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Type I Interferon in Systemic Lupus Erythematosus and Other Autoimmune Diseases

Review

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Summary

Different genetic alterations may lead to type I interferon (IFN) overproduction in human systemic lupus erythematosus (SLE). The increased bioavailability of type I IFN contributes to peripheral tolerance breakdown through the activation of immature myeloid dendritic cells (mDCs). IFN-matured mDCs activate autoreactive T cells. These cells, together with plasmacytoid DCs, help expand autoreactive B cells. IFN-matured DCs also activate cytotoxic $CD8^+$ T cells, possibly increasing apoptotic cell availability. The capture of apoptotic cells by mDCs and of nucleic acid-containing immune complexes by plasmacytoid DCs and B cells amplifies the autoimmune reaction leading to disease manifestations. Genetic alterations in lineages other than B cells might explain other autoimmune syndromes where type I IFNs appear to be involved.

Introduction

Interferon (IFN) was shown to act on cells of the immune system more than 40 years ago. However, its role on the pathogenesis of human disease, especially SLE, has only recently been established. In 1979, interferon activity was found in the serum of patients suffering from several autoimmune diseases (Hooks et al., 1979), a finding that was subsequently confirmed mainly in SLE (Preble et al., 1982). Further inference of the role of type I IFN (refer to here as IFN-$\alpha\beta$ for both IFN-α and IFN-β) in SLE came from the induction of autoimmunity during IFN-$\alpha\beta$ therapy (Ronnblom et al., 1991) and the presence of circulating inducers of IFN-$\alpha\beta$ in SLE blood. We proposed in 2001 that a key pathogenic event in SLE might be a break in peripheral tolerance mechanisms after activation of myeloid dendritic cells (mDCs) in response to an excess of IFN-$\alpha\beta$ (Blanco et al., 2001). This proposal was based on three pieces of information: (1) the critical role of immature mDCs in the maintenance of peripheral tolerance, as opposed to mature mDCs that induce antigen-specific immunity (Banchereau et al., 2000; Steinman et al., 2003), (2) the remarkable capacity of plasmacytoid DCs (pDCs) to secrete large amounts of IFN-$\alpha\beta$ upon exposure to viruses (Siegal et al., 1999), and (3) the ability of IFN-$\alpha\beta$ to activate immature mDCs (Blanco et al., 2001).

Systemic Lupus Erythematosus

There is a spectrum of human lupus ranging from solely skin involvement to systemic disease, the common denominator being tissue damage resulting from the deposits of immune complexes (IC) that fix complement. Systemic disease is characterized by a relapsing and remitting course with flares of high morbidity. SLE patients are predominantly women who present with chronic nonspecific symptoms such as fever, weight loss, and fatigue often associated to lymphadenopathy and especially lymphopenia. Some patients may present with severe acute illness characterized by seizures, psychosis, renal failure, profound anemia, pulmonary hemorrhage, or sepsis. Confirming the diagnosis of SLE requires the fulfillment of 4 out of 11 criteria, one of those being the presence of anti-nuclear antibodies (ANA), which are detected in >95% of patients. Loss of tolerance to nuclear antigens is restricted to chromatin components (such as dsDNA, histones, and nucleosomes) and U-rich ribonucleoproteins (such as RNP and Sm), which are Toll-like receptor 9 (TLR9) and TLR7 ligands, respectively (Martin and Elkon, 2005). Antibodies against phospholipids (complexed to β2-glycoprotein) exposed on the surface of dying cells are also frequently detected in SLE patients and correlate with the development of thromboembolic complications. Autoantibodies directed to cell-surface molecules, especially those expressed on cells of hemopoietic origin, cause hemolytic anemia, neutropenia, and thrombocytopenia. Antibodies against antigens expressed in target organs like the kidney (glomerular extracts) are also found in patients with severe kidney disease (Li et al., 2005). In addition to the direct damage caused by cellular and/or tissue antigen-antibody interactions, many lupus symptoms result from indirect damage through the deposition of IC on tissues (i.e., nephritis, arthritis, and vasculitis).

Genetic defects in molecules involved in the removal of anti-DNA-nucleosome complexes (i.e., complement proteins, C-reactive protein, etc.) are among the strongest genetic factors predisposing to SLE. However, most SLE patients do not have mutations in this particular set of genes. Multicase family-based genome scans with microsatellite markers have identified more than 12 candidate genes (those that encode FcγR, CD2, CR2, FCRL3, CD45, HF1, Ro, PARP, PDCD1, HLA, TNF, C4) distributed along 6 chromosomes (Alarcon-Riquelme, 2005). Genetic association studies with single-nucleotide polymorphisms have confirmed the involvement of genes that encode FcγR and complement while disclosing other novel candidates. A common haplotype of interferon regulatory factor 5 (IRF5), a transcription factor expressed in pDCs and B cells, is associated with increased risk of SLE. IRF5 acts downstream of the TLR-MyD88 signaling pathway in the induction of proinflammatory cytokines (Takaoka et al., 2005). The association of SLE with IRF5 polymorphisms giving rise to unique IRF-5 isoforms has been confirmed across different ethnic backgrounds (Graham et al., 2006).

