Autoimmune Rheumatic Diseases 3

Immunopathogenic mechanisms of systemic autoimmune disease

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Systemic lupus erythematosus, Sjögren's syndrome, and dermatomyositis are systemic autoimmune diseases that develop after environmental triggering of genetically susceptible individuals. The precise cellular and molecular mechanisms leading to autoimmune disease, and what factors determine which organs are involved, remain poorly understood. Recent insights into genetic susceptibility now make obvious that environmental triggers often act via cellular pathways containing disease-associated polymorphisms. In the breaking of tolerance, the initiating tissue—including dendritic cells—provides a decisive microenvironment that affects immune-cell differentiation, leading to activation of adaptive immunity. Type 1 interferon produced by innate immune cells has a central role in systemic autoimmunity and activates B cells and T cells. In turn, B-cell-derived autoantibodies stimulate dendritic cells to produce type 1 interferon; thus, a positive feedforward loop is formed that includes both the innate and adaptive systems. New treatments could simultaneously and specifically target several such vital pathways in autoimmunity.

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This is the third in a **Series** of three papers about autoimmune rheumatic diseases

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Introduction

Our immune system developed to protect us against invading pathogens and to aid tissue healing after injury. In systemic autoimmune diseases, mechanisms that regulate the balance between recognition of pathogens and avoidance of self-attack are impaired. Furthermore, control of inflammation is lost, resulting in continuous immune activation without any overt infection, with different amplitudes during flares and quiescent disease. Adaptive immunity providing immune memory remains of central interest, but the role of the innate immune system in the pathogenesis of systemic autoimmunity is also currently under intense investigation. Study findings suggest genetic risk loci are shared in systemic autoimmune diseases and, therefore, that pathogenic mechanisms may be similar.[1] However, distinct loci have also been identified that are specific to individual diseases, indicating that various immunopathogenic pathways are present.[2] Concordance rates of 20–30% in monozygotic twins emphasise that environmental components that interact with the host genetic factors are important to our understanding of autoimmunity.

Two hypotheses for systemic autoimmune inflammation have been suggested. First, barrier control between innate and adaptive immunity could be disturbed, fuelling continuous inflammation by a positive feedforward loop, consistent with interferon effects. Second, impaired reactivity of adaptive immunity with reactivated (auto)reactive memory by lymphocytes could result in persistent inflammation and include defects of tolerance checkpoints. These two ideas are not mutually exclusive, and both innate and adaptive mechanisms seem operational in systemic autoimmunity.

Systemic lupus erythematosus, Sjögren's syndrome, and dermatomyositis are systemic autoimmune diseases that share clinical, immunological, and genetic features but have disease-specific traits. In people with systemic lupus erythematosus, almost any organ of the body could be a target for autoimmune inflammation, whereas Sjögren's syndrome is restricted mostly to exocrine glands; in dermatomyositis, proximal muscles and skin are affected predominantly. In this Review, we describe our current understanding of the immunopathogenic mechanisms underlying systemic autoimmunity, with particular reference to systemic lupus erythematosus, Sjögren's syndrome, and dermatomyositis.

Induction of autoimmunity

The idea that we are predisposed genetically to either an increased or diminished risk of developing systemic autoimmunity stems from observations of disease prevalence within families and in the general population. Most autoimmune disorders have a frequency in the general population of 0·1–1·0%, whereas prevalence in first-degree relatives is around five times higher, with a further five times increase in monozygotic twins of affected individuals. Although risk is obviously raised with increasing genetic similarity to an affected

Search strategy and selection criteria

We searched the Cochrane Library, Medline, and Embase (date last accessed March 15, 2013) with the terms: "autoimmunity", "T cell", "B cell", "regulatory", "Th1", "Th2", "Th17", "autoantibodies", "dendritic cell", "lymphoid organs", "germinal centers", "follicles", "cytokines", "organ damage", "lupus", "autoimmune myositis", "dermatomyositis", "Sjögren", and "co-stimulation" in different combinations. Because of the comprehensive topic, we selected the most relevant publications from the past 5–7 years but considered highly regarded and appropriate older publications. We restricted our search to articles published in English.

individual, the concordance rate of most autoimmune diseases in monozygotic twins is still not more than 20–30%, indicating that additional elements—eg, environmental factors—play a part in whether clinical disease develops in a genetically predisposed individual. Furthermore, systemic lupus erythematosus, dermatomyositis, and Sjögren's syndrome all affect women mainly (80–90%), suggesting hormonal factors have a role in pathogenesis. This idea is emphasised further by the peak in incidence of systemic lupus erythematosus during childbearing ages.[3]

