, but also includes products of related genes, including IFN β . To determine which of these type I IFNs was most responsible for expression of the IFN-inducible genes, a functional assay of type I IFN activity in plasma or serum was developed and preferential inhibition of that activity in SLE plasmas by neutralizing antibodies to IFN α was observed.¹⁶ In contrast, only modest inhibition of type I IFN activity was seen when antibodies to IFN β or IFN θ were included in the cultures. The data lead us to suggest that IFN α represents the major type I IFN active *in vivo* in SLE patients, but it is likely that other isoforms contribute a small fraction of the type I IFN activity that alters immune system function in lupus patients.

The proportion of lupus patients demonstrating the IFN signature has varied from one report to another. In some studies of unselected adult patients less than 50% show this gene expression pattern while a study of pediatric lupus patients, most of whom had recently been diagnosed and many of whom had not yet been treated aggressively, saw the IFN signature in nearly all patients.^{3,13} An association of IFN pathway activation with several clinical features of lupus, particularly a history of renal disease and anemia, has been demonstrated in several cohorts, and a relative underrepresentation of IFN pathway activation has been seen in patients with antiphospholipid antibodies.^{2,13,14,17} In view of the acknowledged diversity of disease manifestations in patients with lupus, along with the fluctuating course of disease, it is not surprising that there are differences in prevalence of the IFN signature in cross-sectional studies of lupus patients.

The demonstration of near universal activation of the IFN pathway in pediatric lupus patients, with fewer adult patients showing this pattern, raises a question of whether the production or response to IFN α is a function of age. In that regard, a study characterizing plasma type I IFN activity in SLE patients and healthy first-degree relatives based on age of the subjects showed similar patterns in female and male patients but distinct levels of activity based on age.¹⁸ Interestingly, the age at which plasma IFN activity was greatest corresponded to the peak reproductive years, with females between 12 and 22 showing higher levels than those younger than 12 or older than 22. Female lupus patients and their first-degree relatives showed the lowest levels of type I IFN activity after age 50. Males showed a similar pattern, but with the peak age range several years older (16–29) than the females. IFN levels were not significantly different between females and males in either the patients or relatives. Taken together these data suggest that age of the pediatric lupus cohort, in and of itself, likely contributed to the higher prevalence of IFN signature among those patients compared to studies of adult patients. The molecular basis of this interesting age-related pattern of IFN pathway activation is not known.

Association of IFN α with disease activity in SLE

The first studies of IFN α in SLE from the 1970's indicated that the circulating levels of the cytokine were associated with serologic activity of SLE.¹ As microarray data from studies of PBMC of carefully characterized patients emerged from several laboratories in the early 2000's, the relationship between clinical disease activity, as measured by standard tools such as SLEDAI, and a quantitative real-time PCR measurement of IFN-inducible gene expression clearly showed a relationship to disease activity.^{2,13,14,17} What has been less certain is the degree of fluctuation of IFN pathway activation over time in individual patients. The question remains, do patients who do not demonstrate IFN-inducible gene expression in their PBMC or increased plasma type I IFN activity represent a distinct subset of lupus, a reflection of low disease activity, or based on chronic disease that was earlier characterized by IFN pathway activation but is now "burned-out"? Arriving at an answer to this question has been difficult due to the inherent challenges of longitudinal clinical research studies as well as the technical difficulties and expense of quantifying IFN pathway activation. Validated disease activity measures are rarely applied to patients followed in the routine course of clinical care. While several clinical investigator teams have established large cohorts of well characterized lupus patients and have followed those patients over several years, appropriate biologic samples are rarely available on those patients and the clinical investigators who collect that data are rarely the same investigators who perform the laboratory analyses of IFN-inducible gene expression or plasma type I IFN activity. Most commercially available ELISA platforms for measurement of IFN α have not been useful in gaining insights into patterns of disease activity, most likely due to the limited range of IFN isoforms measured or possibly due to the presence of inhibitors or other plasma or serum components that obfuscate accurate measurement of the functionally active IFN.¹⁹ The current available data do not provide a definitive answer to whether and how IFN pathway activation relates to changes in immune function or disease activity, but our group does document fluctuations in IFN-inducible gene expression in PBMC over time, in some cases, with close parallel to fluctuations in disease activity scores or response to therapy.²⁰ Additional longitudinal data will be helpful in determining whether IFN pathway activation can in some cases precede flares in disease activity and whether a causal link between those two events can be established. Characterizing the changes in gene expression, serologic activity or immune function that bridge a discrete increase in IFN pathway activation and a flare in disease activity could provide invaluable novel insights into lupus pathogenesis.