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. SLE is a remitting disease characterized by flares often associated to viral infections, which

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might trigger unabated production of IFN-αβ. Even though the effects of IFN-αβ may explain many SLE features, not every SLE patient displays elevated serum IFN-αβ. However, peripheral blood mononuclear cells (PBMCs) from all active pediatric SLE patients upregulate IFN-induced genes as defined in vitro by culturing healthy PBMCs with IFN-αβ (Bennett et al., 2003). The expression of a subset of these IFN- as well as neutrophil-related genes correlates better with disease activity than titers of dsDNA antibodies. Furthermore, treating SLE patients with high-dose intravenous steroids, which induce clinical remission, abrogates the IFN-αβ signature in PBMCs (Bennett et al., 2003). This effect might result from pDC depletion (Shodell et al., 2003), supporting the role of pDCs and IFN-αβ in disease pathogenesis. Leukocytes from most adult SLE patients also display a remarkable IFN signature that correlates with disease severity (Baechler et al., 2003). The expression of the same spectrum of IFN-regulated genes is induced by culturing healthy blood cells with SLE plasma samples, and this activity is >90% inhibited by IFN-α antibody, but not by IFN-β or IFN-γ antibodies (Hua et al., 2006).

Systemic Lupus Erythematosus Animal Models

Several murine lupus models exist, though none of them appears to fully reproduce the human disease. They can be divided into spontaneous, congenic, and engineered models (Liu and Mohan, 2006). The best known spontaneous models arise on New Zealand Black (NZB), New Zealand White (NZW), MRL, BXSB, and SWR backgrounds. Hybrids of some of these background strains, like the NZB/W F1, NZM2410, and the SWR/NZB F1 develop ANA, glomerulonephritis, and other features of human disease. The *lpr* (Fas) or *gld* (FasL) mutations on the MRL background give rise to mice with features of human lupus. Yet, the massive degree of lymphoproliferation that occurs in these mice is not found in humans. Additionally, although MRL-*lpr*/*lpr* mice spontaneously develop arthritis, it displays more features of rheumatoid arthritis than of the arthropathy seen in human SLE. Conversely, humans carrying mutations in the Fas/FasL genes do not develop SLE. The Yaa mutation, which accelerates disease on the BXSB background, is due to a translocation of the TLR7 gene into the Y chromosome, explaining the predominantly male predisposition to disease in this particular model (Pisitkun et al., 2006; Subramanian et al., 2006), which is at striking difference with the 9:1 female to male ratio in human SLE.

Congenic mice bearing individual predisposing loci on lupus-resistant strains have been generated. The best-studied models bear the NZM2410-derived Sle1, Sle2, and Sle3/5 intervals on the B6 background (Liu and Mohan, 2006). These models underscore the importance of epistatic interactions among different loci. Sle1, for example, is a genetic interval responsible for breaking B cell tolerance to chromatin. However, this interval does not lead to disease manifestations unless in epistasis with Sle2 (B cell hyperactivity), Sle3/5 (APC hyperactivity), Yaa (TLR7 duplication), or *lpr* (Fas mutation).

Many lupus-like syndromes arise in mice deficient in single genes. They fall into two main categories: clearance of apoptotic cells and lymphocyte activation and survival. Genes encoding proteins that fall within the first (C1q, C2, C4, and Dnase I) and second (CD45, CTLA-4, PD1, and FcγRIIb) groups are also candidate susceptibility genes in humans according to linkage and/or association studies (Alarcon-Riquelme, 2005).

Studies in lupus-prone mice have confirmed the critical role of IFN-αβ in SLE pathogenesis. In vivo delivery of IFN-αβ to preautoimmune NZB/W F1 mice rapidly results in severe SLE. dsDNA antibodies appear as early as 10 days after initiation of IFN-α treatment, demonstrating a critical role for IFN-α in the selection and expansion of autoreactive clones. 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 (Mathian et al., 2005). Conversely, the cross of both NZB and B6 *lpr*/*lpr* mice with a type I IFN receptor-deficient strain substantially decreases morbidity and prolongs the survival of these animals (Braun et al., 2003; Santiago-Raber et al., 2003). As opposed to the NZB/W model, IFN-γ but not IFN-αβ seems to mediate disease in the MRL*lpr* mice. Indeed, deficiency of the IFN-γ or the IFN-γ receptor genes leads to a delay in both the onset of disease and the severity of glomerulonephritis (Balomenos et al., 1998), whereas these symptoms are enhanced when the mice are rendered deficient in the type I IFN receptor (Hron and Peng, 2004). These data, together with the expression of a strong IFN-γ gene signature in the spleen (Liu et al., 2006), support the role IFN-γ in the *lpr* model. IFN-γ, however, has not yet been linked to any human autoimmune disease.

Cellular Aspects of SLE: Dendritic Cells

DCs are the initiators and regulators of immune responses. The recognition that DCs control immunity and tolerance (Hawiger et al., 2001; Steinman et al., 2003) led to the hypothesis that SLE may be driven by unabated DC activation. Classically, two main DC differentiation pathways are recognized (Banchereau et al., 2000): myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) (Liu, 2005).