Inherited susceptibility

In the rare families with multiple cases of systemic autoimmunity, and in case-control studies of sporadic cases of these disorders, findings support that genetic variation predisposes for systemic autoimmune diseases. These genetic variations fall into three main categories: rare (<1%) genetic polymorphisms and copy-number variants; common (>1%) single-nucleotide polymorphisms (SNPs) and copy-number variants; and epigenetic modifications. Few polymorphisms or mutations that directly cause autoimmune diseases have been pinpointed, and most identified polymorphisms rather represent markers of genetically linked sequences conferring the disease-related traits. Genes associated with disease, therefore, commonly represent a best guess based on the position and strength of the genetic signal and function of the gene.

Panel 1: Emerging concepts

Quantitative thresholds of immune signalling

Ligand concentrations, receptor densities, and the sum of activity of intracellular factors in a pathway will determine the signalling strength, which in turn affects cellular responses. In systemic autoimmune diseases, the genetic polymorphisms—individually conferring a low odds ratio—may interact to overcome or lower thresholds in immune signalling.

Time to phenotypic manifestation

On the basis of current understanding of gene–environment interactions in the pathogenesis of systemic autoimmune diseases, this idea considers diseases from the perspective of time-to-phenotypic-manifestation rather than defining a point of disease onset.

Type 1 interferon accelerates an autoimmune feedforward loop

The many roles of type 1 interferon in regulation of immunity—including regulation of MHC expression, increased amounts of interferon α, and presence of an interferon signature in systemic autoimmunity—propose type 1 interferon as a central factor in the enhanced interaction between innate and adaptive immunity, driving the positive feedforward loop in the autoimmune disease process.

Tissue microenvironment and infiltrating immune cells

The affected organ could have a more active role in the autoimmune process than previously thought. The tissue microenvironment—including resident dendritic cell—affects infiltrating immune cells actively and can contribute to maturation and induce cross-differentiation between the T-cell subset through secreted cytokines. The tissue microenvironment is also important for development of ectopic germinal centres, which in turn contribute to formation and selection of autoreactive clones.

In genome-wide association studies, SNPs have been identified that suggest both shared and distinct pathogenic pathways to development of autoimmune disorders.[1] The strongest associations have been found within the MHC locus, which contains several signals from both classic (antigen-presenting) MHC class I and II genes and non-classic (non-antigen-presenting) MHC class III genes. Although separation and pinpointing of signals has been difficult because of gene density and high linkage disequilibrium within the locus, association with the HLA class II molecule HLA-DR3 is firmly linked to systemic lupus erythematosus, Sjögren's syndrome, and subtypes of autoimmune myositis.[4] In systemic lupus erythematosus, the HLA-DR3 haplotype associates with production of antibodies against DNA,[5] in myositis it associates with anti-Jo1 production,[6] and in Sjögren's syndrome the association is between *HLA-DRB1*03* and production of autoantibodies against Ro and is even stronger than the HLA association with disease itself.[7] Within the MHC class III region, several genes—including those encoding complement factor 4 (*C4A*) and tumour necrosis factor (*TNF*)—are implicated.[8] The dominant association between systemic autoimmune disease, specific autoantibodies, and distinct MHC haplotypes that play a part in antigen presentation indicates a vital role of antigen-presenting cells and lymphocytes in translating signals from innate immune activation into specific autoimmune responses and establishing (auto)immune memory.

MHC disposition and interferon production are linked

Several non-MHC susceptibility genes have been identified by genome-wide association studies in systemic lupus erythematosus, typically relating to pathways of B-cell and T-cell activation or innate receptor signalling. The most significant and consistent associations are with *IRF5*, *STAT4*, *PTPN22*, C8Orf13-*BLK*, *TNFSF4*, *TNFAIP3*, *PRDM1-ATG5*, and *ITGAM-ITGAX*.[9] Preliminary reports from genome-wide association studies in Sjögren's syndrome and dermatomyositis unsurprisingly show that SNPs within the MHC locus are implicated for both diseases.[10,11] In Sjögren's syndrome, genome-wide significance was also recorded for *IRF5*, *STAT4*, *BLK*, and *IL12A*. Although minor allele frequencies and odds ratios of non-MHC polymorphisms are generally much lower than for the MHC locus, they contribute towards the idea of quantitative thresholds for immune-cell signalling (panel 1).[2] This emerging concept takes into account how several genetic factors with a fairly small effect individually could combine to enhance susceptibility to systemic autoimmunity. For example, *IRF5* and *STAT4* risk alleles have been shown to interact in both systemic lupus erythematosus and Sjögren's syndrome, resulting in increased susceptibility.[12] Functional interaction between MHC-encoded genes and the interferon signature observed in systemic autoimmunity is also an idea that has developed recently. This notion is based on the role

