Published in final edited form as:

Transl Res. 2015 February ; 165(2): 283–295. doi:10.1016/j.trsl.2014.10.002.

Interferon Regulatory Factors: Critical Mediators of Human Lupus

Mark A. Jensen, PhD and Timothy B. Niewold, MD

Department of Immunology and Division of Rheumatology, Mayo Clinic, Rochester, MN

Abstract

The pathogenesis of systemic lupus erythematosus (SLE) is multi-factorial, and the interferon regulatory factors (IRFs) play an important role. Autoantibodies formed in SLE target nuclear antigens, and immune complexes formed by these antibodies contain nucleic acid. These immune complexes can activate anti-viral pattern-recognition receptors (PRRs), resulting in the downstream activation of interferon regulatory factors (IRFs), which can induce type I interferon and other inflammatory mediators. Genetic variations in IRFs have been associated with susceptibility to SLE, and current evidence supports the idea that these polymorphisms are gain-of-function in humans. Recent studies suggest that these genetic variations contribute to the break in humoral tolerance that allows for nucleic acid binding autoantibodies, and that the same polymorphisms also augment type I IFN production in the presence of these autoantibody immune complexes, forming a feed-forward loop. In this review, we will outline major features of the PRR/IRF systems and describe the role of the IRFs in human SLE pathogenesis.

Introduction

Systemic lupus erythematosus (SLE) is a chronic complex multisystem autoimmune disease that often arises during early adulthood, resulting in great morbidity. Clinical symptomatology varies between individuals and within individuals over time, as inflammation often affects multiple organs and tissues. Almost any organ system can be involved, and sites commonly impacted by disease include the joints, skin, lungs, neurological system, kidneys, and hematological systems¹. Antibodies reactive to nucleic acids and nuclear proteins are prominent in lupus subjects and most individuals have circulating antibodies to numerous autoantigens. Antinuclear antibodies found in the disease can be reactive to nucleosomes, double-strand DNA, Ro/SSA, Smith, small nuclear ribonucleoproteins, and La/SSB, with up to 90% of patients having reactivity against nucleosomes¹. Disease is thought to result from a break in immune tolerance to self-proteins, including DNA and other nuclear proteins,

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Corresponding author: Timothy B. Niewold, MD; Mayo Clinic, Division of Rheumatology, Department of Immunology, 200 1st St. SW, Guggenheim Bldg. 3-42, Rochester, MN 55905; Phone: 507-284-8450; Fax: 507 284 0564; Niewold.Timothy@mayo.edu.

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The authors have no conflicts of interest and have read the journal of Translational Research authorship agreement. The manuscript was prepared by and approved by both authors.

with both genetic and environmental factors contributing to disease susceptibility and symptomology.

Though the etiology of SLE is unknown, many individuals with SLE show defects in their ability to clear apoptotic cells and cellular debris, suggesting that an excess of nucleic material contributes to autoreactivity to nuclear antigens²⁻¹⁰. Reactivity to nuclear material leads to the formation of circulating immune complexes that serve as a stimulus for the production of type I interferon by plasmacytoid dendritic cells (PDCs)¹¹⁻¹⁴. Immune complexes are internalized in PDCs via surface expressed Fc receptors and traffic to endosomal compartments where the nucleic acid contained within them binds to toll like receptors (TLRs) that recognize RNA (TLR7, TLR8) and DNA (TLR9). TLR7, TLR8, and TLR9 triggering activates members of interferon regulatory factor (IRFs) family of transcription factors including IRF1, IRF3, IRF5, IRF7, and IRF9, that are positive regulators of type I interferons (IFN-1)¹⁵¹⁶⁻²¹. Uptake of immune complexes is thought to trigger IFN-1 production by PDCs, and elevated levels of type I IFNs are detectable in the blood of roughly half of SLE individuals²²⁻²⁶. This concept is supported by the fact that the strongest predictor of high type I IFN levels in human SLE patients is the presence of antibodies targeting DNA and RNA-binding proteins²⁷. Interestingly, these autoantibodies are not sufficient for high serum type I IFN²⁸, suggesting that there are also disease-associated factors that modify the association between nucleic acid ICs and high IFN in SLE. One likely factor is intrinsic over-activity within the TLR signaling system. Human genetic variants in IRF5, IRF7, and IRF8 have been implicated in SLE susceptibility²⁹⁻³⁶, suggesting that gain-of-function polymorphisms in these molecules augment TLR signaling. Many studies have placed the IRF family as central mediators in human SLE³⁷. In this review, we will summarize the TLR/IRF/type I IFN pathways and the role of this system in human SLE pathogenesis.

