Systemic lupus erythematosus: all roads lead to type I interferons
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In recent years, the study of systemic lupus erythematosus (SLE) patients has revealed a central role for type I interferon (IFN) in disease pathogenesis. IFN induces the unabated activation of peripheral dendritic cells, which select and activate autoreactive T cells rather than deleting them, thus failing to induce peripheral tolerance. IFN also directly affects T cells and B cells. Furthermore, immune complexes binding to FcγR and Toll-like receptors provide an amplification loop for IFN production and B-cell activation in SLE. Polymorphisms in genes that control IFN production or its downstream signaling pathway, such as IRF5, might be responsible for some of these alterations. This novel information is leading to the development of IFN antagonists as a potential therapeutic intervention in SLE, thus bringing hope to SLE patients.

Introduction
Patients with systemic lupus erythematosus (SLE) have long been known to display elevated levels of type I interferon (IFN) in their serum. The key role that this cytokine family plays in disease pathogenesis has only emerged, however, in the past few years. We proposed in 2001 that an excess type-I IFN might break peripheral tolerance through the activation of myeloid dendritic cells (DCs). Since then, the roles of type I IFN in two key SLE pathogenic events (i.e. human plasma cell differentiation and activation of CD8 T cells able to generate nucleosomes) have been described. It has also become evident that antigen–antibody complexes containing RNA and DNA activate DCs and B cells through interaction with TLR7/8 and 9, respectively. Thus, lupus-specific autoantigens might act as TLR ligands and contribute to IFN and autoantibody production.

In this article, we will review recent evidence regarding the role of type I IFN in human SLE. We will discuss controversial data on the role of IFN and TLR activation in different murine lupus models. Finally, we will summarize some recent genetic studies that further link the type-I IFN pathway to the pathogenesis of human SLE.

Beyond B and T cells: dendritic cell alterations in SLE
One of the hallmarks of SLE is the loss of tolerance to nuclear antigens and the development of immune complexes that deposit in tissues and cause widespread inflammation. Alteration of T cell–B cell interactions has therefore been proposed as the common pathogenic mechanism that leads to disease [1]. However, the recognition of DCs as efficient stimulators of B and T lymphocytes as well as key controllers of immunity [2] and tolerance [3,4] has led to the hypothesis that SLE might be driven by unabated DC activation.

Monocytes represent the circulating pool of macrophage and DC precursors. These cells have limited antigen-presenting capacity, as they cannot initiate primary immune responses unless they are triggered to differentiate into myeloid dendritic cells. However, blood monocytes from SLE patients behave like DCs, as they are able to induce the proliferation of allogeneic CD4 T cells [5]. Furthermore, exposure of normal monocytes to SLE serum results in the rapid generation of cells that have DC morphology and function, which suggests that SLE blood represents a DC-inducing environment [5]. Unabated DC maturation could lead to the activation and expansion of autoreactive T cells that have escaped central tolerance, which explains many of the features of the disease (reviewed in [6]).

IFN-α dysregulation at the center of SLE pathogenesis
Several cytokines have been shown to allow the differentiation of precursor cells into DCs. In particular, IFN-α together with granulocyte-macrophage colony-stimulating factor drive monocytes to become DCs [7–9]. Accordingly, the DC-inducing property of SLE serum is dependent on IFN-α and correlates with disease activity [5]. Thirteen different IFN-α subtypes together with IFN-β, IFN-ω, IFN-κ and IFN-ε/τ (reviewed in [10]) constitute the type I IFN family. A new class of type I IFN-like molecules (IFN-λ 1–3 [11] or IL-28 and IL-29 [12]) that signal through a receptor different from the common type I IFN receptor (IFNAR) have also been
identified recently. Type I IFNs can be produced by numerous cell types. Plasmacytoid DCs (pDCs), however, are equipped to quickly secrete large amounts of type I IFN [13,14] and probably contribute IFN-α in SLE patients. pDC numbers are reduced in SLE blood [5,15], but these cells massively infiltrate inflamed lupus skin [16,17]. The decrease in SLE blood pDCs might thus result from their accelerated migration to inflammation sites, as demonstrated in allergen-challenged nasal mucosa [18].

Type I IFNs also act directly on cells of the adaptive immune system. Through their effect on B cells, type I IFNs directly enhance primary antibody responses to soluble proteins and induce the production of all subclasses of IgG in mice [19]. In humans, pDCs triggered with virus induce CD40-activated B cells to differentiate into plasma cells; they do this through two pDC cytokines, which act sequentially. First, IFN-α generates non-Ig-secreting plasmablasts, and, second, IL-6 induces the differentiation of these plasmablasts into Ig-secreting plasma cells [20]. Thus, IFN-α might contribute to increased autoantibody secretion and immune complex formation in SLE by directly activating B cells.

