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TLRs and interferons: a central paradigm in autoimmunity

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Abstract

Investigations into the pathogenesis of lupus have largely focused on abnormalities in components of the adaptive immune system. Despite important advances, however, the question about the origin of the pathogenic process, the primary disease trigger, and the dominance of autoantibodies against nuclear components, remained unanswered. Discoveries in the last decade have provided some resolution to these questions by elucidating the central role of nucleic acid-sensing TLRs and the attendant inflammatory response, particularly the production of type I interferons. These priming events are responsible for initiating the adaptive responses that ultimately mediate the pathogenic process.

Introduction

Discoveries underpinning current understanding of the basic pathophysiology of systemic lupus erythematosus (SLE) have begun to dissect fundamental pathways and branches and provide an explanation for the common presence of antinuclear antibodies (ANAs). This has focused attention on two major innate immune system factors, the type I interferons (IFN-I) and the nucleic acid-sensing Toll-like receptors (NA-TLRs). Here, we will review this area focusing on recent publications.

Type I interferons in SLE

It is now widely accepted that IFN-I are a driving pathogenic force in the majority of SLE patients based on substantial clinical, epidemiologic, and genetic data (reviewed in [1•, 2,3,4]) as well as direct evidence from animal models using IFN-I receptor-deficient lupus mice or anti-IFN- α/β R antibody treatment [5,6•]. Additional studies in these models have also documented: (a) the existence of IFN-I-independent lupus in MRL-*Fas*^{lpr} mice due to background genes and not Fas deficiency [7,8]; (b) a requirement for IFN-I in mouse lupus models despite the absence of elevated IFN- α or interferon-stimulated genes (ISGs, so called ‘IFN-I signature’) [5], consistent with the recent finding that IFN-I expression even at very low concentrations modulates immune homeostasis by affecting tonic signaling [9]; (c) IFN- α induction of clinically-significant lupus required genetic susceptibility [10], which could explain the infrequent occurrence of lupus in patients treated with high dose IFN-I; and (d) inhibition of lupus was most effective when IFN-I signaling was blocked in early

disease stages, implying IFN-I is mainly important at this innate stage, but not after the pathogenic adaptive autoimmune response has been established [6•].

Production of IFN in lupus

Plasmacytoid dendritic cells (pDCs) are considered the main source of IFN-I in SLE because of their capacity to produce 100–1000-fold greater amounts of IFN- α than other cell types and evidence of pDC activation in SLE patients [1•]. The importance of these cells in disease pathogenesis is supported by the finding that, in lupus mice, significant disease suppression occurred either with IRF8 deficiency, which arrests development of predominantly pDCs, or with the *feeble* mutation in the endosomal histidine transporter, *Slc15a4*, which blocks NA-TLR-induced cytokine production, including IFN-I, selectively in pDCs [11•]. Other cell types, such as macrophages and nonhematopoietic cells, have been implicated as the primary source of aberrantly elevated IFN-I in certain monogenic diseases as well as in pristane-induced lupus [12•,13,14•,15].

Cellular mechanisms of IFN-I production by nucleic acid sensors

Nucleic acid pattern recognition receptors (PRRs) that mediate production of IFN-I can be divided into the endosomal TLRs and several types of cytosolic sensors. The NA-TLRs include TLR3, TLR7, TLR8, and TLR9, recognizing dsRNA, ssRNA, and DNA (reviewed in [16]). All, except TLR3, signal through MyD88 and IRAK4 to activate IRF3/7 and IKK pathways leading to IFN-I production or NF- κ B activation. TLR3 signaling occurs through TRIF and activation of TBK1 and IKK pathways.