Type I IFN in SLE

Type I IFNs are classically involved in viral defense, and induce activation of antigen presenting cells and increased expression of MHC and co-stimulatory molecules. Type I IFN has been implicated as a primary pathogenic factor in human SLE by various lines of evidence³⁸. High type I IFN levels are clustered in SLE families as a heritable trait^{39, 40}, and this clustering supporting heritability is not observed with other SLE-associated cytokines such as tumor necrosis factor alpha^{41, 42}. Many of the genetic factors associated with SLE function within the type I IFN pathway⁴³, with clear over-representation of the IRF family as noted above (three of the nine IRF family members have genetic variants associated with SLE). When SLE-risk polymorphisms in IFN pathway genes have been investigated functionally in humans, they have been associated with increased type I IFN pathway signaling⁴⁴⁻⁴⁶, supporting the idea that gain-of-function variants in the type I IFN pathway underlie human SLE^{47, 48}. Additionally, human subjects given recombinant human type I IFN in the form of IFN- α 2 as a treatment for malignancy or chronic viral infection have developed de novo SLE⁴⁹, which has generally resolved when the IFN- α was discontinued⁵⁰. These data all support a causal role for type I IFN in human SLE. There are two major categories of type I interferons, alpha and beta, with the majority of circulating IFN activity in SLE attributable to the IFN- α isoforms^{39, 51, 52}. A prominent IFN gene

signature, comprised of genes strongly activated by type I IFNs (interferon stimulated genes (ISGs)), is present in circulating peripheral blood mononuclear cells (PBMC) in a sub group of SLE patients and is associated with the presence of anti-dsDNA antibodies, elevated IFN levels, and worse disease activity^{23, 24, 51, 53}. Type I IFNs also induce members of the STAT and IRF transcription factor families, including IRF5 and IRF7, and which can then influence the expression of extended gene networks⁵⁴.

Type I IFNs (IFN- α and IFN- β) bind to a single receptor; a heterodimer (IFNAR) consisting of IFNAR1 and IFNAR2 chains. Additionally, IFN- β has the capacity to bind to and trigger signaling via the IFNAR1 chain in the absence of the IFNAR2 chain⁵⁵. IFNAR signaling activates the receptor-associated kinases Janus kinase I (JAKI) and tyrosine kinase 2 (TYK2) that then phosphorylate STAT1 and STAT2 (pSTAT1 and pSTAT2)⁵⁶. Heterodimers of pSTAT1 and pSTAT2 translocate to the nucleus and conjoin with interferon regulatory factor 9 (IRF9) to form IFN-stimulated gene factor 3 (ISGF3)^{56, 57}. ISGF3 binds to DNA-sequences called IFN-stimulated response elements (ISREs; consensus sequence TTTCNNTTTC) present in the promoters of many ISGs inducing their transcription. Most cells are competent to express low levels of early or immediate IFN-1 genes, including IFN- α 4 and IFN- β , which then bind to the IFN receptor, an autocrine or paracrine manner, to activate the JAK-STAT pathway leading to ISGF3 formation⁵⁸. IRF1, IRF3, and IRF5 have each been shown to play essential roles in different in vitro assays in the induction of this early IFN response⁵⁸. ISGF3 induces the expression of IRF7 which is required for the induction of high levels of late or delayed type I IFNs by plasmacytoid dendritic cells (PDCs) downstream of multiple PRR pathways⁵⁸⁻⁶⁰. Thus the following sequence of events is thought to occur in SLE, leading to a positive feedback loop. Nucleic acids bind to cytoplasmic or transmembrane expressed PRRs, resulting in the induction of early type I IFNs, which bind to the type I IFN receptor. This primes the cells by inducing IRFs such as IRF5, IRF7, IRF9, as well as STAT family members, and any further triggering of PRRs in the cells leads to induction of high levels of late IFN-1s. Multiple IRFs, including IRF3, IRF5, and IRF7 are integral to the pattern-recognition receptors (PRRs) response and the IFN response within this cycle⁶¹.