IFN also acts directly on T cells. In mice, type I IFNs prevent activated T-cell death during inflammatory responses [21], and IFN signaling in CD8 T cells is crucial for the generation of effector and memory cells in response to viral infection [22]. In humans, DCs generated in the presence of SLE patient sera promote the differentiation of CD8+ effector T lymphocytes. These CD8+ T cells can kill target cells and generate nucleosomes and SLE autoantigens in a granzyme-dependent manner [23]. Nucleosomes might be uptaken by DCs and presented to T and B cells. Indeed, administration of DCs loaded with apoptotic cells consistently triggers autoimmune responses in mice, although clinical autoimmunity only develops in genetically susceptible recipients [24,25].

Even though the effects of IFN-α can explain many SLE features, not every SLE patient displays elevated serum IFN-α. However, peripheral blood mononuclear cells (PBMCs) from all active pediatric SLE patients [26] and most adult SLE patients display a remarkable IFN signature [27,28]. Furthermore, treatment of SLE patients with a high intravenous dose of steroids abrogates the IFN signature in PBMCs [26]. This effect might result from pDC depletion [29], which further supports the role of pDCs and type I IFN in disease pathogenesis.

Clinical observations revealed the potential role of IFN in the development of SLE. Thus, IFN therapy in cancer and viral infections induces autoantibody formation in 4–19% of patients, and a variety of SLE symptoms have been reported in 0.15–0.7% of them (reviewed in [30]). Furthermore, patients who have rheumatoid arthritis or Crohn’s disease can develop anti-dsDNA antibodies and reversible SLE when treated with anti-TNF agents [31,32]. The PBMCs of these patients indeed display an IFN signature, and in vitro studies reveal that TNF antagonists increase IFN secretion by pDCs [33].

Recent studies in lupus-prone mice confirm the crucial role of IFN in SLE pathogenesis. In vivo delivery of IFN-α to pre-autoimmune NZB/W F(1) rapidly results in severe SLE. Anti-dsDNA antibodies appear as early as 10 days after initiation of IFN-α treatment. Proteinuria and glomerulonephritis-induced death occurred in all treated mice at 9 and 18 weeks, respectively, a time when untreated mice did not show any sign of disease [34]. Conversely, the cross of both NZB and B6 lpr/lpr mice with a type-I IFN receptor KO strain significantly decreases morbidity and prolongs the survival of these animals [35,36]. Finally, B6.Nba2 (congenic for the Nba2 interval derived from the NZB mice) display an increased expression of Ifi202 [37] — an interferon-inducible gene that inhibits p53-mediated apoptosis [38].

**IFN-α and immune complexes: the TLR connection and disconnection**

The mechanism(s) that leads to unabated production of IFN is currently the subject of debate. There are probably two paths to consider: first, a genetic alteration that prevents the prompt shutdown of IFN production by pDCs; and second, an amplification loop that results from immune complex (IC) activation of pDCs and B cells. We will review the recent studies on the role of ICs and nucleic acids that activate pDCs and B cells by way of Toll-like receptor (TLR)-dependent and -independent mechanisms.

**TLRs and pDCs**

Cell-derived DNA gives rise to ICs that can trigger pDCs in vitro to secrete type I IFN [39,40]. Chromatin-containing ICs are internalized by pDCs by way of FcγRIIa, reach the endosomal compartment and activate TLR9 to induce IFN-α production [14*,41,42*,43*,44]. Specific highly conserved RNA sequences within snRNPs — a classic lupus autoantigen — can directly stimulate TLR7 and TLR8 and activate pDCs, which then secrete high levels of type I IFN as well. Furthermore, ICs containing RNP from SLE patients are taken up through FcγRII and efficiently stimulate type I IFN production through TLR7 and TLR8 activation of pDCs as well [43*,45].

**TLRs and B cells**

TLRs 7–9 are also expressed in B cells. The contribution of IC and TLR signaling to the generation of autoantibodies characteristic of SLE has been the subject of numerous studies in mice. Contradicting results have been reported, which could depend on the genetic background of the animals.

