Type I Interferon and Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease associated with multiple immunologic abnormalities. Prominent among these is upregulation of type I interferon (IFN)—a powerful immune adjuvant. IFN is, in part, produced in SLE in response to autoantigens in the form of self-nucleic acids and their associated nuclear proteins. Sources of these autoantigens include apoptotic and necrotic cells as well as neutrophils undergoing a specific form of cell death called NETosis. Although plasmacytoid dendritic cells are the main producers of IFN- α , other cells are important regulators of this process. Both genetic and environmental risk factors play a role in the development and pathogenesis of SLE. Further highlighting the importance of IFN, candidate gene and genome-wide association studies have identified a number of genes involved in type I IFN pathways associated with SLE. In this review, 3 monogenic deficiencies that result in lupus-like phenotypes and several polygenic variants that have been consistently associated with SLE are highlighted, and the relationship of these genes to IFN- α production is discussed. Clinical associations of the type I IFN pathway and the use of IFN-blocking agents as therapeutic agents in SLE are also reviewed.

Systemic Lupus Erythematosus

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE or lupus) is a complex autoimmune disease with diverse clinical manifestations ranging from mild rash and arthralgia to severe or life-threatening forms of the disease affecting the kidneys and central nervous system (Rahman and Isenberg 2008). Multiple abnormalities contribute to the pathogenesis of SLE. These include abnormal clearance of apoptotic cells and immune complexes (ICs) and low thresholds of activation of B and T lymphocytes leading to loss of self-tolerance and autoantibody production. These autoantibodies are directed against nucleic acids (DNA) and associated nuclear proteins as well as ribonuclear proteins (RNP) such as Ro, La, and Sm (Tan 1989). Tissue damage is mediated by deposition of pathogenic autoantibodies and ICs in the affected organs, followed by activation of downstream inflammatory pathways mediated by complement and FcR engagement of innate immune cells (Rahman and Isenberg 2008).

Both environmental and genetic risk factors are important in the development of SLE. The female to male ratio is 9:1, suggesting that hormones may be important in the development of the disease. Exposure to UV light is known to trigger SLE, and several viruses and bacterial infections have also been implicated in disease pathogenesis and development. Drug-induced lupus is a well-described syndrome in which lupus-like symptoms develop after exposure to certain medications, including hydralazine, procainamide, isoniazide, and interferon- α (IFN- α) (Sarzi-Puttini and others 2005). Twin

studies reveal a 25% to 40% concordance rate among monozygotic twins and a 2% concordance rate among dizygotic twins (Harley and others 2009), emphasizing the strong genetic component in SLE. HLA associations and complement deficiencies, especially of early complement components C1q, C2, and C4 have long been known to be associated with SLE. To date, 3 monogenic deficiencies—C1q, 3 prime repair exonuclease 1 (TREX1), and tartrate-resistant acid phosphatase (TRAP)—have been identified that result in clinical phenotypes consistent with lupus. In addition, more than 100 common genetic variants (only 8 consistently replicated) that confer increased risk of SLE susceptibility but with small effect have been identified by genome-wide association studies (GWAS) (Harley and others 2009). Of note, all 3 monogenic syndromes and many of the genetic variants identified by GWAS are involved in the type I IFN pathway, further emphasizing the importance of this pathway in SLE (Deng and Tsao 2010; Sestak and others 2011).

IFNs: Types and Effect on Immune Function

There are 3 types of IFNs: type I (alpha, beta, omega, and other less common subtypes), type II (IFN- γ), and type III (IFN- λ) [interleukin 28 and 29 (IL-28 and IL-29)] (reviewed in Theofilopoulos and others 2005). Of the type I IFNs, IFN- β can be produced by almost any cell and release of this cytokine serves to prime or amplify type I IFN by other cells, especially plasmacytoid dendritic cells (pDCs) that are the main producers of IFN- α (reviewed in Swiecki