Pattern Recognition Receptors

Pattern recognition receptors (PRRs) were initially characterized as sensing receptors that recognized pathogen associated molecular patterns (PAMPs) associated with fungi, bacteria, flagella, and viruses, and are now understood to also recognize damage associated molecular patterns (DAMPs) released by damaged cells and tissues⁶². PRRs may be broadly subdivided into membrane bound types that include the toll like receptors (TLRs) and c-type lectin receptors (CLRs); cytoplasmic sensors that include NOD like receptors (NLRs), pyrin, and HIN domain-containing members (PYHIN), RIG-I-like receptors (RLRs), and additional cytoplasmic and nuclear nucleic acid sensing receptors⁶³. Several PRRs that recognize nucleic acids (cytosolic RLRs, cytosolic DNA sensors, and several members of the TLRs) are of particular interest in understanding the pathogenesis of SLE in that nucleic acids (and the proteins that bind nucleic acids, nucleosomal proteins) are targets of the autoreactive response in SLE patients, and triggering these PRRs induces type I IFN production⁶⁴. Events that lead to the initial break in tolerance and reactivity to nuclear

matter in SLE and also to the ongoing reactivity to nucleic acid in lupus patients are thought to likely involve multiple types of nucleic acid sensing PRRs that signal through members of the IRF family ⁶⁵.

Nucleic Acid Sensing TLRs

Nucleic acid sensing TLRs are expressed within endosomes and include TLR3 and TLR7-9 ⁶⁶. TLR3 binds dsRNA and synthetic dsRNA analogues such as poly I:C. TLR7 and TLR8 recognize single strand viral RNA, self RNA bound in immune complexes, and imidazoquinoxaline derivatives such as resiquimod (R848). Though TLR7 and TLR8 both bind ssRNA, TLR7 preferentially binds ssRNA rich in GU while TLR8 is activated with ssRNA rich in AU. TLR9 binds dsDNA and oligonucleotides that have a high content of unmethylated CpG motifs resembling bacterial DNA. The CpG motifs have been thought to be key PAMP structures associated TLR9 sensing, however mammalian DNA low in CpG motifs can also potentially activate TLR9 when efficiently translocated to endosomes ⁶⁷. Some studies indicate that the key structure in DNA associated with TLR9 recognition appears to be the 2' deoxyribose phosphate backbone ⁶⁷⁻⁶⁹. TLR3 signals through the adaptor TRIF, which associates with TRAF3. TRAF3 associates with TBK1 and IKK- ϵ which phosphorylates IRF3 leading to IFN- β production. TLR7-9 signal through the adapter MyD88 and IRAK4 which complex with TRAF6, TRAF3, osteopontin and the kinases IRAK1 and IKK- α that phosphorylate IRF7 in PDCs and IRF5 in macrophages and myeloid dendritic cells (MDCs).

Cytosolic Pattern Recognition Receptors

The two main cytosolic RNA sensing PRRs that sense intracellular RNA are the RLRs, retinoic acid-inducible gene 1 (RIG-1) and melanoma differentiation factor 5 (MDA5) ⁷⁰. RIG-1 recognizes single-stranded RNA (ssRNA) that contains a 5'-triphosphate moiety and short double stranded RNA (dsRNA) while MDA5 is efficiently activated by long dsRNA ⁷¹. Both sensors signal through adapter mitochondrial antiviral signaling (MAVS; also known as IPS-1/CARDIF/VISA) that leads to activation of the kinases, TANK-binding kinase 1 (TBK1) and inducible kinase I κ B kinase (IKK- β ; also known as IKK- ϵ) that then phosphorylate IRF3 and IRF7 which induce IFN-1 genes ^{72, 73}. IRF5 also appears able to activate the early IFN-1 response downstream of MAVS independent of IRF3 and IRF7 ^{20, 74}. Importantly, gain of function mutations in MDA5 lead to a lupus like disease in mice ⁷⁵. Furthermore, polymorphisms in or near IFIH1, the gene encoding MDA5, leads to increased susceptibility to lupus and patients with anti-dsDNA antibodies have an heightened IFN gene signature that is associated with increased responsiveness to type I IFNs ^{46, 76}. Additionally, loss-of-function variants in MAVS result in lower type I IFN levels in SLE patients ⁷⁷, further supporting the importance of the cytosolic PRR pathways in human SLE.