Tissue-resident mDCs that encounter microbes at mucosal surfaces or at sites of tissue damage migrate to the lymph node via afferent lymphatics. Importantly, mDC express receptors, such as TLRs, nucleotide binding oligomerization domain (NOD) proteins, and lectins, to sense these pathogens. Mouse mDCs express TLR7 and TLR9, whereas human mDCs lack TLR9 but express TLR7 upon exposure to IFN-αβ (Mohty et al., 2003). mDCs can also be activated by ICs through the activating FcγRIIa (Boruchov et al., 2005; Dhodapkar et al., 2005).

pDCs circulate in the blood and lymphoid organs. Upon viral exposure, these cells secrete large amounts of IFN-αβ as well as other cytokines (Liu, 2005). They also differentiate into cells with DC morphology and function (Grouard et al., 1997). Both mouse and human pDCs express TLR7 and TLR9. Chromatin-containing and snRNPs-containing ICs are internalized by pDCs via FcγRIIa and reach the endosomal compartment where they activate TLR9 and TLR7, respectively, leading to secretion of cytokines including IFN-αβ (Figure 1; Barrat et al., 2005; Bave et al., 2003; Boule et al., 2004; Honda et al., 2005; Means et al., 2005). The classic lupus autoantigens snRNPs can also be directly internalized

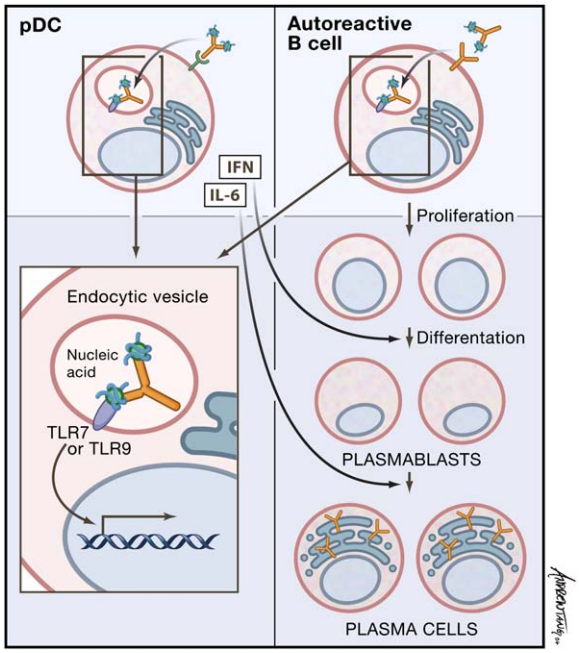

Figure 1. Immune Complexes Activate pDCs and B Cells

Immune complexes are formed when autoantibodies are engaged with either (1) chromatin whose DNA can interact with TLR9 inside pDC and B cell endosomes or (2) ribonucleoproteins whose RNA can interact with TLR7 also inside pDC and B cell endosomes. TLR signaling in pDC results in the secretion of IFN-αβ and IL-6. The combined triggering of the autoreactive BCR and TLR results in autoreactive B cell proliferation; IFN-αβ induce their differentiation into plasmablasts and IL6 induces their differentiation into autoantibody-secreting plasma cells.

within endosomes where they stimulate TLR7 and TLR8 (Vollmer et al., 2005).

Autoreactive T cells that are not purged in the thymus are controlled in the periphery by immature DCs (peripheral tolerance). In the steady state, immature mDCs capture apoptotic cells and migrate, without maturing, to the draining lymph node. There, they present self-peptide-MHC complexes, in the absence of costimulation signals, to the circulating naive autoreactive T cells, resulting in their anergy or deletion. Immature DCs may also control peripheral tolerance through induction and maintenance of regulatory T cells. Such tolerance mechanisms prevent or reduce the development of autoimmunity when dying cells are generated and processed at the time of infection. It can thus be easily imagined that "inappropriate" activation of what should otherwise be an immature, tolerogenic DC may lead to the break of peripheral tolerance and launch immunity to self-antigens.

Monocytes represent a major source of precursor DCs under inflammatory conditions, and a combination of IFN-αβ and GM-CSF drives them to differentiate into DCs. Indeed, blood monocytes from SLE patients behave like mDCs, and exposure of normal monocytes to SLE serum results in the generation of DCs (Blanco et al., 2001). Thus, SLE blood, through its IFN-αβ content, represents a DC-inducing environment. Unabated DC maturation could lead to the activation and expansion of autoreactive T cells, thus explaining many of the features of the disease (Banchereau et al., 2004).

DCs generated in the presence of SLE sera also drive the differentiation of $CD8^+$ T cells toward fully active cytotoxic effector T lymphocytes able to generate nucleosomes (Blanco et al., 2005) and granzyme B-dependent autoantigens (Casciola-Rosen et al., 1999). These autoantigens could be captured and presented by mDCs, thereby further broadening the autoimmune process. Indeed, administration of DCs loaded with apoptotic cells triggers autoimmune responses in mice, although clinical autoimmunity develops only in genetically susceptible recipients (Bondanza et al., 2003).

pDC numbers are reduced in SLE blood (Blanco et al., 2001), but these cells massively infiltrate inflamed lupus skin (Farkas et al., 2001). The decrease in SLE blood pDCs might thus result from their accelerated migration to inflammation sites, as demonstrated in allergen-challenged nasal mucosa (Jahnsen et al., 2000).

Cellular Aspects of SLE: T Cells

Alterations in SLE T cells may originate from defects in T cell subset representation, activation, and senescence (Kyttaris and Tsokos, 2004). T and B cell lymphopenia are common in SLE patients. This defect may be due to an excess of IFN-αβ, as indicated by the fact that administration of IFN-αβ in neonatal mice results in lymphopenia (Lin et al., 1998). SLE $CD4^+$ T cells express high amounts of CCR4, which might drive their migration into kidneys (Yamada et al., 2002). Increased expression of perforin by SLE $CD4^+$ T cells might also contribute to increased cytotoxic properties. Indeed, our preliminary observations suggest that IFN-αβ-stimulated DCs might favor the generation of these cells.