and Colonna 2010). The 13 type I IFNs, each encoded by a separate gene on chromosome 9, as well as IFN- β signal through a common heterodimeric receptor, IFN- α receptor (IFNAR). The IFNAR is composed of 2 chains (IFNAR1 and IFNAR2) that signal through Jak1-Tyk2 and signal transducer and activator of transcription 1 protein (STAT1) (Uze and others 2007). The type I IFNs are important for protection against viral infections with powerful effects on both the innate and adaptive immune responses, but also have roles in cell growth regulation (Fitzgerald-Bocarsly and others 2008). Acting as an immune adjuvant, IFN- α increases the survival and activation of DCs and induces up-regulation of the major histocompatibility complex (MHC) and costimulatory molecules such as CD40 and CD86. IFN also induces DCs to promote the secretion of BlyS and APRIL, which, in turn, lead to increased B-cell survival, differentiation, and isotype class-switching. Type I IFNs directly affect T cells by increasing survival, activation, and cross-priming as well as promoting Th1 differentiation (Reviewed in Baccala and others 2007).

Type I IFN: Cells, Sensors, and Pathways

As the dominant alarm signal responding to virus infection, type I IFN (mostly IFN- β) can be produced by most cells, including macrophages, DCs, and epithelial cells (Fig. 1). In addition to virus infection, IFNs (mostly IFN- α) may be triggered by ICs containing nucleoproteins and, in some cases, by necrotic debris as discussed below. In most cells, a variety of cytosolic receptors or sensors exist to detect (foreign) nucleic acids. These sensors include Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLR) comprising RIG-I, and the melanoma differentiation-associated gene 5 (MDA-5) helicases and pyrin and HIN200 domain-containing protein receptors (reviewed in Swiecki and Colonna 2010; Barber 2011). Cytoplasmic DNA-sensing receptors are not yet fully characterized, but activation of

stimulator of IFN gene appears to be a common adapter in the non-TLR IFN pathway stimulated by DNA, and is also implicated in the RNA-stimulated RIG-I pathway (Barber 2011) (Fig. 1).

In contrast to the universality of IFN- β production, the main IFN- α producing cell among human leukocytes is the pDC. Although pDCs are present in very low frequency in human peripheral blood, accounting for only 0.2%–0.5% of human peripheral blood mononuclear cells (PBMC), they have the ability to produce 10–100 times more type I IFNs than other cell types. pDCs are unique in that they constitutively express high levels of the endosomal TLRs 7 and 9 as well as IFN regulatory factors (IRFs) 5 and 7, allowing them to rapidly produce type I IFNs in response to viral infection. Activation of TLR7 or 9 induces signaling through the myeloid differentiation factor 88 (MyD88) pathway leading to phosphorylation of IRFs 5 and 7 (Fig. 1), which in turn translocate to the nucleus and stimulate expression of IFNs (reviewed in Cao 2009).

Type I IFNs in SLE Pathogenesis

It has been known for some time that there are elevated levels of IFNs in the serum of patients with SLE, and that this elevation correlated with disease activity (Hooks and others 1979; Ytterberg and Schnitzer 1982). Additional evidence that IFNs may have a pathogenic role in the development of SLE came from the observation that some patients who were treated with type I IFNs for either malignancy or chronic infections developed a lupus-like syndrome that was indistinguishable from spontaneous SLE (Ronnblom and others 1991; Sanchez Roman and others 1994). More recently, several groups have shown through microarray analysis that peripheral blood mononuclear cells (PBMCs) from SLE patients overexpress a large number of IFN-stimulated genes (ISGs) (Baechler and others 2003; Bennett and others 2003). This discovery dramatically focused attention on type I IFN in SLE.

FIG. 1. Innate signaling pathways leading to type I interferon (IFN) production. Various triggers—viruses, nucleic acid-containing immune complexes, and necrotic debris associated with antimicrobial peptides—are sensed by pattern recognition receptors in cells, including endosomal Toll-like receptors (TLRs) and cytoplasmic RIG-I-like receptors (RLRs) and pyrin and HIN200 domain-containing protein (PYHIN) receptors and lead to IFN production. TREX1 negatively regulates interferon stimulation by cytoplasmic DNA. Yellow boxes represent adapter molecules necessary for downstream signal transduction. IPS-1, interferon-beta promotor stimulator 1; IRF, interferon regulatory factor; MyD88, myeloid differentiation factor 88; STING, stimulator of interferon gene; TRIF, Toll-receptor associated activator of interferon; TBK-1, TANK binding kinase1.