of plasmacytoid dendritic cells in antigen presentation in systemic lupus erythematosus, Sjögren's syndrome, and dermatomyositis and the fact that these cells produce large amounts of type 1 interferon, which in turn up-regulates HLA expression. These inter-relations suggest that autoimmune specificity is linked closely to initiation and propagation of autoimmunity by type 1 interferon.

Action of environmental triggers via genetically polymorphic pathways

Several environmental factors that trigger and precipitate systemic autoimmunity have been identified (figure 1). These triggers act via pathways in which gene polymorphisms associated with disease cluster.

Chemicals (eg, aromatic amines and organophosphates) and drugs (eg, thiazides, hydralazines, calcium-channel blockers, proton-pump inhibitors, and interferon α) can induce reversible lupus-like disorders.[13] Interferon α binds to the interferon (α, β, ω) receptor (IFNAR1 and IFNAR2), which triggers a downstream signalling and regulating cascade that includes several genes polymorphic in systemic lupus erythematosus, including *TYK2*, *IKZF1*, and *STAT4* (figure 1).

Ultraviolet radiation can exacerbate skin manifestations in patients with systemic lupus erythematosus and dermatomyositis. It might trigger systemic autoimmunity via immune pathways or by induction of apoptosis. During apoptosis, self-antigens are exposed and, with defective uptake of apoptotic material in systemic lupus erythematosus, prolonged contact with autoantigen-specific B cells may occur. Downstream signalling of the B-cell receptor includes molecules such as LYN, PTPN22 (LYP), BLK, and BANK1, all of which carry polymorphisms that have been associated with systemic lupus erythematosus (figure 1). Furthermore, apoptosis can generate autoantigens containing nucleic acids that might activate endosomal toll-like receptors. With more advanced characterisation of the processes, these pathways could be used to develop novel treatments.

Smoking is another risk factor for systemic lupus erythematosus, Sjögren's syndrome, and myositis and might contribute to disease development via several pathways (figure 1). Similar to rheumatoid arthritis,[14] smoking interacts with the HLA haplotype for development of autoantibodies in autoimmune myositis,[15] suggesting that a specific risk factor will