DNA sensing PRRs are less well understood than are the RNA sensors but several candidate sensors have been identified and include DNA-dependent activator of interferon regulator factors (DAI, also known as ZBP1), DEAD box polypeptide 41 (DDX41), interferon- γ inducible protein 16 (IFI16), cyclic guanosine monophosphate-adenosine monophosphate

synthase (cGAS), meiotic recombination homolog 11a (MRE11), and absent in melanoma (Aim2)⁷⁸⁻⁹⁰. Signaling through DAI, DDX41, IFI16, MRE11, and cGAS leads to IRF3-dependent IFN production while NLRs and Aim2 signaling leads to inflammasome activation. In addition to the sensors listed above, cytosolic double stranded DNA (poly dA-dT) is detected by RNA polymerase III which transcribes the dsDNA into poly (A-U) RNA that results in IFN production by the RIG-1/MAVS pathway described above⁹¹.

IFI16, DDX41, cGAS, MRE11, and likely DAI, all induce type I IFN expression in a stimulator of IFN genes (STING) dependent manner^{89, 92}. Importantly, DNA-bound cGAS synthesizes cyclic GMP-AMP which binds STING and acts as intermediary between cGAS and STING and possibly for other DNA sensors as well^{82, 83}. STING (also known as MITA/MPYS/ERIS) provides a scaffold for bringing IRF3 in close proximity to tank binding kinase (TBK1) which phosphorylates IRF3 leading to its activation and downstream activation of type I IFN gene expression. Triggering of these sensors by poorly cleared cytoplasmic DNA appears to be linked to lupus type diseases in humans and in mice in studies involving mutations in the exonuclease Trex1. Trex1 digests single-stranded DNA in the cytoplasm arising from the retrotranscription of endogenous retroelements^{93, 94}. In humans, rare loss of function mutations in Trex1 cause Aicardi-Goutieres syndrome (AGS) and chilblain lupus, while other more common variants are associated with SLE⁹⁵⁻⁹⁹. TREX1 deficient mice show increased levels of cytoplasmic DNA, develop myocarditis and lupus-like symptoms including multi-organ inflammation and renal IgG deposition that are dependent on host expression of IRF3 and associated with anti-nuclear antibodies and an IFN gene signature^{94, 100}. An IFN gene signature is still detectable in heart tissue of Trex1^{-/-} Rag2^{-/-} mice suggesting that an increased type I IFN response precedes the autoreactive adaptive immune response in these mice^{94, 100}.

Interferon Regulatory Factors

Interferon regulatory factors (IRFs) comprise a family of transcription factors that include nine members, IRF1, IRF2, IRF3, IRF4 (also known as LSIRE, PIP or ICSAT), IRF5, IRF6, IRF7, IRF8 (also known as ICSBP), and IRF9 (also known as ISGF γ) in humans and mice (reviewed in^{65, 101}). IRFs play prominent roles in the signaling cascades of pattern-recognition receptors (PRRs) such as TLRs that induce type I IFNs, cytokine secretion, cellular apoptosis, and cell activation and differentiation. In 1998, X-ray crystallographic studies showed that the N-terminal domain of IRF1, the first IRF family member discovered bound to a sequence 5'-GAAA-3' within the promoter region of IFN β . The N-terminal region of IRF1 forms a helix-turn-helix structure that is common among DNA-binding proteins and is referred to as the N-terminal DNA binding domain (NDB)¹⁰². Further studies on other IRF family members defined the NDB has a region approximately 120 amino acids shared among all IRFs family members that binds to the core recognition sequence, GAAANNGAAAG/CT/C, where N denotes any nucleotide^{103, 104}. IRF members, except for IRF1 and IRF2, also contain a C-terminal IRF association domain (IAD). The IAD allows for homodimeric interactions or heterodimeric binding to other IRF family members. Some IRF members have also been shown to interact with members of the ETS, STAT, and AP1 family members via binding to this region. A conserved auto inhibitory region blocks IAD binding which is relieved by phosphorylation of key residues

within this region in several IRF family members (including IRF3, IRF4, IRF5, and IRF7)¹⁰⁵⁻¹⁰⁸. IRF family members regulate the expression of numerous genes, including the type I IFNs (IFN- β and IFN- α) and genes induced by type I IFNs (IFN-stimulated genes, ISGs), via different signaling pathways in cell specific manners. A diagram outlining interactions between PRRs and IRFs is shown in Figure 1.