Sources of Autoantigens in SLE and Regulation of Type I IFN Production by pDC

Apoptosis and SLE

One mechanism by which nuclear material may become a source of autoantigens and lead to systemic autoimmunity is through defective clearance of dead and dying cells. In healthy individuals, apoptotic cells are rapidly removed by macrophages—a process that is inherently anti-inflammatory in nature. In many mouse models and in human SLE, there is evidence of defective clearance of apoptotic cells (Munoz and others 2010), resulting in a transition to a necrotic form of cell death. It is proposed that release of (modified) nuclear antigens exposes self-antigens (particularly nucleic acids) to intracellular sensors that respond by producing inflammatory cytokines, including IFN- α (Navratil and others 2006), although whether and how this material enters responding cells remains to be determined. The adjuvant effects of IFN promote loss of self-tolerance, production of autoantibodies against nuclear material, and IC formation (Martin and Elkon 2005). The process of IC formation generated by SLE autoantibodies and antigen derived from dead and dying cells can be replicated *in vitro* using apoptotic or necrotic debris. These ICs bind to low affinity Fc γ RIIa receptors expressed on the surface of pDCs and are endocytosed. In the endosome, RNA or DNA present in these complexes activate TLR7 or 9, respectively, and induce IFN- α production by pDCs (Martin and Elkon 2005; Ronnblom and others 2006). The ability of SLE sera to induce IFN- α production appears to be well correlated with the presence of antibodies against small nuclear ribonucleoprotein particles (RNPs) such as Sm or U1RNP (Vollmer and others 2005). In addition, the presence of antibodies specific for Sm, U1RNP, Ro, and dsDNA in serum has been associated with higher expression of ISGs in SLE patients, supporting the clinical significance of these observations (Kirou and others 2005).

Neutrophils and SLE

In addition to the IFN gene signature observed in microarrays when whole blood was analyzed, upregulation of granulopoiesis-related genes was also observed in the arrays obtained from SLE patients with active disease (Bennett and others 2003). These granulopoiesis-related genes include enzymes and enzyme inhibitors as well as bactericidal proteins. It has also been noted that SLE patients have increased numbers of apoptotic neutrophils in peripheral blood that correlated with anti-DNA antibodies and disease activity (Courtney and others 1999). Two recent studies have provided evidence that activation and/or apoptosis of neutrophils may directly contribute to the IFN signature in SLE.

As part of their antimicrobial functions, activated neutrophils release web-like structures called neutrophil extracellular traps (NETs), composed of large amounts of nuclear DNA in a specific type of cell death termed NETosis (Brinkmann and others 2004). Of note, IFN- α can prime mature neutrophils *in vitro* to form NETs (Martinelli and others 2004). Lande and others (2011) reported that SLE serum contains ICs composed of neutrophil-derived antimicrobial peptides and self-DNA. Neutrophil-specific antimicrobial peptides protect DNA from nuclease degradation *in vitro* and are required for pDC acti-