The cytosolic nucleic acid sensors can be grouped by response to RNA or DNA. RNA is recognized by RIG-I-like receptor (RLR) family members, RIG-I and MDA5, that bind 5' triphosphate RNA or long double-stranded RNA, respectively [17]. They both signal through MAVS (encoded by *IFIH1*) located on the mitochondrial membrane to mediate IRF-dependent and NF- κ B-dependent cell activation and transcription of inflammatory genes including IFN-I. The main sensor for cytosolic self-DNA was recently shown to be the cyclase, cGAS, which, upon encountering DNA, produces a second messenger, cGAMP [18•,19•]. The generated cGAMP then dimerizes and activates the adapter protein STING (encoded by *TMEM173*), a five-membrane-spanning receptor on the endoplasmic reticulum and outer mitochondria, resulting in phosphorylation of IRF3 and IKK, and activation of the TBK1 and NF- κ B pathways, respectively, the former inducing IFN- β synthesis [20•]. Several other DNA-recognizing receptors, some signaling through STING, have also been identified, including DAI that binds zDNA, IFI16 (p204 in mice), RNA polymerase III that transcribes A-T rich dsDNA into uncapped 5' triphosphate RNA recognized by RIG-I, several DHX helicases, and LRRFIP1 that senses both RNA and DNA, and activates the transcriptional co-factor β -catenin, which enhances the IFN- β gene promoter activity of IRF3 [21,22]. Although these other receptors were shown to detect cytoplasmic DNA, including those from specific pathogens, their role in the recognition of self-DNA is not known.

Mechanisms for increased IFN-I

The cause of the type I IFN signature has been attributed to several possible acquired and genetic factors [4]. The most prominent of these is probably endosomal NA-TLR-mediated activation of pDCs by phagocytized IgG immune complexes that contain nucleic acid cargos. DNA entry into phagosomes is also facilitated by LL37, a cationic antimicrobial peptide that can bind to DNA and inhibit nuclease activity, and by HMGB1, a proinflammatory nuclear protein that, when bound to DNA, can mediate phagocytosis by binding to the RAGE receptor [23,24]. Although their role in lupus is less certain, both LL37 and HMGB1 coat DNA in neutrophil extracellular traps (NETs), an antimicrobial product which recent evidence suggests is a potential source of self-nucleic acids and is prevalent in SLE because of induction by anti-ribonucleoprotein antibodies [25•,26,27]. The significance, however, of NETosis in SLE is controversial [28].

In terms of genetic causes of type I IFN signature in SLE, a wide range of genes and mechanisms, have been implicated that extend throughout the IFN-I pathway (reviewed in [29•]). These include genetic variants that affect response to stimuli (e.g. *STAT4*, *IFIH1*) [30], regulation of IFN-I (*TRAP*) [31], clearance of cytosolic DNA (*TREX1*, *DNaseII*, *ADAR1*) [13,32–34], expression of IRGs (*IRF5*) [35], clearance of apoptotic material (*C1Q*), and regulation of pDCs (*C1Q*) [36,37].

IFN-I enhancement of lupus

IFN-I have pleiotropic effects on the immune system, many of which have the potential to promote lupus. In addition to maintaining immune homeostasis and development of certain immune cell populations, these include upregulation of TLR7 [16], activation of cDCs, induction of T-dependent immune responses [38], and impairment of germinal center selection [39]. Importantly, IFN-I promote a feed-forward amplification process by inducing genes responsible for the production of IFN-I, enhancing the production of interferogenic immune complexes, and increasing expression of TLR7 [23,40].

NA-TLRs are central mediators of ANAs and lupus

Similar to IFN-I, there is substantial evidence, primarily in studies using overexpression or deletion of TLRs in lupus-prone mice, that NA-TLRs are crucial for the development of lupus (reviewed in [23,41–43]). Furthermore, recent studies showed that NA-TLRs are necessary for the production of a broad range of autoantibody specificities, including nuclear antigens, cardiolipin, β 2-GPI, myeloperoxidase, and red blood cells, all self-antigens that to varying extents contain or are associated with nucleic acids, but are dispensable for autoantibody specificities not associated with nucleic acids [44••]. Thus the requirement for NA-TLRs appears to tie together the many diverse autoantibodies, manifestations, and genetic heterogeneity that constitute SLE.