Advances in research into mechanisms of IFN pathway activation in SLE

Genetic contributions to type I IFN production or response

The Major Histocompatibility Complex (MHC) provides the most significant contribution to the genetic variations that result in increased risk of developing SLE.²¹⁻²² It seems likely that alleles of MHC-encoded class II molecules are involved in the capacity to generate autoantigen-specific immune responses and production of autoantibodies. Candidate gene studies developed in recent years have identified several additional lupus-associated gene variants, and large scale genome-wide association studies (GWAS) and their follow-up investigations, particularly two seminal collaborative studies published in 2008, have demonstrated additional lupus-associated variants that identify up to twenty-five more genes, intronic regions or gene loci.²³⁻²⁵ The SNPs identified in the published GWAS datasets represent common variants that are widespread in the population and confer a very modest increased risk of SLE. Additionally, several rare genetic variants that are associated with much greater risk of lupus-like disease have been found.²⁶ Together, the growing body of data on common variants conferring low risk and rare variants conferring higher risk of lupus are pointing to the most important molecular pathways that are involved in lupus pathogenesis.

When the list of lupus-associated gene variants is considered in the context of their known biologic function, the aggregate data collected so far strongly support the essential role of the immune system in disease pathogenesis.²⁷ Characterization of the precise functions that are altered by the nucleic variations enriched among lupus patients is the next major challenge and will require use of the tools of genetics, molecular biology and cell biology to gain new understanding of lupus disease mechanisms and identify new therapeutic targets. Yet the data generated so far can be synthesized to propose several essential components of the disease, all of which reflect genetic factors which might augment likelihood of disease development. They include increased generation or impaired clearance of self-antigens, particularly nucleic acids, and capacity to activate autoantigen-specific immune response, including T cell and B cell activation and differentiation to plasma cells.^{6,27} Most relevant to this review are a growing number of lupus-associated genetic variants, both common and rare, that impact type I IFN production or response.

A role for genetic variation in the increased production of type I IFN seen in many SLE patients was first supported by a family study in which plasma type I IFN activity was quantified in SLE patients, their first-degree relatives and unrelated individuals.²⁸ A significant increase in IFN level was documented in healthy first-degree relatives of patients compared with unrelated subjects. Moreover, high IFN levels tended to cluster in families. The conclusion that increased plasma type I IFN was a heritable trait led to subsequent efforts to relate lupus-associated genetic variants to activation of the IFN pathway and publication of a series of papers by Timothy Niewold identifying contributions of specific gene variants to activation of that pathway.^{29–32}