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In *vivo*, chromatin-containing ICs activate transgenic autoreactive B cells by way of sequential engagement of the B-cell antigen receptor (BCR) and TLR9 [46]. In *vivo*, TLR9 appears to be crucial for the development of anti-dsDNA antibodies, because lupus-prone (Fas-deficient) mice that lack TLR9 on the mixed MRL/B6/129 background fail to generate these antibodies [47]. TLR9, by contrast, seems to deliver protective signals in the pure MRL/lpr background, because TLR9 knockout mice develop more autoantibodies and severe lupus-like disease [48]. Furthermore, the absence of type I IFN receptor in these mice also worsens disease [49]. On a different background (B6 mice that lack the inhibitory FcγRIIB [50]) TLR9 signaling is required for anti-DNA and polyreactive IgM+ B cells that have escaped central tolerance to switch to pathogenic isotypes [51]. Overall, the contradictory mouse results regarding the contribution of TLR9 signaling to SLE pathogenesis should be cautiously extrapolated to humans. First, the expression of TLR9 on immune cells differs in mice and in humans. Second, the various murine lupus models might not faithfully reproduce the human disease. For example, mutations in Fas and Fas-L, which are the hallmark of the MRL/lpr models extensively studied as surrogates to human SLE, do not cause this disease in humans. Instead, these mutations give rise to a unique syndrome characterized by lymphoproliferation and autoimmunity that is easy to differentiate from SLE (reviewed in [52]).

TLR7 signaling also appears to contribute to SLE-like syndrome in mice. As shown for pDCs, immune complexes that contain RNA and RNA-associated autoantigens activate autoreactive B cells in *vivo* [53]. In *vivo*, FcγRIIB−/− mice develop enhanced autoimmunity when crossed to the Y-linked autoimmune accelerator (Yaa) locus [54], which harbors a duplication in the TLR7 gene [55]. Finally, although others have shown a protective role for TLR9 and IFN-α, injection of the TLR7 ligand imiquimod increases serum levels of IL-12p70, IFN-α and IL-6 and aggravates lupus nephritis in MRL/lpr mice [56]. Naturally occurring differences in expression of the TLR7 gene, as well as environmental factors that induce TLR7 expression (i.e. IFN-α [57,58]) and/or TLR7 responses, could therefore result in increased pDC and B-cell responses to RNA-containing self antigens and could contribute to SLE pathogenesis.

The recognition of nucleic acid-derived antigens might depend on receptors other than TLRs. For example, IFN production in response to SLE ICs is only partially dependent on TLR9 and MyD88 [44]. Indeed, the existence of a TLR-independent cytosolic DNA receptor that recognizes endogenous DNA has been proposed recently (reviewed in [59,60]). Whether these TLR-independent pathways contribute to the pathogenesis of autoimmune diseases such as SLE remains to be explored.

**From biology to genomics and back**

The genetic basis of human SLE has been the subject of extensive study for more than 20 years. Indeed, genomewide linkage studies have identified several genetic loci associated to SLE in multiplex families. The multiplicity and complexity of these loci, however, represent a formidable challenge for lupus geneticists. The development in recent years of high-density single nucleotide polymorphism genotyping is allowing both study of previously identified candidate loci and development of genome-wide association studies. Alleles within the major histocompatibility complex (Fcγ receptors, Ig receptor homologues, molecules involved in cell-signalling, cytokines and chemokines, opsonins etc.) have been found associated to SLE patients from different ethnic groups in the past (reviewed in [61]).

A recent genetic analysis of Swedish, Finnish and Icelandic patients focused on 13 genes of the type I IFN pathway. It revealed two novel strong associations: three polymorphisms within the flanking and intronic regions of the IFN regulatory factor 5 (IRF5) gene, and two within the coding region of the tyrosine kinase 2 (TYK2) gene [62]. The transcription factor IRF5 is involved downstream of the TLR–MyD88 signaling pathway in the induction of pro-inflammatory cytokines [63]. IRF5 is expressed in pDCs and B cells; it appears to be a crucial mediator of TLR7 signaling, because it is activated by TLR7–TLR8 ligation and because its ectopic expression enables type I IFN production in response to TLR7 ligands [64]. Transcription of IRF5 is regulated by two distinct promoter regions that respond differentially to viral infection and IFN stimulation. Indeed, the IRF5 transcription pattern is quite complex, as at least nine distinct alternatively spliced mRNAs have been identified [65]. The association of SLE with IRF5 polymorphisms that give rise to unique IRF5 isoforms has been confirmed [66]. These studies support a causal role for type I IFN pathway genes in human SLE. They also represent a successful example of biology leading genetic studies at a time when high-throughput analysis of the human genome is becoming available.

**A unified view of SLE pathogenesis**

SLE is a remitting disease characterized by flares that progressively result in deterioration of the patient. These flares are often associated with environmental triggers such as viral infections. Infection could trigger the unabated production of IFN-α in SLE patients. We contend that this increased bioavailability of IFN-α is fundamental to SLE pathogenesis. It induces and maintains the generation of mature DCs, tilting the fate of autoreactive T lymphocytes that have escaped central tolerance from deletion to activation. These mature DCs activate cytotoxic CD8+ T cells to generate nucleosomes that can be captured and presented by DCs generated in the presence of interferon (IFN-DCs) [23**]. Together with
IL-6, IFN promotes the differentiation of mature B cells into plasma cells [67]. Thus, the effects of IFN-α on DCs, B cells and T cells could explain the breakdown of tolerance to nuclear antigens, autoantibody secretion and IC formation characteristic of SLE. Chromatin-containing ICs activate B cells through the co-engagement of BCRs and TLRs and trigger pDCs to secrete more IFN-α through the co-engagement of FcγR and TLRs. This results in amplification of this pathogenic loop (Figure 1).