Very little is known about the status of regulatory T cells in SLE, though IFN-α strongly enhances IL-10-induced differentiation of functional $CD4^+$ T regulatory cells (Tr1) ($IL\text{-}10^+$, $IFN\text{-}\gamma^+$, $IL\text{-}2^{-/lo}$, $IL\text{-}4^-$) (Levings et al., 2001). This Tr1 population, however, has not been studied in SLE patients. Likewise, alterations of the follicular B helper T cell population (T_{Fh}), which plays a crucial role in high-affinity antibody responses and B cell memory within germinal centers, may occur in SLE. The T_{Fh} cells are expanded in two murine lupus-like models: the sanroque mice carrying a mutated ubiquitin ligase (roquin) gene (Vinuesa et al., 2005) and the B6.Sle1.yaa mice carrying a polymorphic cluster encoding SLAM and CD2 in addition to a duplication in TLR7 (Subramanian et al., 2006). No information is available on the status of the T_{Fh} cells in human SLE.

Despite the overall lymphopenia, $CD8^+$ T cells are increased in frequency, particularly in patients with severe lupus nephritis (Matsushita et al., 2000). Additionally, an increased proportion of SLE $CD8^+$ T cells express HLA-DR, a marker of activation (Viallard et al., 2001). The $CD8^+$ T cell compartment is remarkably skewed with a prominent depletion of the naive repertoire ($CCR7^+$ $CD45RO^-$) to the benefit of the central memory (CM) T cells ($CCR7^+CD45RO^+$) that spontaneously secrete IL-4 and IL-5 (Sen et al., 2004). SLE patients with disease flares also display higher proportions of perforin- and/or granzyme B-positive lymphocytes with a differentiated effector phenotype ($CCR7^-$ and $CD45RA^+$) (Blanco et al., 2005). Interestingly, administration of IFN-αβ to metastatic melanoma patients also results in an increase of circulating terminally differentiated effectors

(Di Pucchio et al., 2006). DCs generated in vitro with SLE serum promote the differentiation of $CD8^+$ effector T cells, which can kill target cells and generate nucleosomes and SLE autoantigens in a granzyme-dependent manner (Blanco et al., 2005).

In mice, IFN-αβ prevents activated T cell death during inflammatory responses (Marrack et al., 1999), and IFN signaling in $CD8^+$ T cells is critical for the generation of effector and memory cells in response to viral infections (Figure 2; Kolumam et al., 2005). IFN-αβ also directly stimulates $CD4^+$ T cells to enhance antigen-specific B cell responses (Le Bon et al., 2006b) and prolongs the proliferation and expansion of antigen-specific $CD8^+$ T cells during crosspriming (Le Bon et al., 2006a).

Cellular Aspects of SLE: B Cells

The key role that B cells play in the pathogenesis of SLE is supported by the clinical improvement of patients treated with the B cell-depleting CD20 antibody (rituximab) (Isenberg, 2006). Indeed, profound alterations in blood B cell compartments have been described in SLE. Conventional naive and memory B cell numbers are decreased whereas $CD38^+$ B cells, which include oligoclonal plasma cell precursors (PCPs), are expanded. The high expression of CD38 on B cells might actually be due to IFN-αβ exposure (Jego et al., 2003). PCPs display a phenotype similar to that of PCPs found in the blood during secondary immune responses, including isotype-switched and mutated Ig genes (Arce et al., 2001). Whether these SLE PCPs will become short-lived or long-lived plasma cells is not clear yet. Studies in mice suggest that they may be only short lived (our unpublished data).

Large numbers of developing B cells in the bone marrow and recent emigrants in the blood express self-reactive B cell receptors (BCRs). They represent, however, only 5%–20% of healthy naive blood B cells, but as many as 25%–50% of the SLE blood naive B cells, suggesting that there is an alteration of a tolerance check point in these patients (Yurasov et al., 2005). Further evidence for altered B cell tolerance checkpoints in SLE comes from the analysis of B cells expressing the VH4-34 gene, which encodes autoantibodies of different specificities. In healthy individuals, $VH4\text{-}34^+$ B cells are excluded during the early stages of the germinal center (GC) reaction, thus representing a second tolerance checkpoint in the life of a B cell. In SLE, but not in rheumatoid arthritis (RA), patients, $VH4\text{-}34^+$ cells progress through this checkpoint, participate in GC reactions, and are expanded within the post-GC IgG memory and plasma cell compartments (Cappione et al., 2005). The gene(s) responsible for the control of human B cell tolerance checkpoints in SLE patients are not known. However, in the Sle1z/Sle1bz mouse, an SLE susceptibility locus on chromosome 1 associated with production of chromatin antibodies has been shown to impair B cell anergy, receptor revision, and deletion. The Ly108.1 isoform of the Ly108 gene, a member of the SLAM costimulatory family, influences the ability of immature B cells to undergo deletion and RAG reexpression and represents a potential controller of tolerance checkpoints in this model (Kumar et al., 2006).