Mechanism of TLR-mediated autoimmunity

NA-TLRs can respond to virtually all forms of self-nucleic acids, including non-CpG DNA, as natural DNA with phosphate backbone activates TLR9 regardless of sequence motif, in

contrast to synthetic phosphorothioates [45]. To prevent their deleterious activation, several mechanisms have evolved. These include limiting expression to certain cell types (e.g. selective expression of human TLR9 in pDCs and B cells), sequestering NA-TLRs inside endosomes and restricting the active form of TLR7/9 to this compartment, the abundant presence of nucleases, and inhibition of TLR activation when phagocytosis of apoptotic material is mediated by scavenger and complement receptors [16,46,47]. In lupus, these barriers can be overcome in two related ways. For B cells, antigen-receptor engagement leads to transport of antigenic material containing nucleic acids to the endosomal compartment where binding to the relevant NA-TLR is followed by cell activation. For pDCs, DCs, and other cell types, entry of IgG immune complex-containing nucleic acids into the endosome is mediated by FCGR2A (Fcgr3 in mice) [23,48]. IgM immune complexes, on the other hand, lack FcR binding, but instead deposit C3 split products via C1q and mannose-binding lectin, which, by engaging complement receptors, induce an inhibitory signal [46]. In B cells, IgG immune complexes are less stimulatory than antigen alone because the IgG on immune complexes binds to the inhibitory FcγRIIb, the only FcγR expressed in B cells in both humans and mice [49].

TLRs on B cells mediate autoantibody production

Recent studies using different animal models have shown that expression of TLRs in B cells is required for the development of most lupus-associated autoantibodies [44•,50,51•,52]. Lack of NA-TLRs, however, has minimal effect on antibody response to certain foreign antigens [53,54]. Taken together this suggests that NA-TLRs have a specific role in the loss of tolerance to nucleic acid-containing self-antigens or in the expansion of corresponding B cells and plasma cells. On the basis of this conclusion, it was suggested that targeting NA-TLR signaling specifically in the B cell population could potentially result in a largely lupus-specific therapy [44•]. In addition to B cells, TLR signaling in pDCs was also recently shown to play a crucial role in the development of lupus, as discussed above.

TLR7 and TLR9 in lupus

Of the NA-TLRs, TLR7 and TLR9 appear to be the most important based on studies of TLR-deficient lupus-prone mice [55]. Furthermore, consistent with the requirement for TLRs in B cells, mice lacking TLR7 are unable to produce anti-RNA, while loss of TLR9 to varying degrees, depending on the study, impairs the anti-nucleosome response [41,55]. However, although TLR3 was not required for spontaneous lupus [56], administration of poly(I:C), a TLR3 agonist, was reported to induce lupus in MyD88-deficient (lacks TLR7/9 signaling) MRL-*Fas^{lpr}* mice, suggesting that under certain circumstances this sensor can also mediate disease [57]. Accordingly, in human SLE, it is possible that TLR8, which binds ssRNA in contrast to mouse TLR8, which does not, may also play a role [58].

Despite the strong association of SLE with anti-double stranded DNA (dsDNA), several lines of evidence suggest TLR7 may be more important than TLR9. This was first suggested by an early experiment showing that the interferogenic activity of nucleic acid-containing IgG immune complexes (generated by combining SLE sera with apoptotic or necrotic cells) for pDCs was more sensitive to RNase than to DNase [59]. Lupus-prone mice lacking only

TLR7 had a substantial reduction in disease, albeit not as great as TLR7/9 double deficiency, whereas absence of TLR9, contrary to expectations, resulted in greater severity [55]. Although an initially perplexing result, subsequent studies have attributed this to the absence of competition from TLR9 for UNC93B1-mediated endoplasmic reticulum to endosome trafficking resulting in increased transport and activation of TLR7 [41,60•]. Similarly, knockout of TLR8, which in mice does not bind nucleic acids but still relies on UNC93B1 for trafficking to the endosome, also leads to the development of systemic autoimmunity [61], presumably by the same mechanism. It should be mentioned that lupus-prone mouse strains produce, in addition to conventional ANAs, species-specific anti-gp70 autoantibodies to circulating RNA-containing endogenous retroviral particles, and this specificity is TLR7-dependent and associated with disease development [62,63]. It is possible that this mouse-specific response may be a factor in the TLR7 predominance in murine lupus.