vation and IFN production. Peptide/DNA complexes were released by neutrophils undergoing NETosis and were able to trigger IFN- α production in pDCs through activation of TLR9 (Lande and others 2011). Significantly, SLE serum contains antibodies against the antimicrobial peptide LL37 and self-DNA, which can induce NET formation by neutrophils isolated from healthy donors. Interestingly, neutrophils from SLE patients release more NET DNA than healthy donors, even in the absence of *in vitro* stimulation (Lande and others 2011). In the study by Garcia-Romo and others (2011) neutrophils isolated from SLE patients were shown to undergo accelerated cell death *in vitro* and express genes involved in type I IFN and TLR signaling pathways. This same expression pattern was seen in neutrophils from healthy donors after exposure to SLE serum *in vitro*. Neutrophils from patients with SLE, but not healthy donors, underwent NETosis upon exposure to anti-RNP antibodies and caused IFN- α production by pDCs in an Fc γ RII, NADPH, and TLR7-dependent manner (Garcia-Romo and others 2011). SLE NETs released large amounts of extracellular DNA, LL37 (an antimicrobial peptide), and HMGB1 (a protein that leads to type I IFN secretion by binding to DNA and targeting cytosolic DNA receptors). Healthy neutrophils exposed to IFN- α are susceptible to NETosis induced by anti-RNP antibodies *in vitro* (Garcia-Romo and others 2011). Together, these data identify a pathogenetically important link between neutrophils, pDCs, IFN- α production, and autoantibodies in SLE, and describe a cycle of chronic inflammation that may be important in disease pathogenesis (Fig. 2).

Regulation of IFN- α production by pDC

A recent study by Eloranta and others (2009) explored the regulation of IFN- α production by RNA-containing IC-stimulated pDCs. PBMCs or pDCs primed with IFN- α 2b and granulocyte-macrophage colony-stimulating factor before stimulation increased subsequent IFN- α production—suggesting that pDCs themselves may enhance their own production of IFN- α . Monocytes inhibited IFN- α production in PBMC cultures, but had no effect on IFN- α production in isolated pDC cultures. CD56+ natural killer (NK) cells enhanced IFN- α production by pDCs, an effect that is lost with addition of monocytes to the cultures. Interestingly, monocytes isolated from SLE patients were less effective at inhibiting RNA IC-stimulated pDCs. The inhibitory effects of monocytes on IFN- α production by RNA-IC-stimulated pDCs could be mediated through production of prostaglandin E2, tumor necrosis factor alpha (TNF- α), or reactive oxygen species (Eloranta and others 2009). The mechanism by which CD56+ NK cells promoted IFN- α production by pDC after RNA-containing IC was subsequently shown to be via secretion of macrophage inflammatory protein 1 beta (MIP-1b) and cell-cell contact through lymphocyte function-associated antigen 1. Perhaps counter-intuitively, NK cells from SLE patients showed lower capacity to promote IFN- α production by RNA-containing IC-stimulated pDCs, although capacity was fully restored with the addition of exogenous IL-12 and IL-18 (Hagberg and others 2011).

Genetics, IFN, and SLE

SLE has a strong genetic predisposition as evidenced by the high concordance rates in mono- and dizygotic twins

FIG. 2. Stimulation of type I interferon by cell debris and immune complexes. Immune complexes (ICs) containing ribonucleoproteins (RNP) can be generated by the binding of autoantibodies to RNP containing antigens released by apoptotic or necrotic cells (*top right*). In addition, ICs containing deoxyribonucleoproteins (DNP) can be generated by the binding of autoantibodies to DNA or antimicrobial peptides, such as LL37, after NETosis of neutrophils (*bottom right*). Ultimately, these ICs are internalized by FcγR on plasmacytoid dendritic cells (pDC) and activate TLR7 or 9 resulting in the production of type I interferon in systemic lupus erythematosus (SLE; *top left*).

(Harley and others 2009). Although many mouse models have been generated by manipulation of single genes, the lupus phenotype in many cases depended upon background lupus prone genes present in C57BL/6×Sv129 mice (Carlucci and others 2007). Similarly, single-gene disorders predisposing to SLE in the human population are very uncommon—the majority of patients have multiple genetic variants, each of which has a small effect that combine to contribute to disease (Harley and others 2009). Below we will discuss the only 3 known, highly penetrant, monogenic deficiencies involving the first component of complement, C1q; an intracellular DNA exonuclease, TREX-1; or an intracellular acid phosphatase, TRAP. Provocatively, all of these syndromes are associated with alterations in type I IFN production. In addition, data from increasingly powerful GWAS have identified variants in >100 genes that have been reported to confer increased risk for the development of disease through several different pathways. In the following sections, we briefly highlight mono- and polygenic alterations that involve the type I IFN pathway.