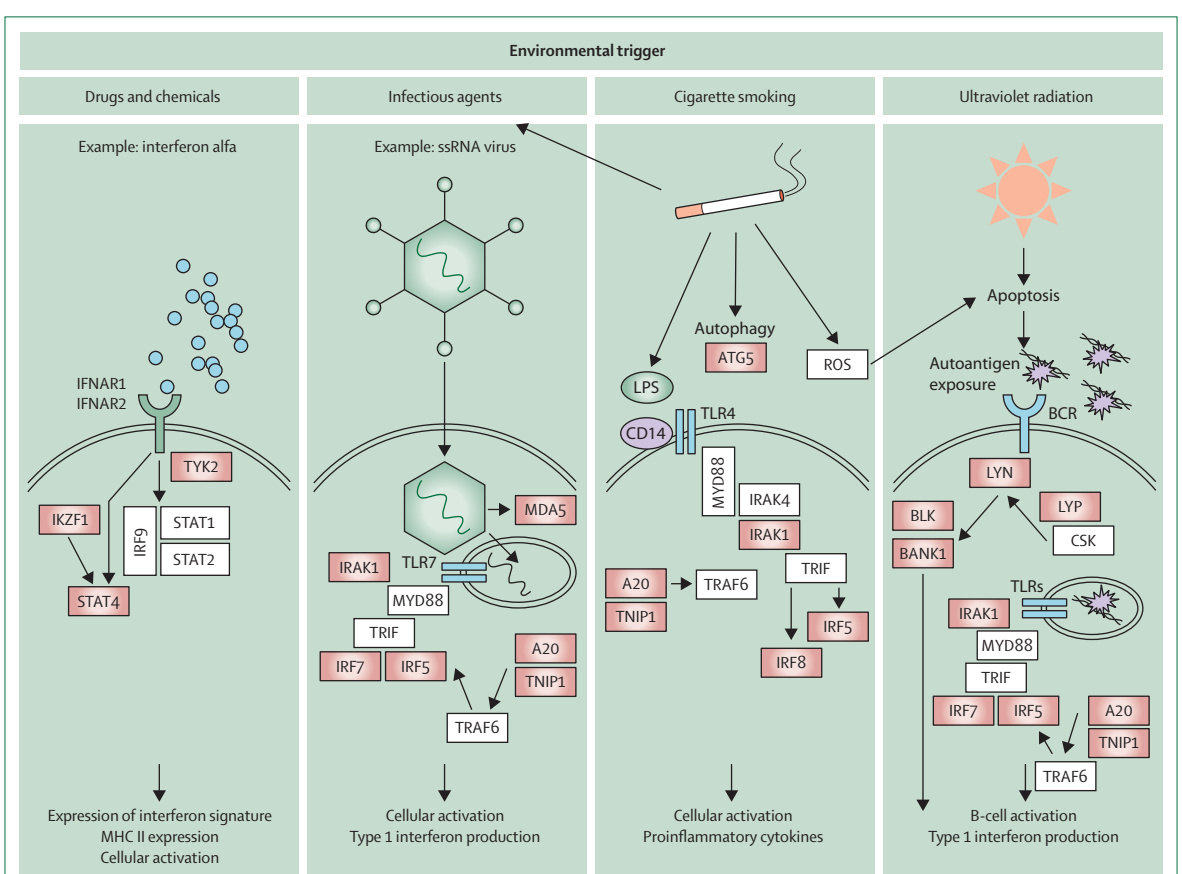

Figure 1: **Mechanistic pathways in gene–environment interactions**
Molecules shown in red are encoded by loci with a confirmed genome-wide association with systemic lupus erythematosus ($p<5\times10^{-8}$). LPS=lipopolysaccharide. A20=TNFAIP3. BCR=B-cell receptor. LYP=PTPN22. MDA5=IFIH1. ROS=reactive oxygen species. ssRNA=single-strand RNA. TLR=Toll-like receptor. TRIF=TICAM1

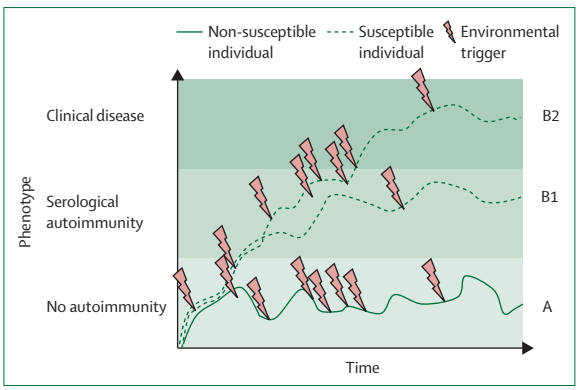

Figure 2: **Time to clinical manifestation**
Environmental triggers establish how individuals who are genetically different (A and B) and genetically identical (B1 and B2) progress into autoimmunity and show clinical signs of systemic autoimmune disease. Individual A, not genetically predisposed for autoimmunity, might encounter similar environmental challenges as B without developing autoimmunity. Genetically susceptible and identical individuals (B1 and B2) might progress (or might not progress) into autoimmunity and systemic autoimmune disease depending on the environmental factors they are exposed to.

predominantly increase disease risk in individuals with a given genetic background. Cigarette smoke elicits innate immune responses and contains several toll-like receptor-stimulating compounds, including lipopolysaccharide, a TLR4 agonist that activates TLR4 signalling directly and triggers proinflammatory pathways. Signals are passed via molecules with genetic polymorphisms associated with systemic immunity, providing a direct connection and mechanistic basis for gene–environment interactions. Smoking also increases the risk for respiratory-tract infections, which might trigger toll-like receptor pathways that promote inflammation. Furthermore, smoking leads to autophagy in the respiratory tract, a process in which ATG proteins are central. Polymorphisms in *ATG5* are associated with systemic lupus erythematosus.[9] Finally, cigarette smoke induces apoptosis in the lung via reactive oxygen species, providing additional ways of triggering and sustaining autoimmune responses.