Abundant data have supported a complex set of SNPs in the interferon regulatory factor 5 (*IRF5*) gene that are associated with SLE.^{33–35} Dissection of that association points to a role for particular autoantibody specificities, such as anti-Ro and anti-DNA, in the association with the *IRF5* risk haplotype.²⁹ Moreover, that risk haplotype shows increased association with SLE and with increased type I IFN activity in plasma of lupus patients who express those autoantibodies. Together those data link autoantibodies that target nucleic acids or nucleic acid-binding proteins, *IRF5* and type I IFN production. With the knowledge that *IRF5* is a signaling molecule downstream from several of the intracellular TLRs, the data support the concept that TLRs activated by DNA and RNA signal through *IRF5* to induce type I IFN. Additional lupus-associated gene variants that might modulate the TLR pathway and IFN production include *IRF7* and *TNFAIP3*, encoding A20, an inhibitor of the TLR pathway.^{27,36} An association between the lupus risk variants of *PTPN22*, a lymphocyte phosphatase, and secreted phosphoprotein 1 (*SSP1*; osteopontin) and plasma type I IFN activity have also been reported although the exact mechanisms by which those variants impact IFN production have not been elucidated.^{30,32} A relationship between polymorphisms in *FCGR1A*, one of the first lupus-associated genes to be identified, and IFN production has not been investigated, although that issue might be a productive research direction in view of the role of that Fc receptor in internalization of immune complexes that induce IFN through TLRs.^{9,37}

The response to type I IFN depends on sequential interaction of the cytokine with the two chains of IFNAR, the type I IFN receptor, and activation of a series of kinases, including members of the signal transducer and activator of transcription (STAT) and Janus kinase (Jak) families. Although STAT1 has been most often implicated in signaling downstream of IFNAR, the STAT4 gene has been associated with SLE in several GWAS. A study of SLE patients with the risk allele of *STAT4* showed normal or even increased levels of plasma type I IFN activity but a significant association with increased IFN-inducible gene expression in the PBMC of those patients.³¹ That is, for a given amount of type I IFN those patients with the *STAT4* risk allele appeared to have augmented transcription of genes that are regulated by type I IFN.

It should also be noted that in some individuals the genetic deck may be stacked in such a way that autoantibody production is favored over IFN pathway activation.²² A study of serum from mothers of babies with the neonatal lupus syndrome in whom anti-Ro antibodies were universally present in high titer showed that those antibodies were accompanied by high IFN activity only in those with clinical features of SLE or Sjogren's syndrome.³⁸ These clinical data further support the concept that there are several prerequisites for development of clinical lupus. Some individuals have a genetic load for activation of the IFN pathway and others have increased capacity to form autoantibodies. It is those individuals who engage both arms of the immune system, the production of IFN by the innate immune response and the production of autoantibodies by the adaptive immune response, who are most likely to develop clinical disease.²⁵

It is possible that the third important prerequisite, generation or impaired clearance of cell-derived self-antigens, is required to complete the requirements for disease pathogenesis. In that regard, while the lupus-associated genetic variants described above represent common variants that result in a modest increased risk of SLE and it is likely that at least several of those risk variants must act together to achieve the threshold needed to initiate disease, several recently described rare variants are associated with a greater risk of disease and appear to generate increased self-antigen. One of these genes, *TREX1*, encodes a DNase and another encodes an RNase.^{26,39} Normal function of those gene products is required to dispose of endogenous nucleic acids that might otherwise stimulate an innate immune response. That concept is supported by studies in mice deficient in *TREX1* which show increased production of type I IFN.⁴⁰

In summary, the data accumulated so far implicate gene variants involved in the intracellular nucleic acid-response TLR pathways, as well as at least one gene that might contribute to signaling through the type I IFN receptor pathway, in susceptibility to SLE. A role for genetic variation in components of the TLR-independent cellular pathways in induction of type I IFN production is currently under investigation. In addition, rare variants are being identified that could result in generation of stimuli for immune responses that result in increased type I IFN. Additional research will be required to determine whether analysis of lupus risk allelic variants and/or type I IFN activity or the IFN signature will prove practically useful in predicting increased susceptibility to lupus in individuals otherwise at risk, e.g. sisters of lupus patients.