Monogenic Deficiencies Associated with SLE

C1q deficiency

An extremely strong genetic risk for SLE is conferred by defects in the early complement components (C1q, C4, and, to a lesser extent, C2). To date, deficiency in C1q, the first component of the classical pathway, is the strongest known susceptibility factor identified in humans. More than 90% of individuals who have a homozygous deficiency of C1q develop SLE, usually with more severe disease manifestations (Pickering and others 2000). Although complete C1q deficiency is very rare, reduced production, increased consumption, and autoantibodies to C1q may contribute to acquired lowering of C1q levels (Trendelenburg 2005). C1q not only has a role in clearance of apoptotic cells, but can also

affect cytokine production by other immune cells (Fraser and others 2009).

Kirou and others (2005) reported that low complement levels are associated with the activation of the type I IFN pathway. Although these observations could be explained by increased disease activity, our group (Santer and others 2010) and Lood and others (2009) recently observed that C1q itself plays a direct role in the regulation of IFN-α stimulation by SLE ICs. The mechanisms whereby C1q protects against IFN-α production differed in these 2 studies. Whereas Lood and others reported that IC containing C1q were less stimulatory on isolated pDC, we observed that the presence of C1q in ICs diverted ICs away from pDC and favored binding to monocytes (that do not make IFN-α). Regardless of the mechanism involved, we demonstrated the clinical significance of these observations in that patients with C1q deficiency had increased serum and, in one case, CSF concentrations of IFN-α.

Our observation that C1q deficiency leads to defective suppression of IFN-α in response to nucleoprotein containing ICs raises the question of how IFN-α production is initiated in individuals with low C1q. There is good evidence to suggest that normal individuals are exposed to circulating ICs presumably after minor injury or infection and that one function of natural antibodies is to help facilitate the removal of tissue debris (Elkon and Casali 2008; Madi and others 2009). We propose that in C1q-sufficient individuals, monocytes and macrophages rapidly clear small amounts of ICs containing self-antigens. However, in C1q-deficient patients, these complexes would be less efficiently cleared by monocytes and would be more likely to engage pDCs with resulting induction of IFN-α. Once pDCs release IFN-α, this cytokine not only primes other cells of the immune system, but also renders pDCs more resistant to C1q-mediated inhibition (Santer and others 2010). Combined, these effects lead to a positive feedback loop that sustains the autoimmune response, as also evidenced by the reports of SLE

induced by type I IFN therapy discussed above. Whether a similar series of events occurs in patients with antibodies to C1q remains to be determined.

Deficiency of TREX1 and other intracellular nucleases

Mutations of any 1 of 3 genes encoding the intracellular nucleases, TREX1 (the most abundant 3' to 5' DNA exonuclease), RNase H2 (digests DNA:RNA hybrids), and SAMHD1 (a putative nuclease), cause the Aicardi Goutieres syndrome (AGS) in children (Crow and others 2006a, 2006b). This syndrome is characterized by mental retardation, cerebral atrophy (white matter destruction), and calcification of the basal ganglia, and is associated with CSF lymphocyte pleocytosis. Elevated levels of IFN- α is seen in the CSF of patients with AGS, and some patients also develop lupus-like features with hypocomplementemia and antinuclear autoantibodies (Ramantani and others 2010). Of note, *TREX1* mutations were found in 0.5%–2.7% of SLE patients (Lee-Kirsch and others 2007; Namjou and others 2011). In a recent analysis of over 8,000 multi-ancestral lupus patients (Namjou and others 2011), a *TREX1* risk allele was associated with neurologic manifestations, especially seizures in patients of European descent. In addition, a strong association between a *TREX1* single-nucleotide polymorphism and anti-nRNP antibodies was seen (Namjou and others 2011).