Through their effect on B cells, IFN-αβ directly enhance primary antibody responses to soluble proteins and induce the production of all subclasses of IgG in mice (Le Bon et al., 2006b). Furthermore, IFN-αβ promotes the differentiation of mDCs that can directly act on the growth and differentiation of B cells (Dubois et al., 1997; Garcia De Vinuesa et al., 1999). mDCs trigger B cell growth and differentiation through IL-12 and IL-6 (Dubois et al., 1998) as well as B cell-activating factor (BAFF) (Litinskiy et al., 2002). BAFF is induced by IFN-αβ and contributes to the survival of peripheral B cells, inlcuding those expressing autoreactive B cell receptors (Thien et al., 2004). Virus-stimulated pDCs can induce the maturation of activated B cells into plasma cells. First, IFN-αβ promotes the differentiation of activated B cells into plasmablasts (Figure 2), and then IL-6 permits plasmablasts to become antibody-secreting plasma cells (Jego et al., 2003).

The contribution of IC and TLR signaling to the generation of autoantibodies characteristic of SLE has attracted numerous studies in mice, but less is known in humans (Figure 1). In vitro, chromatin-containing IC activate transgenic autoreactive B cells via sequential engagement of the B cell antigen receptor (BCR) and TLR9

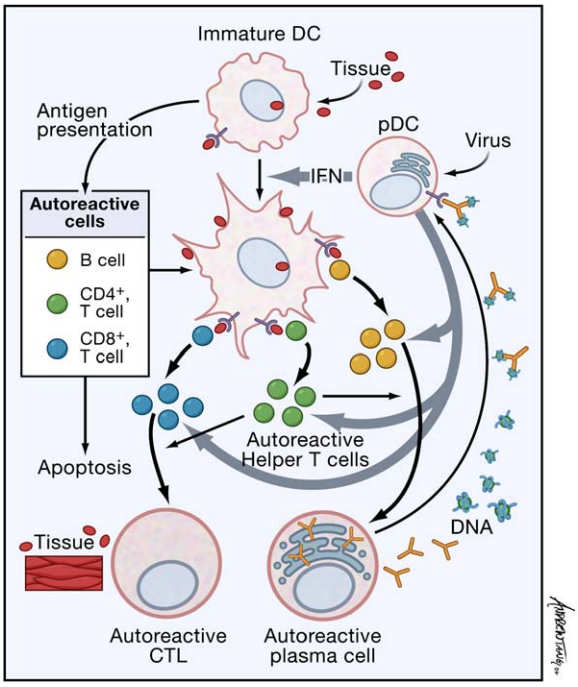

Figure 2. The Four Immune Targets of IFN

Under steady state, immature myeloid DCs capture apoptotic bodies and present their autoantigens, without costimulatory molecules, to autoreactive lymphocytes, which are then deleted (left arm). Upon viral infection, SLE pDCs produce IFN-αβ in a sustained fashion. IFN activates myeloid DCs, which now present nucleic acid-containing autoantigens from apoptotic bodies. The high expression of costimulatory molecules triggers the expansion and differentiation of autoreactive lymphocytes. Autoreactive $CD4^+$ T cells provide help to differentiate autoreactive $CD8^+$ T cells and B cells into autoreactive effectors. Autoreactive CTLs contribute to tissue damage and apoptotic body overload. Autoreactive plasma cells secrete autoantibodies, which will generate nucleic acid-containing immune complexes. These can activate pDCs to make more IFN-αβ, and autoreactive B cells to proliferate and differentiate in response to pDC-derived IFN and IL-6. These activated B cells might also engage into a germinal center reaction that will increase the affinity of autoreactive antibodies. Thus, low-affinity nonpathogenic might become high-affinity pathogenic autoantibodies.

(Leadbetter et al., 2002). In vivo, TLR9 contributes to the development of dsDNA antibodies, as shown by the fact that lupus-prone (Fas-deficient) mice that lack TLR9 on the mixed MRL-B6-129 background fail to generate these antibodies. Yet, the production of cardiolipin and RNA-reactive antibodies and the development of nephritis are not affected in these mice (Christensen et al., 2005). On a different mouse background (B6 mice lacking the inhibitory FcγRIIb), TLR9 signaling is required for anti-DNA polyreactive IgM$^+$ B cells that have escaped central tolerance to switch to pathogenic isotypes (Ehlers et al., 2006). Yet, in a different mouse lupus-like model, the pure MRL/*lpr*, TLR9 seems to deliver protective signals, because TLR9-deficient MRL/*lpr* mice develop more autoantibodies and severe lupus-like disease (Wu and Peng, 2006). As discussed earlier, this model seems to be mediated by IFN-γ and not IFN-$\alpha\beta$: the absence of type I IFN receptor worsens disease in these mice (Hron and Peng, 2004), which is at variance with the NZB model (Santiago-Raber et al., 2003).

Conclusions drawn from lupus-like models should therefore be extrapolated to humans only with caution. First, the expression of TLR9 on immune cells differs markedly in mice and humans. Second, the various murine lupus-like models may not faithfully reproduce the human disease. For example, mutations in Fas-FasL, which are the hallmark of the MRL, *lpr*, or *gld* 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.

TLR7 signaling also seems to contribute to SLE-like syndrome in mice. As shown for pDCs, immune complexes containing RNA and RNA-associated autoantigens activate autoreactive B cells in vitro (Lau et al., 2005). In vivo, FcγRIIb-deficient mice develop enhanced autoimmunity when crossed to the Y-linked autoimmune accelerator (Yaa) locus, which harbors a duplication in the TLR7 gene (Pisitkun et al., 2006). Finally, injection of the TLR7 ligand Imiquimod increases serum amounts of IL-12p70, IFN-α, and IL-6 and aggravates lupus nephritis in MRL/*lpr* mice (Pawar et al., 2006). Naturally occurring differences in expression of the TLR7 gene, as well as environmental factors that induce TLR7 expression (i.e., IFN-$\alpha\beta$ [Mohty et al., 2003]) and / or TLR7 responses, could therefore result in increased pDC and B cell responses to RNA-containing self antigens and contribute to human SLE pathogenesis as well.