NA-TLR centric model of SLE autoantibody production

Both IFN-I and NA-TLRs are central to the current model of lupus pathogenesis wherein it is postulated that autoantibody production results from a positive feedback loop (Figure 1). This loop consists of several steps: (a) autoreactive B cells recognizing nucleic acid-containing self-antigens are activated following engagement of their antigen-receptors and NA-TLRs; (b) B cells subsequently produce IgG anti-nuclear antibodies; (c) autoantibodies form nucleic acid-containing immune complexes such as those with apoptotic material; (d) such complexes are transported by the FCGR2A receptor into the endosomal compartments of pDCs, conventional DCs (cDCs), and potentially macrophages and other cell types where release of nucleic acids activates NA-TLRs; (e) activated pDCs produce copious amounts of IFN-I as well as other cytokines; (f) activated cDCs produce proinflammatory cytokines including IFN-I and are potent APCs for T cells; (g) IFN-I in particular have been shown to promote lupus in many ways, including enhancing B cell response and loss of tolerance; (h) these processes then further enhance autoantibody production leading to amplification of the autoimmune state. The sequence of events that lead to the initial production of IgG antinuclear antibodies responsible for triggering the forward feedback loop has not yet been deduced, but evidence from animal models indicate that DCs are not required, thus implying a B cell costimulation of CD4 T cell mechanism [64••].

Altered cytosolic nucleic acid regulation elicits IFN-mediated autoinflammatory and autoimmune diseases

Failure to regulate cytosolic self-nucleic acids has been shown to cause aberrant and destructive responses mediated primarily by IFN-I. Mice deficient in DNase II (endonuclease located in lysosomes) die in utero because of IFN- β -induced apoptosis of erythroid precursors in the liver [13]. Double DNase II/IFN-I deficiency allows survival, but adults develop a TNF- α -mediated TLR9-independent inflammatory arthritis with anticitrullinated peptide antibodies, rheumatoid factor, and low titers of anti-dsDNA [65]. STING reverses all manifestations, including arthritis [66] suggesting that disease is caused by the accumulation of undigested DNA in the cytosol.

Defects in TREX1 (Dnase III) leads to Aicardi-Goutieres syndrome (AGS), chilblain lupus (CLE), and retinal vasculopathy with cerebral leukodystrophy (RVCL) [14•,67]. AGS also occurs with mutations in *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, and *ADARI* [34,14•]. Most cases of AGS are not associated with lupus, although features commonly found in SLE were observed in 60% of patients in one small study [67,68]. Similarly, with CLE, association with SLE does not exist in the few reported familial forms [69]. Nevertheless, TREX1 variants although rare in the population are associated with SLE [14•, 67]. Taken together, this suggests that TREX1 defects may act more as a facilitator of SLE, perhaps as a result of overproduction of IFN-I and other cytokines. In mice, deletion of TREX1 leads to a STING-dependent autoinflammatory/autoimmune syndrome, primarily affecting the heart and muscles [33]. This is mediated by overproduction of IFN-I caused by inadequate degradation of single stranded DNA from endogenous retroelements [12••,33] or aberrant replication intermediates [32]. Initial production of IFN-I occurs locally in nonhematopoietic cells, which drives T cell-mediated inflammation and autoantibodies to target tissue antigens [12••]. Deletion of B cells does not reduce tissue inflammation, but significantly extends survival by an undetermined mechanism.

The cytosolic RNA sensing-related adaptor, MAVS, is also associated with enhanced IFN-I production and SLE. *IFIH1* is linked to SLE, with loss of function variants linked to resistance and high expression variants to susceptibility [30,70–72]. Transgenic overexpression of MDA5 resulted in chronic elevation of IFN-I associated with IFN signature and resistance to viral infection, but no autoimmunity unless combined with lupus-predisposing FcγR2b deficiency [73].