Molecular pathways mediating production of IFN α

As suggested above, numerous laboratories have supported an important capacity of nucleic acid-containing immune complexes to activate immune responses, particularly the innate immune response but also B cell proliferation, through TLR pathways.^{9,41–44} Ronnblom and colleagues were the first group to study the circulating factors in lupus serum or plasma that induced IFN α production *in vitro*.^{9,40} They showed that apoptotic or necrotic cell debris, when associated with SLE serum, could induce IFN. That response was inhibited by blockade of Fc receptors as well as chloroquine. At least one proposed mechanism of chloroquine's effects *in vitro* is its modification of the acidification of intracellular vesicles, a mechanism that is likely to impact signaling downstream of TLRs engaged by nucleic acid ligands. A similar mechanism is likely to be operative in patients treated with hydroxychloroquine. Studies from other groups have refined the mechanisms that account for immune complex stimulation of IFN production and have implicated TLR7, responsive to single stranded RNA, and TLR9, responsive to hypomethylated CpG-rich DNA, in the induction of IFN by nucleic acid-containing immune complexes.^{41–44} Although this mechanism is difficult to document *in vivo* in lupus patients, a striking association of IFN pathway activation with the presence of RNA-binding protein-specific autoantibodies (anti-RBP), such as anti-Ro, La, Sm or RNP has

been observed.¹⁴ The data noted above in studies of the IRF5 lupus risk haplotype also supports a functional link between anti-RBP and induction of IFN through the TLR7 pathway.²⁹

Of course the induction of IFN by immune complexes cannot fully account for the increased production of type I IFN seen in SLE, as increased plasma IFN is observed in lupus relatives who do not express lupus autoantibodies, and some lupus patients without measurable anti-RBP or anti-DNA antibodies do have an IFN signature.²⁸ Yet the documented capacity of chloroquine to inhibit immune complex-mediated IFN production *in vitro* and the convincing data showing reduced frequency and severity of lupus flares in patients maintained on hydroxychloroquine support an important contribution of TLR signaling to clinical disease activity.^{45–47} Our current view is that induction of IFN α by nucleic acid containing immune complexes represents an important mechanism of augmenting type I IFN production that is influenced by genetic factors. But it does not fully account for type I IFN produced early in the course of pre-clinical disease, prior to the development of autoantibodies. Additional studies addressing a role for environmental triggers, including virus infection, that act on a susceptible genetic substrate should provide more detailed understanding of lupus pathogenesis.

IFN α in murine lupus models

In contrast to many aspects of lupus pathogenesis in which murine models have led the way in defining important disease mechanisms, in the case of type I IFN the most significant milestones in elucidating the role of this pathway in disease have derived from studies of lupus patients. In fact, data from the standard murine lupus models pointed to a predominant role of IFN γ rather than IFN α in lupus pathology, based on knock-out and transgenic studies as well as documentation of increased IFN γ in kidneys of mice with glomerulonephritis.⁴⁸ A role for IFN γ in many patients with lupus is also supported, but the body of data from the human system emphasize type I rather than type II IFN as the major player.

With attention focused on the type I IFN pathway in human lupus, new data from murine experiments are now supporting an important contribution of IFN α to mouse lupus. This has been demonstrated by administering IFN α to mice in the form of an adenoviral construct that leads to sustained increased levels of that cytokine.^{49–51} The result is accelerated development of autoimmunity, renal pathology and death. In addition, a proposed effect of adenoviral IFN or type I IFN induced by poly (I-C), a surrogate double stranded RNA ligand for TLR3, on recruitment of activated monocytes to kidneys may suggest additional mechanisms of tissue fibrosis and damage that could be modified by targeting IFN α .⁵¹

A murine model with very high fidelity to human lupus, or at least the aspects of human lupus associated with IFN α , has been studied in detail by the laboratory of Westley Reeves.⁵² Administration of 2,6,10,14-tetramethylpentadecane (pristane) to healthy mice results in activation of immature monocytes that produce type I IFN that is dependent on signaling through TLR7. Typical lupus autoantibodies are produced and the mice go on to develop lupus nephritis. While the precise mechanisms that account for activation of the monocyte targets by pristane are not yet described, this model is arguably the ideal experimental system for gaining new understanding of a potential role for environmental triggers in initiating IFN pathway activation and disease in a manner highly similar that that observed in human patients.