Cellular Aspects of SLE: Neutrophils

Microarray analysis of SLE blood reveals a striking signature of neutrophil-specific transcripts whose expression correlates with disease activity and with the presence of lupus nephritis (Bennett et al., 2003, and our own unpublished data). Interestingly, the presence of neutrophils on lupus kidney biopsies has been long considered a marker of active nephritis, and these cells may be also be associated to vasculitis in SLE. Neutrophils could thus contribute to end-organ damage through the release of proteases, possibly after IC triggering. Furthermore, immature neutrophils may be dying outside their natural microenvironment and thus may represent a major source of nuclear autoantigens. IFN-$\alpha\beta$ might be responsible for the mobilization of neutrophils, as indicated by the fact that it induces G-CSF secretion (Fukuda et al., 2000). G-CSF-stimulated neutrophils, which release BAFF as efficiently as activated monocytes or DCs (Scapini et al., 2003), might also play an unsuspected role in the regulation of B cell homeostasis. Neutrophils may also contribute to DC activation through several mechanisms, including the production of proinflammatory cytokines, chemokines, and defensins (Yang et al., 1999), and interactions between the integrin Mac-1 and DC-SIGN (van Gisbergen et al., 2005). Accumulation of neutrophils has been described in the spleen of B6.yaa.Sle1 mice (Subramanian et al., 2006) and of NZB/W F1 mice after IFN-α administration (our unpublished data).

Interferon in SLE

We have proposed a unified model where pDCs are considered to be the main producers of IFN-$\alpha\beta$ in SLE. Indeed, pDCs accumulate in the skin at the site of SLE rashes where they might produce the IFN-$\alpha\beta$ in excess. The disease might depend on an excessive production of pDCs because of Flt3L, which is found increased in SLE serum (Gill et al., 2002). Upon viral infection, healthy pDCs secrete IFN for a few hours, and then they secrete other cytokines including TNF, which shuts down autocrine IFN-$\alpha\beta$ production. Genetic alterations in SLE might prevent the shutdown of IFN production, as illustrated by the SLE-like syndrome of SOCS-deficient mice (Hanada et al., 2003). Second, there might be alterations in the expression of molecules that can control IFN secretion. For example, the increased amounts of soluble TNF receptors in SLE serum (Gill et al., 2002) might contribute to sustained IFN-$\alpha\beta$ production by blocking TNF (Palucka et al., 2005). Alternatively, the presence of immune complexes containing either RNA or DNA may sustain IFN production by pDCs through triggering of TLR7 and TLR9, respectively (Barrat et al., 2005; Lau et al., 2005; Vollmer et al., 2005). Other cell types may also contribute to the overall increased bioavailability of IFN-$\alpha\beta$ in SLE. For example, IFN-$\alpha\beta$ triggers TLR7 expression on monocytes and DCs, which can then be induced to produce IFN-$\alpha\beta$ in response to TLR7 agonists (Mohty et al., 2003), such as virus-derived single-stranded RNA.

Under steady state, immature mDCs capture apoptotic bodies and present their autoantigens without costimulatory molecules to autoreactive lymphocytes, which are then deleted (see Figure 2). Upon viral infection, SLE pDCs produce IFN-$\alpha\beta$ in a sustained fashion. IFN-$\alpha\beta$ activates mDCs, which could then present nucleic acid-containing autoantigens from apoptotic bodies. The autoantigens might be presented on MHC class I and class II molecules. The high density of costimulatory molecules allows these mDCs to trigger the expansion and differentiation of lymphocytes into autoreactive CTLs and autoreactive helper T cells. The activated mDCs are also likely to present autoantigens to B cells (Macpherson and Uhr, 2004; Qi et al., 2006; Wykes et al., 1998), leading to B cell maturation and isotype switch (Caux et al., 1997). Activated mDCs and autoreactive helper T cells will induce autoreactive plasma

cells to secrete autoantibodies, which will generate nucleic acid-containing IC. Activated pDCs might contribute to plasma cell generation as well, because they induce the differentiation of proliferating B cells into plasma cells through IFN and IL-6 (Jego et al., 2003). One unresolved issue is the distinction between autoreactive B cell priming and reactivation of autoreactive memory B cells. The priming may be dependent on mDCs, whereas the memory activation may be dependent on mature pDCs (our own unpublished data). The role of affinity maturation also remains to be understood. In this respect, the number and size of germinal centers, which are the sites of somatic mutation, B cell selection, and isotype switching, dramatically increase in the lymphoid organs of NZB/W mice treated with IFN-α (our unpublished data). These may represent sites where autoreactive antibodies increase their affinity, transforming a low-affinity, nonpathogenic autoantibody into a high-affinity pathogenic one.