TREX1 is known to preferentially bind single-stranded DNA, and its homology to other editing enzymes suggested that it plays a role in DNA repair. However, TREX1 is most strongly implicated in interaction with an endoplasmic reticulum-associated DNA repair complex (the SET complex) that is involved in the caspase-independent, granzyme A-mediated form of apoptotic cell death (Chowdhury and others 2006). TREX1-knockout mice develop a lethal inflammatory myositis (Morita and others 2004). This finding was explained in a study by Stetson and others (2008), where TREX1 was shown to function as a negative regulator of the cytosolic IFN stimulatory DNA response (Fig. 1). Mice deficient in TREX1 had an increase in IRF3-mediated type I IFN production that caused heart inflammation. TREX1-deficient mice lacking the type I IFN receptor were

protected from disease and also did not develop autoantibodies (Stetson and others 2008). These data emphasize the pathogenic contribution of IFN- α in TREX1-mediated systemic autoimmunity.

TRAP deficiency

Although the immuno-osseous dysplasia spondyloenchondrodysplasia (SPENCD) has been regarded primarily as a skeletal dysplasia, SPENCD patients may also show immune dysfunction, with a particular susceptibility to the development of SLE. Two groups (Briggs and others 2011; Lausch and others 2011) have recently confirmed a high frequency of autoimmune phenotypes, including lupus, in SPENCD patients. Both studies identified loss-of-function mutations in the *ACP5* gene, encoding TRAP, as causative of the disease. Increased type I IFN activity and the presence of an IFN signature provided a pivotal link between this mutation and lupus—but why?

Osteopontin (OPN) is a glycoprotein that exists as both a secreted OPN and intracellular OPN (iOPN) protein. Post-translational modification and proteolytic cleavage indicate that OPN has a diversity of biological functions. In mouse pDC, iOPN has been described to regulate IFN- α secretion via MyD88 and TLR9 pathways. Since OPN is a recognized substrate for osteoclast-derived TRAP *in vivo* (Andersson and others 2003), it is possible that in the absence of TRAP in pDC, OPN would remain phosphorylated and maintain persistent stimulation of IFN- α through TLR9/MyD88. Lausch and others (2011) studied OPN and demonstrated that compared with controls, SPENCD patients have higher urinary levels of phosphorylated OPN. These results suggest that TRAP is responsible for dephosphorylating OPN and that this function is defective in patients with SPENCD. In summary, extracellular phosphorylated OPN results in osteoclast overactivation and the bony phenotype of SPENCD, whereas increased intracellular phosphorylated OPN enhances IFN- α levels, via TLR9 and MyD88 in pDCs (Fig. 3). Interestingly, variants in the *OPN* gene have been associated with SLE, and patients who carry risk alleles for this gene have been found to have elevated IFN- α activity (Kariuki and others 2009b).

FIG. 3. Hypothetical 2-step model depicting how tartrate-resistant acid phosphatase (TRAP) deficiency may regulate IFN- α production in pDCs. In step 1, the presence of cytoplasmic DNA leads to phosphorylation of intracellular osteopontin (iOPN). This in turn facilitates binding to MyD88/TLR9, activation of IRF7, and IFN- α production. In step 2, in normal cells (top right) TRAP dephosphorylates iOPN leading to reduction in IFN- α production (negative regulation). In the absence of TRAP (bottom right), iOPN remains phosphorylated, resulting in persistent activation of the TLR9/MyD88 pathway and continued IFN- α production.

Polygenic Variants in SLE

Candidate gene studies and GWAS in SLE

The importance of the MHC region in SLE susceptibility has long been known. Among class II genes, *HLA-DR2*, *HLA-DR3* (common in Europeans and Asians), and *HLA-DR8* (more common in Hispanics) were identified. Important Class III genes include *TNF*, *C2*, *C4A*, and *C4B* (Deng and Tsao 2010). Since the discovery of the IFN gene signature in SLE, there have been a number of candidate gene studies performed analyzing genes involved in the type I IFN pathway. In addition, there have been 7 GWAS performed on lupus patients (5 European and 2 Asian) who have confirmed the association of many candidate genes and have also identified a number of associations with new genetic variants. These studies are comprehensively reviewed in (Deng and Tsao 2010; Lee and Bae 2010; Sestak and others 2011). Here, we focus on genes and pathways relevant to IFN- α stimulation or signaling.