Genetic polymorphisms in several molecules of the nucleic acid sensing PRR pathways have been associated with SLE susceptibility. As noted above, three members of the IRF family have been implicated as genetic risk factors for in SLE, and these will be dealt with in detail in later sections. Additionally, genetic polymorphisms in other genes involved in PRR pathways have been reported, and these are summarized in Table 1. These PRR pathway gene polymorphisms include a variant of the RNA sensor, IFIH1, tagged by rs1990760 that shows genetic association for the presence of anti-dsDNA antibodies and also for lower levels of type I IFN levels in individuals with anti-dsDNA antibodies (i.e. dsDNA antibody positive and IFN low)^{34, 109}. Though these individuals have lower IFN levels, the IFIH1 variant also associates with increased cellular response to type I IFNs as measured by IFN-induced gene expression in peripheral blood mononuclear cells, suggesting an effect on sensitivity to IFN signaling¹⁰⁹. A STAT4 variant shows a similar association with low IFN levels and increased cellular response to type I IFNs in SLE patients as does the IFIH1 variant, and these two genetic effects are independent^{46,109}. SLE-risk genes not directly associated with PRR signaling pathways may also influence the type I IFN pathway in SLE patients. For example, methyl CpG binding protein 2 (MECP2) represses gene transcription by affecting DNA methylation and histone acetylation, but may also augment gene expression by recruiting CREB1 to chromatin. An SLE-risk polymorphism in the MECP2 gene is associated with altered transcript levels in PBMC of lupus patients¹¹⁰⁻¹¹². This variant appears to modulate gene expression differently than the non-risk MECP2 variant and its presence is linked to increased expression of a wide range of genes, many of which are induced by type I IFNs^{111, 112}. Collectively, these studies emphasize the importance of PRR pathways in human SLE pathogenesis, and ways in which different genetic polymorphisms may be tuning the PRR and IFN pathways in SLE patients. Studies of the IRF family of transcription factors in humans provide further support for this idea, and we will discuss the SLE-associated IRFs in detail next.

IRF5

IRF5 is a key regulator of numerous biological functions that contribute to the pathogenesis of SLE. Early studies using IRF deficient mice showed a definite role for IRF5 in TLR-mediated production of pro-inflammatory cytokines including IL-6, TNF- α , and IL-12^{60, 125}. High expression levels of IRF5 are associated with macrophage polarization and conversion from M2 to M1 macrophages¹²⁶. In vitro studies of virally infected or TLR7 triggered human cells showed that IRF5 plays a role in induction of type I IFNs and that IRF5 homodimers or heterodimers formed between IRF5 and other IRF members bind to promoter regions of specific IFN genes and selectively induced the transcription of different IFN gene members including IFN- β ¹²⁷. In vivo, IRF5 contributes to IFN production in virally infected mice in an MAVS dependent manner²⁰. IRF5 also influences B cell responses by regulating transcription of B-lymphocyte-induced maturation protein

(Blimp-1) crucial for B cell maturation and plasma cell formation, promoting B cell switching to the pathogenic IgG2a isotype, and possibly through induction of increased circulating levels of Blys¹²⁸. Finally, IRF5 appears to be strongly proapoptotic and promotes DNA damage-induced apoptosis¹²⁹⁻¹³¹.