As shown in different murine models of SLE, however, excessive IFN production only induces disease in certain genetic backgrounds, and epistatic interactions among several genes might be necessary for the disease to occur [68]. The recently described polymorphisms in the IRF5 gene [62,66] might predispose to SLE by increasing the ability of pDCs to release type-I IFN and pro-inflammatory cytokines upon activation and by enhancing B-cell responses to these cytokines. SLE patients might display other genetic defects that lead to alterations in autoreactive B-cell checkpoints that might be independent from IFN and DCs. Indeed, only a small fraction of patients treated with IFN develop anti-nuclear and anti-dsDNA antibodies and even fewer patients develop clinical SLE. Defects in B cell tolerance check points that might allow the survival of autoreactive clones into the peripheral compartment have been described in children who have SLE [69] and in murine lupus models. Indeed, B6.Sle1 congenic mice display intrinsic B-cell alterations that lead to the development of anti-chromatin antibodies in the absence of T-cell help. A polymorphic isoform of the Ly108 gene, a member of the SLAM family, has been

Figure 1

The central role of type I IFN in SLE pathogenesis. Several genetic alterations predispose to SLE. For example, genes involved in IFN production and/or signaling pathway, B cell tolerance checkpoints or removal of apoptotic cells can be altered in SLE patients. SLE flares are associated with environmental factors such as viral infections that trigger the unabated production of type I IFN. Type I IFN induces the generation of mature DCs, which expand rather than delete the autoreactive T lymphocytes that have escaped central tolerance. These mature DCs activate cytotoxic CD8+ T cells that damage tissues to yield large numbers of nucleosomes. These nucleosomes can be captured by mature DCs, further amplifying the autoreactive process. Together with IL-6, IFN promotes the differentiation of mature B cells into autoantibody-secreting plasma cells. Chromatin-containing immune complexes activate B cells through the co-engagement of BCR and TLRs and induce pDCs to secrete more IFN-α through the co-engagement of FcγR and TLRs. Red arrows indicate direct effects of type I IFN on DCs and B cells. Black arrows indicate indirect effects, either as the result of IFN-induced DC activation or through the generation of immune complexes.
recently found to impair the ability of immature B cells from these mice to undergo deletion and RAG re-expression — two key mechanisms in the implementation of tolerance [70**]. Even though they display high autoantibody titers, these mice do not develop further SLE manifestations unless crossed with mice carrying other lupus-prone genetic intervals (reviewed in [68]).

In humans, healthy relatives of SLE patients often display antinuclear but not anti-chromatin antibodies. Consequently, most of these relatives do not develop SLE.

Altering the type I IFN system in SLE were described more than 20 years ago but were forgotten or diluted within the plethora of mouse lupus-like models. The study of SLE patients, however, has recently brought the role of this old cytokine family in the pathogenesis of the disease back into the spotlight. With the help of high throughput technologies, more pieces of the puzzle are being put together. Gene expression profiling, for example, has revealed the almost universal expression of blood IFN-inducible transcripts and their correlation with disease activity in SLE patients [26–28]. Single nucleotide polymorphism analysis focusing on IFN-related genes has found a common IRF5 haplotype as an important genetic risk factor for this disease [62*,66].

Altogether, these studies support a causal role for the type I IFN pathway in human SLE. Indeed, blocking a single cytokine might be the answer to control this genetically complex and clinically heterogeneous disease that continues to be one of the major challenges in rheumatology.

Conclusions

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


15. This study demonstrates that IFR7 is a fundamental factor in TLR9-induced IFN production by pDCs.


6 Autoimmunity


In this study, the authors demonstrate that the expansion of antigen-specific CD8+ T cells that occurs in response to viral infection is crucially dependent on the direct action of type I IFN on CD8+ T cells.


This study shows that DCs generated in the presence of sera from SLE patients who have active disease promote the differentiation of CD8+ effector T cells that are fully functional and that are able to generate SLE autoantigens.


26. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, Pascual V, Banchereau J: Antigen-specific CD8+ T cells that are fully functional and that are able to generate SLE autoantigens.


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This study shows that a genetic duplication leading to increased expression of TLR7 is responsible for the Yaa defect.


This article describes a polymorphic isoform of the Ly108 as a potential regulator of immature B cell tolerance checkpoints.