IFN α and its targets as lupus biomarkers

The body of data supporting IFN α as a central pathogenic mediator in lupus has contributed to interest in investigating IFN α or expression of its gene targets as candidate biomarkers of severe disease, active disease, predictors of disease flare, or for identification of patients likely to respond in clinical trials of agents that might modify the IFN pathway. The rationale for

these studies is strong, but as noted, the practical considerations involved in quantitatively assessing IFN protein, IFN activity or IFN-inducible gene expression have been challenging. Moreover, the limited number of registries of lupus patients who have been longitudinally followed with collection of disease activity data using validated instruments and with paired biologic samples stored has provided a hurdle to biomarker discovery in SLE that has not yet been fully overcome, in spite of excellent biomarker candidates for further study.

Based on our experience, real-time PCR quantification of a small panel of IFN-inducible genes in RNA isolated from PBMC lysates provides the most sensitive and specific measure of IFN pathway activation. Data being developed by Kyriakos Kirou and colleagues has demonstrated fluctuations in IFN-inducible gene expression over up to three years of follow-up of individual lupus patients pre-selected to enrich for those with IFN pathway activation based on the presence of anti-RBP autoantibodies. Approximately half of those patients show a parallel pattern of fluctuations in disease activity with changes in IFN score, consistent with prior cross-sectional data. Another group of patients shows an intriguing pattern of IFN-inducible gene expression that peaks months prior to clinical disease flare. Additional data will be required to determine whether the relationship of these two events – IFN score and disease activity score – over time has functional significance or is an arbitrary concurrence of an immunologic response and a clinical presentation. We have utilized an assay of type I IFN functional activity in plasma or serum, based on induction of type I IFN-inducible gene expression in the WISH epithelial cell line that is highly responsive to IFN, to identify those patients with increased production of IFN.¹⁶ This assay has proved highly useful in relating levels of IFN activity to various lupus-associated gene alleles and to monitor IFN production over time. Because this assay measures IFN present in the circulation but does not reflect the contribution of expression of IFN receptors or the efficiency of signaling and new gene transcription downstream of IFNAR in patient cells, it does not fully reflect the many determinants of IFN pathway activation and may be less useful as a biomarker of disease activity than the IFN score, which measures expression of IFN-inducible genes in PBMC. Bearing this supposition in mind, any conclusion regarding the optimal experimental approach to assessing IFN pathway activation will await a comprehensive comparison of IFN activity and PBMC IFN score in a well characterized lupus cohort followed regularly for at least two or three years, allowing for sufficient examples of disease flare to assess the relationship of the candidate biomarkers to disease activity.

With the challenges of accurately measuring IFN pathway activation, some investigators have addressed the hypothesis that some of the chemokines that are induced by type I IFN and other stimuli might serve as a more reliable and useful biomarker of lupus disease activity or future flare.^{54–56} Several chemokines are highly induced by type I IFN but many of those are also induced by IFN γ , other cytokines or by microbial or endogenous stimuli of TLRs. Regardless of issues regarding specificity of chemokines as biomarkers of disease activity, several studies suggest that measuring serum chemokines, or alternatively PBMC chemokine transcripts by PCR, might reflect IFN pathway activation, along with other inflammatory triggers, in a manner helpful in predicting generalized lupus flares or occurrence of lupus nephritis. Most promising is a recent study showing the capacity of a chemokine score to predict future flares of lupus nephritis.⁵⁶ Whether an assay is based on IFN activity, its specific gene targets or a broader array of chemokine targets, additional studies based on collaborations among several centers and investigators will be required to arrive at a practically useful biomarker that can aid patient management.

Promise and risk of therapies that target the IFN pathway

With this strong case for type I IFN's important role as a heritable risk factor, a correlate of disease activity and a global immune response modifier central to the pathogenesis of SLE

(and some other systemic autoimmune diseases), many groups have concluded that this cytokine is an appropriate target for therapeutic modulation. Current efforts are directed at understanding the impact of currently available therapies on IFN pathway activation and development of new agents to inhibit the pathway, directly or indirectly.