Several genetic polymorphisms in IRF5 are associated with SLE susceptibility, and with increased circulating type I IFN levels^{132, 133}. The IRF5 gene structure has 4 non-coding first exons (in sequence order of exon 1d, 1a, 1b, and 1c) that are alternatively used and 8 coding exons (exons 2 – 9). Each first exon has its own promoter that directs transcription of the IRF5 gene to yield more than 14 different mRNA isoforms expressed in cell specific manners, resulting in significant complexity in the IRF5 gene transcriptome¹³⁴. The genetic epidemiology of IRF5 in SLE is similarly complex, as there are multiple polymorphisms which occur together in combinations on various haplotypes that result in graded degrees of SLE risk. Functional polymorphisms on IRF5 haplotypes include a promoter insertion/deletion, a splice site variation that allows exon 1b to be spliced in, two coding change variations in exon 6, and a 3' UTR polymorphism that results in alternate polyadenylation¹³⁵⁻¹³⁸ (Table 2). These functional genetic variations come together on chromosomes in particular combinations (haplotypes), and various combinations are associated with risk of SLE the formation of anti-Ro, anti-La, and anti-dsDNA autoantibodies³⁰ (Table 3). Interestingly, when type I IFN levels are examined in the context of IRF5 genotype, elevations in type I IFN related to IRF5 genotype are only observed in patients who have one of these associated autoantibodies³⁰. This suggests a model in which Gene + Autoantibody = High IFN, and would fit an induced model of function for SLE-associated variants in IRF5, in which the autoantibodies provide a chronic endogenous stimulus which along with IRF5 variants is sufficient to result in dysregulation of circulating IFN levels³⁷. When case-control genetic data for IRF5 are re-analyzed with respect to autoantibodies, >70% of the genetic risk of SLE related to IRF5 is carried within these two serologic subgroups of patients, which compose approximately half of the SLE population³⁰. And it is notable that this gene is one of the strongest overall case-control genetic associations in SLE, despite this strong subgroup effect. While this subset effect could relate to the effect of the gene-antibody interaction upon IFN levels, IRF5 variants could also predispose to autoantibody production, and this hypothesis could fit the data as well. TLRs are important in B cell differentiation, and it is plausible that IRFs could augment TLR signaling in B cells resulting in an increased propensity to autoantibody production. In a study that explores this question in humans, Cherian et al studied IRF5 genotypes in a group of subjects who had SLE-associated autoantibodies but were otherwise healthy to assess whether IRF5 was associated with serologic immunity in the absence of SLE disease and high IFN¹³⁹. They found that IRF5 haplotypes were enriched in serologically positive healthy individuals to the same degree as SLE patients, suggesting that IRF5 is involved in serologic autoimmunity in humans¹³⁹. This supports a model in which the SLE-associated variants in IRF5 predispose to autoantibodies that can bind nucleic acid, and then these same autoantibodies form immune complexes which trigger the TLR system in innate immune cells, resulting in over-production of IFN in the setting of the same genetic variants. This would be an example of the same variant functioning in the TLR system in two different immune cell types, with a synergistic or feed-forward effect in which the

product of the hyperactive TLR system in B cells provides a chronic TLR stimulus to innate immune cells with a similarly hyperactive TLR system. The complexity of IRF5 genetics and the functional effects described thus far is impressive, and these are summarized in Table 2.

IRF7

IRF7 functions downstream of the endosomal TLRs via the MyD88 adaptor protein, and is activated by phosphorylation¹⁴¹. In addition to phosphorylation, ubiquitination likely plays a role in IRF7 regulation as well¹⁴¹. IRF7 is a major transcription factor involved in type I IFN production in plasmacytoid dendritic cells, and can cooperate with IRF3 to induce robust IFN production following viral infection¹⁷. Genetic variations in IRF7 have been associated with SLE susceptibility^{142, 143}. The genetic polymorphisms in IRF7 that are associated with SLE are also associated with anti-dsDNA and anti-RNA-binding protein antibodies¹⁴⁴, similar to the above situation with IRF5. In a similar parallel fashion, genetic variations in IRF7 are associated with increased type I IFN only in the presence of these specific SLE-associated autoantibodies. These data further support an induced model of IRFs impacting circulating IFN levels, in which the autoantibody immune complexes presumably act as a chronic endogenous stimulus. This parallel relationship between IRF5 and IRF7 in human SLE strengthens the confidence in this mechanism as a common pathogenic route in SLE. In the case of both IRF5 and IRF7, these associations between genotype and cytokine phenotype extended across difference ancestral backgrounds.