As described for SLE-like murine models, SLE patients may display genetically imprinted alterations in B cell tolerance checkpoints that would allow the survival of autoreactive clones into the peripheral compartment. IFN-αβ and/or IFN-αβ-stimulated DCs might contribute to the survival of these clones into autoantibody-secreting plasma cells. Indeed, healthy relatives of SLE patients often display ANA. Furthermore, only a fraction of patients receiving IFN-α therapy develop ANA. Similarly, B6.Sle1 congenic mice display intrinsic B cell alterations that lead to the development of chromatin antibodies in the absence of T cell help. In spite of the high autoantibody titers, these mice do not develop further SLE manifestations (Liu and Mohan, 2006). In humans, clinical SLE is preceded by the progressive accumulation of autoantibody specificities up to 9 yr before diagnosis. Ro, La, and phospholipid antibodies appear first, followed by dsDNA antibodies and then Sm and nuclear ribonucleoprotein antibodies (Arbuckle et al., 2003). Thus, as it has been observed in lupus-prone mice, the presence of SLE-specific antibodies is not enough to cause overt disease. Other factors, like IFN-αβ, may be necessary to produce the amount and/or quality of autoantibodies that will sustain the formation of IC able to activate B cells and pDCs.

Other Autoimmune Syndromes with a Potential Role for Type 1 IFN

The therapeutic use of IFN-αβ to treat cancer and hepatitis patients is associated with the induction of autoimmunity other than SLE. Although thyroiditis is the most common autoimmune manifestation, diabetes and autoimmune dermatitis have also been described. Indeed, elevated expression of IFN-α was found in the pancreas of recently diagnosed insulin-dependent diabetes mellitus (IDDM) patients (Huang et al., 1995). In dermatomyositis, an autoimmune disease targeting the skin and proximal muscle groups, muscle biopsies reveal infiltration with pDCs as well as IFN-αβ-inducible gene and protein expression (Greenberg et al., 2005; Tezak et al., 2002). Sjogren's syndrome (SS), an autoimmune disease affecting mainly salivary and lacrimal glands, also seems to be associated with alterations in IFN-αβ production, as shown by pDCs infiltrating SS salivary gland biopsies (Gottenberg et al., 2006). IFN-inducible genes are overexpressed in minor salivary glands and ocular epithelial cells, and ICs from the serum of SS patients activate pDCs to secrete IFN-αβ (Bave et al., 2005).

Although psoriasis patients are efficiently treated with TNF antagonists, several observations suggest the potential involvement of IFN-αβ in the pathogenesis of the disease. The induction of psoriasis after injection of recombinant IFN-α (Funk et al., 1991), the presence of an activated IFN-αβ signaling pathway in keratinocytes (van der Fits et al., 2004), and the development of psoriasiform inflammation in IRF-2-deficient mice (Hida et al., 2000) all point to the involvement of IFN-αβ. A functional role of IFN-αβ in the initiation of psoriasis has recently been demonstrated. Although IFN-αβ is not elevated in fully established psoriatic plaques, it is produced at early stages. Indeed, pDCs infiltrate the skin of psoriatic patients and become activated to produce IFN-αβ early during disease formation. In a xenograft model of human psoriasis, blocking IFN-α signaling or inhibiting the ability of pDCs to produce IFN-αβ prevented the T cell-dependent disease development (Nestle et al., 2005). Thus, the initial stages of psoriasis development might be IFN-αβ dependent whereas the late stages are TNF dependent. TNF may also play a role in late-stage end organ damage in SLE patients. Indeed, treatment with TNF blockers led to clinical improvement of arthritis and decreased proteinuria in an open label study including six SLE patients. As expected, autoantibodies to dsDNA and cardiolipin increased in the majority of them (Aringer et al., 2004).

Crossregulation of TNF and IFN-αβ in Autoimmune Diseases

TNF has been the first cytokine whose dysregulation was shown to be associated to an autoimmune disease. Indeed, the clinical success of TNF antagonists demonstrate that TNF plays a critical role in the pathogenesis of diseases such as rheumatoid arthritis and psoriasis (Feldmann and Maini, 2001). However, these antagonists may lead to clinical complications, such as reactivation of tuberculosis and induction of reversible SLE. Anti-TNF therapy may also worsen the clinical symptoms of established SLE. In fact, TNF antagonists enhance the production of IFN-αβ by pDCs exposed in vitro to viruses whereas TNF inhibits it, suggesting that the secretion of TNF by pDCs represents an endogenous mechanism to control IFN production. Treatment of patients suffering from systemic onset juvenile idiopathic arthritis with anti-TNF induces overexpression of IFN-αβ-regulated genes in blood leukocytes (Palucka et al., 2005). The high amounts of soluble TNF receptors found in SLE patients (Gill et al., 2002) may block endogenous TNF. TNF-mediated downregulation of the IFN-αβ pathway could also explain earlier observations in the mouse lupus model NZB/W, which bear a genetic deficiency in TNF. Consequently, these mice benefit from replacement therapy with recombinant TNF (Jacob and McDevitt, 1988).