Arguably the most effective approach to inhibiting production of IFN α is administration of intravenous high dose methylprednisolone. This treatment virtually ablates the IFN signature based on microarray or real-time PCR data from patient PBMC before and after pulse steroid treatment.³ The presumed basis of this effect is death of the major producers of IFN α , pDCs, by the high dose steroids. Recent data suggest that additional mechanisms that modulate the capacity of IRFs to regulate gene transcription might also contribute to reduced IFN pathway activation by high dose steroids.^{57–59} Although the mechanism by which other frequently used therapeutic agents, such as mycophenolate mofetil (MMF), might inhibit IFN production are only recently coming under study, our preliminary data suggest that MMF treatment is associated with reduction of the IFN score derived from lupus patient PBMC.⁵⁸ A recent study implicates a possible effect of MMF on autophagy and the TLR-independent innate immune system pathway, but additional investigation will be required to pursue that suggestion.⁵⁹

As described, hydroxychloroquine inhibits acidification of intracellular vesicles, and *in vitro* studies clearly document the inhibition of IFN production induced by nucleic acid-containing immune complexes by chloroquine. It is likely that additional mechanisms account for the positive impact of hydroxychloroquine therapy on reduction of lupus flares, but the strong rationale for its use based on the recent IFN pathway data have suggested that additional approaches to inhibition of TLR activation might be even more productive in SLE. Among the approaches under investigation are inhibition of nucleic acid-mediated TLR activation by oligonucleotide inhibitors of TLR7, TLR8 and TLR9. This approach is quite attractive if the oligonucleotide inhibitors can be modified to assure adequate delivery to target cells.

The most active area of clinical development of therapeutics targeting the IFN pathway involves current clinical trials of monoclonal antibodies specific for numerous IFN α isoforms. At least three of these monoclonal agents are in clinical development, each presumably slightly different from the others in the range of isoforms targeted. Very promising pharmacodynamic data from MedImmune have demonstrated inhibition of the IFN signature in PBMC and in skin biopsies from at least some lupus patients treated with Medi-545.⁶⁰ At this time the blockade of interaction of IFN α with IFNAR by monoclonal anti-IFN α antibodies appears to be the most feasible and likely to be effective approach to controlling this important innate immune system pathway.

Additional antibodies are available that block the IFN receptor. As the receptor not only binds IFN α but is also activated by the other type I IFNs, including IFN β , IFN θ and others, it would seem that receptor blockade might produce a more complete blockade of downstream gene expression than the anti-IFN α antibodies, for better or worse.

The essential role of type I IFN in host defense against virus infection is clearly evident from the obvious effort expended by the collective human genome over evolutionary time to generate a variety of similar but non-identical type I IFN isoforms. Of those, IFN α has the most variants (thirteen different isoforms). The high impact of this system on generating effective and comprehensive immune responses triggered by virus infection is emphasized when considering the numerous approaches used by viruses to hijack the normal host response.⁶¹ Virus-encoded proteins have been shown to block induction of type I IFN and response to that cytokine. In fact, study of the mechanisms used by viruses to paralyze the host IFN response has provided important insights into the essential components of that response. Blockade of any system that holds such responsibility for maintaining the intactness of the host in the setting of a viral

assault should only be modulated with great care. Development of any of the therapeutic approaches described will be accompanied by careful monitoring for viral infection. Considering the different options, it would seem that blockade of IFNAR might be most risky, while TLR blockade or inhibition of selective type I IFNs (such as inhibition of IFN α with monoclonal antibodies) would allow some other routes for production of IFN or response to other isoforms to be available. Although the role of type I IFN in viral host defense has garnered the most extensive investigation, IFN α is also active in modulating certain hematologic malignancies such as hairy cell leukemia, and the role of the type I IFNs in regulating myeloid differentiation is not fully understood, suggesting a further need for caution as therapeutic trials move forward.

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