IRF8

IRF8 is another member of the IRF family which can be activated downstream of PRRs. IRF8 differs in that it does not interact directly with MyD88, but is important to signals downstream of TLR9, as IRF8 deficiency greatly reduces TLR9-induced cytokine production by murine dendritic cells¹⁴⁵. In humans, IRF8 deficiency results in an immunodeficiency characterized by the loss of monocytes and dendritic cells¹⁴⁶, supporting a role for IRF8 in monocyte and dendritic cell development. IRF8 is involved in transcriptional responses to type I IFNs, and can induce transcription of inflammatory cytokines¹⁴⁷. Genetic variations in IRF8 have been associated with susceptibility to both SLE and multiple sclerosis (MS), although the specific variants associated with each disease differ somewhat^{35, 148}. Type I IFNs play opposite roles in MS and SLE, while IFN- α is elevated and thought to be causal in SLE, type I IFN levels are lower in MS patients than in healthy controls^{149, 150}, and recombinant human IFN- β is commonly used as a therapeutic in multiple sclerosis. While both SLE and MS are considered autoimmune diseases, it seems likely that stark differences exist between the two conditions in the regulation of type I IFNs¹⁵¹. Given this background, it is interesting that genetic variations in IRF8 are associated with both of these autoimmune conditions. Type I IFN levels were not different in SLE patients in the context of the SLE-associated genetic polymorphisms³⁶. Interestingly, the MS-associated genetic variant was associated with decreased type I IFN levels in both SLE and MS, and was associated with anti-dsDNA antibodies and increased IRF8 in B cells in the SLE patients³⁶. These data suggest that the MS-associated allele of IRF8 contributes to autoimmunity in the setting of low type I IFN levels, and may play a

role in humoral autoimmunity given the antibody and B-cell associations. It is possible that the SLE-associated allele exerts a different influence on the immune system which was not assessed in the study referenced above.

Conclusions

These studies illustrate how deeply interwoven the IRF family of transcription factors is within human SLE. IRFs provide a link between the autoantibodies formed in SLE and the characteristic activation of the type I IFN pathway observed in the disease. In fact, the studies also support involvement of the IRFs in the initial break in humoral tolerance, as in each case the IRF variant are associated with specific autoantibodies. The fact that SLE-associated variants in IRF5 are associated with these antibodies in the absence of disease¹³⁹, and that the SLE-associated variant of IRF8 alters IRF8 expression specifically in B cells support the concept that IRFs play a primary role in humoral autoimmunity in addition to aberrant type I IFN production in SLE. This suggests a feed-forward loop, in which the IRF variations predispose to autoantibody formation in SLE, possibly via effects in B cells. Then immune complexes made from these autoantibodies provide a chronic stimulus to PRRs, and the same gain-of-function genetic variations result in increased type I IFN production. This provides a fascinating paradigm in human autoimmune disease pathogenesis, that the same gain-of-function polymorphisms could play multiple roles across different immune cell types, with activation of the TLR/IRF pathway in one cell producing a chronic stimulus to that same pathway in another cell type. This type of feed-forward model could explain the particular relevance of the IRF family of transcription factors in human SLE.

Acknowledgements

Funding Sources: TB Niewold – Research grants from the NIH (AR060861, AI083790, AI071651), Rheumatology Research Foundation, the Mayo Clinic Foundation, and the Lupus Foundation of Minnesota.

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Figure 1.

Pattern Recognition Receptor IFN-α Response to Nucleic Acids MDCs/Macrophages and PDCs. In MDCs and macrophages, nucleic acid receptors are detected by RNA and DNA sensing receptors in the cytoplasm and by Toll like receptors (TLR3, TLR7, TLR8, and TLR9) that detect nucleic acids and signal within endolysosomes. The cytosolic DNA sensors, cGAS, DDX41, and IFI16 signal through STING to activate the kinase TBK1 that then phosphorylates IRF3 that then leads to IFN-1 production. cGAS, once activated induces synthesis of cGAMP which binds to and activates STING. The two cytosolic RNA receptors, MDA5 and RIG1, signal through MAVS to activate the kinases TBK1 and IKKε

that then phosphorylate IRF3 and IRF7 respectively, and also IRF5 under certain conditions. TLR3, TLR7 and TLR8 sense RNA and TLR9 sensed DNA when imported to endolysosomes by absorptive receptors, such as Fc receptors expressed by dendritic cells and macrophages. TLR3 signals through TRIF and TRAF6 to activate IRF3 while TLR7, TLR8, and TLR9 signal through Myd88 to activate IRF5. PDCs have the capacity to produce large amounts of IFN- α when activated through the TLR7 and TLR9 receptors by signaling through IRF7. TLR triggering in these cells also seems able to activate IRF5 leading to production of IFN- β .