IRF5

IRF5 is the gene outside the HLA locus that is most strongly and consistently associated with SLE (reviewed in Kyogoku and Tsuchiya 2007). The IRF family is comprised of 9 genes (*IRF1-9*) that recognize a DNA sequence called the IRF enhancer, which is frequently found in the promoter region upstream of a number of ISGs. *IRF5* and *IRF7* are activated upon TLR7 and 9 signaling, resulting in type I IFN production (Kyogoku and Tsuchiya 2007). Sigurdsson and others (2005) first observed an association with *IRF5* and *TYK2* (tyrosine kinase 2) in a linkage study of SLE patients from Sweden, Finland, and Iceland. Since then, *IRF5* associations with SLE have been reproduced by many other groups, mostly in European populations, but also to a lesser extent in Hispanic, Asian, and African American patients (Lee and Bae 2010; Sestak and others 2011). To date, 4 functional variants of *IRF5* have been identified that define both risk and protective haplotypes in SLE (Graham and others 2006, 2007). In European populations, the variants of *IRF5* with the strongest probabilities of being causal in SLE are an SNP (rs10488631) location 3' of *IRF5*, and a CGGGG insertion-deletion polymorphism located 64 base pairs upstream of the first untranslated exon of *IRF5* (Sigurdsson and others 2008). Several groups reported that SLE patients carrying risk haplotypes for *IRF5* have higher serum IFN- α activity (Niewold and others 2007; Rullo and others 2010), and this effect may be most prominent in patients with either anti-RNA binding protein (RBP) or anti-dsDNA antibodies. It should be noted that *IRF5* is also implicated in B-lymphocyte activation and production of IL-12 by cells of the innate immune system.

Signal transducer and activator of transcription 4 protein

A strong association between SLE and *STAT4*, which encodes for STAT4, has been found in both candidate-gene and GWAS studies (Deng and Tsao 2010). The main action of STAT4 is on helper T (Th) cells—it is induced by IL-12, IL-23, and IFN- α , and promotes Th1 as well as Th17 responses (Korman and others 2008). In SLE, the risk variant for *STAT4* is associated with both lower serum IFN- α levels and higher IFN-induced gene expression in PBMCs (Kariuki and others

2009a). *IRF5* and *STAT4* risk alleles confer additive risk for SLE (Sigurdsson and others 2008).

Tyrosine-protein phosphatase nonreceptor type 22

Tyrosine-protein phosphatase nonreceptor type 22 (PTPN22) is a phosphatase that modulates antigen receptor signal transduction in both T and B lymphocytes (Cohen and others 1999). Two functional variants of the *PTPN22* gene have been identified—one confers a greater risk of SLE, whereas the other is protective (Kyogoku and others 2004; Orru and others 2009). One study has reported that SLE patients who carry the risk allele for *PTPN22* had higher serum IFN- α activity and lower serum TNF levels (Kariuki and others 2008), suggesting that, in addition to effects on lymphocyte signaling, PTPN22 variants may also be involved in type IFN regulation.

IFN: Clinical Aspects

IFN: disease activity and manifestations

Upregulation of ISGs was reported to correlate with disease activity, the incidence of nephritis, and the presence of certain autoantibodies, especially those against RNPs (Baechler and others 2003; Kirou and others 2005). Some longitudinal studies suggest that the IFN signature correlates with disease activity, but there are conflicting results (see below). A pathologic role for IFN- α has also been suggested in neuropsychiatric lupus (NPSLE). Cerebral spinal fluid (CSF) from patients with NPSLE induced higher IFN- α production in *in vitro* bioassays compared with other disease controls (multiple sclerosis and other autoimmune diseases). The interferogenic activity of the CSF is dependent of the presence of autoantibodies, Fc γ RII engagement, as well as endosomal transport of predominantly ribonucleoprotein antigens (Santer and others 2009).