Treatment

On the basis of considerable evidence supporting crucial roles of both IFN-I and NA-TLRs in SLE pathogenesis, efforts are ongoing to apply this information to the clinic. Anti-IFN-α therapy with broadly reactive monoclonal antibodies, including sifalimumab (MEDI-545, Medimmune) [74], rontalizumab (Genentech) [75], and AGS-009 (Argos Therapeutics) (ClinicalTrials.gov website, EULAR Congress 2012), has advanced to early phase II clinical trials for SLE following acceptable adverse effect profiles, inhibition of IFN signature and, for some, a trend toward therapeutic response. IFNα Kinoid (IFNα-K001, Novacs) therapy, a series of immunizations with an IFN-α-carrier protein to induce self-polyclonal antibodies to this cytokine [76], is currently in phase I/II trials. An antagonist anti-IFN-α monoclonal antibody (MEDI-546, Medimmune) gave promising phase I trial results for systemic sclerosis [77] and could also be used in SLE.

Two TLR antagonists are in early phase clinical trials for SLE, DV1179 (inhibits TLR7/9, Dynavax, Glaxo-SmithKline) and IMO-8400 (inhibits TLR7/8/9, Indera Pharmaceuticals, also Psoriasis in phase 2). These agents inhibited lupus manifestations including anti-nuclear antibodies and glomerulonephritis in BWF1 mice [78–80]. As an additional potential benefit, Dynavax TLR antagonist could reverse the glucocorticoid resistance associated with nucleic acid-TLR activation [81].

Concluding remarks

It has frequently been said that deciphering the pathogenesis of lupus, a complex polygenic disorder with multiple affected systems and organs, would be an exercise in futility, and that studies of organ-specific diseases offered a more fruitful endeavor. Recent advances in the definition of nucleic acid sensors as initiators of both normal and pathogenic immune responses strongly suggest that the Cassandras were wrong and that we now have a good handle on defining the basic processes by which this disease is triggered. Furthermore, these findings provide the catalyst to delineate how disease-initiating endogenous self-products, and in some instances exogenous pathogens, might be recognized in other autoimmune diseases. Overall, it can be postulated that a unified principle can be formulated for most autoimmune conditions that connects disease predisposition to the engagement of pathogen pattern recognition receptors. It follows therefore, that treatment based on these sometimes adversarial sensing processes would be a means to intervene in a broad spectrum of autoimmune diseases.

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Figure 1.

Role of nucleic acid TLRs and type I IFN to the pathogenesis of autoantibody production in lupus. Antinuclear autoantibody production in lupus is thought to involve several steps. (1) Secretion of IgG autoantibodies by plasma cells (or B cells). (2) Formation of IgG immune complexes containing nucleic acid material, immune complexes with apoptotic material is depicted as an example. (3) Engulfment of these immune complexes via FCGR2A (*FcγRIIa*) by macrophages (Mac), pDCs, and, immature DCs (3a–c) into endosomes where released nucleic acids bind to the corresponding TLR resulting in cell activation. Each of these cell types can promote inflammation, immune responses, and autoimmunity by different mechanisms including release of proinflammatory factors and BAFF, and maturation into potent antigen presenting cells. Importantly, pDCs produce copious amounts of IFN-I, which drives multiple processes that enhance the development of autoimmunity (red arrows, see text for details). (4) Activated DCs can present self-antigen to autoreactive CD4⁺ T cells. (5) Autoreactive B engage corresponding helper T cells. (6) Activated B cells differentiate into plasma cells or enter germinal centers (GCs) where higher affinity

autoreactive plasma cells and memory B cells are generated. Plasma cells then produce more IgG autoantibodies resulting in promulgation and amplification of this process. The nucleic acid-sensing TLR-dependent steps are circled in red.