Table 1

Proteins involved in IRF signaling pathways that have genetic variants linked to SLE susceptibility

Protein	Gene	Name	IRF	Function/Variant Specific Effects
TNFAIP3 (A20)	<i>TNFAIP3</i>	tumor necrosis factor alpha inducible protein 3	3, 4, 5	ubiquitin-modifying enzyme that limits NF- κ B signaling following TNF- α , TLR, IL-1R, and NOD2 signaling; risk variant associated with reduced transcript and protein levels ^{113, 114}
ABIN1	<i>tnfrif1</i>	A20-binding inhibitor of NF- κ B 1 (also TNFAIP3 interacting protein 1)	3, 4, 5	facilitates IKK α ubiquitination by recruiting A20 to IKK γ (NEMO); limits NF- κ B signaling; two independent risk loci linked to lower TNIP1 transcripts and ABIN1 protein levels ^{31, 115}
IRAK1	<i>irak1</i>	interleukin-1 receptor related kinase-1	5, 7	essential kinase for IRF5 and IRF7 activation in the TLR7/8/9 signaling pathways ^{19, 116} variant linked to increased NF- κ B signaling ¹¹²
TREX1	<i>trex1</i>	three prime repair exonuclease	3, 5, 7,	cytosolic 3'-5' DNA exonuclease' (DNAse III) risk variant = loss of function ^{95, 99} variant may lead to increased levels of cytosolic nucleic acid
STAT4	<i>stat4</i>	signal transducer and activator of transcription-4		risk variant associated with increased response to type I IFN and with ischaemic cerebro-vascular events and antibodies to phospholipids and dsDNA ¹¹⁷⁻¹²⁰
IFIH1 (MDA5)	<i>ifih1</i>	IFN induced with helicase C domain 1	3, 5, 7	cytosolic RNA sensor; variant associates with an increased response to type I IFN in SLE patients that have dsDNA antibody titers ^{34, 46, 121}
PTPN22	<i>ptpn22</i>	protein tyrosine phosphatase nonreceptor 22	3, 7	interacts with TRAF3 to augment type I IFN response; risk variant associates with increased circulating levels of type I IFNs ^{31, 122, 123}
MAVS	<i>mavs</i>	mitochondrial anti-viral signaling	3, 5, 7	signals from RIG1 and from MDA5; loss of function variant associated with reduced protein levels of IFN- α and the lack of antibody titers to RNA associated proteins ^{109, 124}

Table 2

IRF5 variants linked to susceptibility to SLE

SNP or variant	gene effect	disease impact	functional impact
promoter CCGGG/- insertion	CGGGG insertion 64 bp upstream exon 1a that creates a Sp1 binding site; in LD with rs10954213 and rs2004640 ¹³⁵	increased risk of SLE MS, IBD, and Crohn's	increased mRNA and protein levels
rs2004640	novel site that allows exon1b splicing to exon 2 with additional IRF5 mRNA isoforms ¹³⁷	increased risk	increased protein levels and altered IRF5 isoforms ¹³⁴
30 bp in-frame insertion in exon 6	occurs within the PEST domain	risk and protective haplotypes ¹³⁶	affects protein stability; alters binding of TRIM21 to IRF5 ¹⁴⁰
rs10954213	altered polyadenylation signal ¹³⁷ shortened poly A tail and greater mRNA stability	increased risk	increase mRNA levels

Table 3

Major IRF5 haplotypes in European ancestry, showing the combinations of functional genetic polymorphisms present and disease associations in SLE

Promoter indel	Splice Site Variation	Exon 6 indel	Alternate poly-a site	Disease associations
+	+	+	+	Risk of disease, anti-Ro and anti-dsDNA antibodies, high IFN
+	+	–	+	Disease risk in seropositive individuals, anti-dsDNA antibodies, high IFN
–	+	+	–	Anti-La antibodies
–	–	–	+	Protective haplotype
–	–	+	–	Protective haplotype

⁺ means indel present, or novel splice site or alternate poly-A site present