An important consideration is what happens to the IFN gene signature with therapy. Glucocorticoids remain one the mainstays of treatment in SLE, and it has been reported that the ISGs tend to normalize in lupus patients treated with corticosteroids (Bennett and others 2003; Guiducci and others 2010), suggesting that they track with disease activity. The primary anti-inflammatory mechanism of glucocorticoids is not through direct down-regulation of IFN- α , but instead is thought to be through nuclear factor kappa B (NF- κ B) inhibition (De Bosscher and others 2000). Glucocorticoids have also been shown to induce apoptosis in many cell types, including pDCs—a response that is abrogated in the presence of TLR7 and 9 activation (Boor and others 2006). As has been discussed above, one of the main pathogenic mechanisms of IFN production in SLE is through activation of TLR7 and 9 signaling, which also activates the NF- κ B pathway necessary for pDC survival. The effect of glucocorticoids on TLR7 and 9 activation of pDCs and subsequent IFN- α production was analyzed in a recent study by Guiducci and others (2010). In addition to decreasing the amount of apoptotic cell death, TLR7 and 9 stimulation was shown to restore IFN- α production in pDCs exposed to glucocorticoids. In SLE patients (and 2 lupus-prone mouse strains), the inhibitory effects of glucocorticoids on the IFN pathway were reduced. Further, glucocorticoids did not affect NF- κ B activation in TLR7 or 9-stimulated pDCs, thus preventing glucocorticoid-induced

pDC death, with the consequence that systemic IFN- α levels were maintained (Guiducci and others 2010). Therapeutic approaches that attenuate either IFN- α production or its downstream effects are currently under investigation.

Therapy

Three different monoclonal antibodies targeting IFN- α , namely, MeDi-545 (sifalimumab), rhuMab iFn- α (rontalizumab), and nnC 0152-0000-0001, are in clinical trials. Results have been published from the first phase 1 study of sifalimumab (Yao and others 2009). Pairwise comparisons of patients treated with sifalimumab or placebo revealed that treatment caused a significant ($P < 0.05$) dose-dependent inhibition of the IFN signature that was observed for the 3, 10, and 30 mg/kg doses of anti-IFN- α antibody. There was a similar trend of reduction in ISGs in the skin lesions of a small number of SLE patients analyzed. No serious adverse events were reported. Preliminary results of the phase 2a trial have been reported in abstract form (Merrill and others 2011). In this 14-week trial, although ISGs declined by an average of 40%, significant changes in disease activity measures were not observed. Longer term studies are eagerly awaited.

ISGs as biomarkers

Because of the reported association with disease activity, there has been interest in evaluating the IFN gene signature or ISG protein products as biomarkers of disease activity in lupus. Although longitudinal data are limited, 2 recent studies found that while a high IFN gene signature is associated with more active disease at a single point in time, its utility as a possible biomarker is limited (Landolt-Marticorena and others 2009; Petri and others 2009). However, different approaches for evaluating the IFN response are still under investigation. For example, Chaussabel and others (2008) have developed a unique method of analyzing blood transcriptional profiles by grouping gene transcripts with common biological functions into modules. They observed that modular profiles can discriminate between several different disease states—healthy, *Streptococcus pneumoniae* infection, melanoma, post-transplant, and lupus. In SLE, the ISG module, among others, correlated with disease activity. Assays for protein products encoded by ISGs have also been used to evaluate disease activity. For example, it was reported that the combined measurement of serum levels of CXCL10 (IFN γ -inducible 10-kd protein), CCL2 (monocyte chemotactic protein 1), and CCL19 (MIP-3b) was predictive of a lupus flare over the ensuing year (Bauer and others 2009).

Summary and Conclusions

Although long implicated in the pathogenesis of SLE, the pre-eminence of ISGs in microarrays has focused attention on the genesis and pathological role of this cytokine in SLE and related systemic autoimmune disorders. In a remarkably short space of time, this has led to clinical trials that attempt to block the adjuvant and inflammatory properties of this cytokine. Since IFN- α is also thought to be vital to immune defense against viruses, other strategies to interrupt either IFN- α induction or downstream effects will no doubt also be tested.

What is less clear is whether and how IFN- α is generated early in disease—before production of high titer IgG antibodies targeted against nucleoprotein antigens. More exciting discoveries will no doubt follow.

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