Conversely, there is evidence that IFN-αβ may regulate TNF. For example, TNF is implicated in the pathogenesis of multiple sclerosis. In humans, IFN-β inhibits in vitro TNF production by microglia, which might partially explain the beneficial effect of IFN-β therapy in

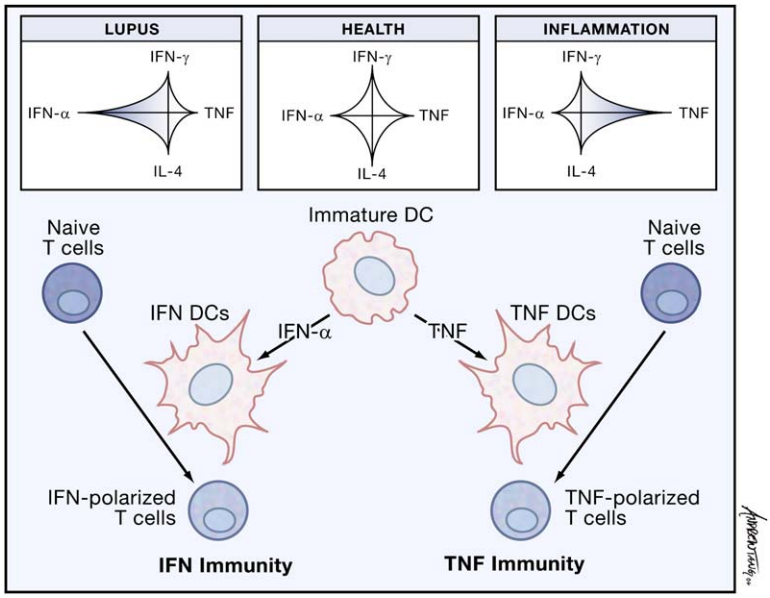

Figure 3. The Compass of Immunity and Immunopathology

Top: In an extension of the Th1-Th2 paradigm, immunity is viewed as a dynamic system driven by two sets of vectors, i.e., TNF and IFN-αβ, IL-4 and IFN-γ. The sum of the vectors yields an equilibrium point, which allows protective immunity when vectors are equal. When one of the vectors prevails beyond a certain threshold, the equilibrium point moves into a zone of immunopathology. When the IFN-αβ vector prevails, then IFN-αβ-mediated autoimmunity, i.e., lupus, occurs. The prevalence of one vector may be due to its absolute strengthening because of the increased production of the cytokine or its relative strengthening because of weakening of the counteracting cytokine. Bottom: The cytokine vectors yield distinct DC subsets that might lead to distinct immunopathologies. Immature DCs exposed to IFN-αβ or TNF will yield distinct DC subsets that will uniquely polarize naive T cells though the expression of different cytokines and costimulatory molecules. It is also proposed that the different DCs, through the expression of distinct proteolytic enzymes, might generate a different repertoire of peptides that will select a different lymphocytic repertoire. IFN-DCs may thus present chromatin/RNP-derived peptides while TNF-DCs may present IgFc.

this disease (Hofman et al., 1989). Furthermore, PBMCs from healthy volunteers injected with IFN-β show markedly decreased secretion of TNF and lymphotoxin as compared to placebo-treated volunteers (Rothuizen et al., 1999). We have proposed an extension of the Th1-Th2 (inflammation-allergy) paradigm (Mosmann and Coffman, 1989), which integrates autoimmune responses (Figure 3). Here immunity is viewed as a dynamic system driven by two sets of opposite vectors, i.e., TNF-IFN-αβ and IL-4-IFN-γ. The sum of the vectors yields an equilibrium point, which allows protective immunity when vectors are equal. This dynamic system can accommodate the prevalence of either vector to a certain extent. However, when one of the vectors prevails beyond a certain threshold, the equilibrium point moves into a zone of immunopathology, such as autoimmunity, allergy, or inflammation. Thus, when the TNF vector prevails, TNF-mediated autoimmunity such as arthritis will occur. When the IFN-α/β vector prevails, IFN autoimmunity such as SLE will occur. This might be due to increased bioavailability of one cytokine or decreased bioavailability of the other one. The unabated production of a given cytokine will affect the cells of the immune system and most particularly DCs. Thus, IFN-stimulated DC will polarize naive T cells differently than TNF-stimulated DCs.

Conclusions

In summary, just like many roads lead to Rome, we contend that many genetic alterations might lead to the sustained overproduction of IFN-αβ in human SLE. The resulting increased bioavailability of IFN-αβ induces the activation of immature mDCs that control peripheral tolerance by deleting autoreactive lymphocytes. IFN-matured DCs activate and expand autoreactive T cells, both helping autoreactive B cell development. In addition to its indirect effect through DCs, IFN-αβ also directly allows the expansion and survival of $CD4^+$ and $CD8^+$ T cells as well as the differentiation of B cells into plasma cells. The increased frequency of autoreactive B cells depends on a second set of genetic alterations targeting B cell tolerance checkpoints. These early events create a first level of autoimmune injury, which is clinically silent but might generate apoptotic cells and nucleic acid-containing immune complexes. The capture of these apoptotic cells by mDCs and nucleic acid-containing ICs by pDCs and autoreactive B cells broadens the autoimmune reaction, thereby leading to disease manifestations.

The past 5 years have highlighted the pathogenic role of IFN-αβ and nucleic acid-containing immune complexes in SLE. Research within the next 5 years will hopefully help us to understand the basic genetic alterations leading to IFN-αβ overproduction and B cell tolerance breakdown in SLE, thus eventually allowing us to identify individuals with predisposition; establish the role of other cells like neutrophils, T_{Fh} cells, and Tr1 cells in the pathogenesis of human SLE, thus eventually allowing us to uncover new avenues of treatment; determine whether, like TNF antagonists, which have brought considerable relief to RA patients, IFN antagonists will bring relief to SLE patients; and apply new tools, e.g., transcriptome analysis, to diagnose the disease during its preclinical stage. Inasmuch as SLE often takes years to develop after the generation of the first wave of autoantibodies, we foresee with enthusiasm the day when blocking IFN during the preclinical phase of SLE might prevent the appearance of clinical symptoms and thus avoid the terrible morbidity associated with this disease.

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