Infectious agents have been proposed repeatedly as risk factors for development of systemic autoimmune disease. Initial hypotheses focused on the possibility of cross-reactivity between proteins of infectious agents and epitopes of endogenous antigens, an idea known as molecular mimicry. With our current understanding of the genetic prerequisites and the central role of type 1 interferon in systemic autoimmunity, focus is now rather on how infectious agents can trigger autoimmunity by interaction with pattern recognition receptors such as toll-like receptors. Downstream signalling from cell-surface or endosomal toll-like receptors (TLR7, TLR8, and TLR9) includes several genetic variants in systemic lupus erythematosus, including IRAK1, TNFAIP3 (A20), TNIP1, IRF5, and IRF7 (figure 1).

Time to phenotypic manifestation

Study findings show that in systemic autoimmune diseases autoantibodies arise long before clinical symptoms develop and a diagnosis is confirmed.[16,17] Anti-Ro/SSA were noted in biobanked tissue samples from individuals who later developed systemic lupus erythematosus, which preceded disease diagnosis by several years. This finding also confirms observations made in mothers of children with congenital heart block. Although some mothers are already diagnosed with systemic lupus erythematosus or Sjögren's syndrome when the fetus develops congenital heart block, many are asymptomatic yet positive for Ro/SSA autoantibodies. Follow-up of these mothers shows that most later develop clinical features of Sjögren's syndrome or systemic lupus erythematosus.[18] Similarly, the risk of thromboembolic complications is increased 12–15 years after women positive for phospholipid antibodies had recurrent spontaneous abortions.[19,20] These observations highlight that even in genetically susceptible individuals, the process from an initiated autoimmunity to clinically overt disease takes many years (figure 2).

Little is known about triggering events that drive the disease process forward: what they are, whether they need to present in a specific sequence or at a certain frequency, and what steps in the deterioration towards autoimmune disease they correspond with. A longitudinal perspective of systemic autoimmune diseases—thinking of disease as a process and time to phenotypic manifestation rather than as a distinct disease onset—might be more productive in terms of understanding the pathogenesis (panel 1).

The innate system in systemic autoimmunity

The innate immune system—consisting of physical mucosal barriers, proteins, and cells such as granulocytes, natural killer cells, macrophages, and dendritic cells—serves as the front line against infectious agents and other environmental challenges. Dendritic cell homoeostasis is implicated directly in systemic autoimmunity, and dendritic cells contribute to disease both as antigen presenters and as major producers of type 1 interferon.[21] Different subsets of dendritic cells regulate humoral and cellular adaptive immunity in human beings (figure 3).[22] Humoral immunity is regulated mainly by CD14+ dendritic cells producing interleukin 12 with direct effects on B cells and follicular B helper T cells. Cellular responses in the skin are managed predominantly by Langerhans cells producing interleukin 15 and activating Th cells supporting CD8+ cytotoxic T lymphocytes. Finally, CD141+ dendritic cells support not only responses of cytotoxic T lymphocytes but also humoral responses via interleukin 12 secretion. Moreover, direct activation of B cells by dendritic cells might increase humoral autoimmunity.

The distinct immunoenhancing profiles and tissue-specific characteristics of dendritic cells may account for heterogeneity of organ manifestations and immune

findings between individual patients. As a common denominator, interferon α is produced, which fuels immune activation (figure 4); however, it also might act to superimpose underlying immunological differences.

Plasmacytoid dendritic cells and type 1 interferon in autoimmunity

Type 1 interferon has a central role in systemic lupus erythematosus, Sjögren's syndrome, and dermatomyositis (panel 1). Systemic lupus erythematosus-like disease was reported after therapeutic use of interferon α to treat cancer and hepatitis.[23] Furthermore, increased amounts of interferon α have been noted in patients with systemic lupus erythematosus, and concentrations correlate with both disease activity and severity.[21] Several manifestations—eg, skin rash, fever, and leucopenia—and markers of immune activation accord with amounts of interferon α in serum. Peripheral blood mononuclear cells from patients with active lupus upregulate interferon-induced genes, and this so-called interferon signature (figure 4) correlates with disease severity. Similar expression of these interferon-regulated genes can be induced by culturing healthy blood cells with systemic lupus erythematosus plasma samples. Greater than 90% inhibition of gene expression can be achieved with anti-interferon α, but not by antibodies to interferon β or interferon γ, indicating that interferon α is the primary type 1 interferon driving systemic autoimmunity.[21,24] Use of high-dose intravenous steroids in patients with systemic lupus erythematosus, which can induce clinical remission, abrogates the interferon signature in peripheral blood mononuclear cells.[24] In Sjögren's syndrome, peripheral blood mononuclear cells and minor salivary glands express genes of the interferon signature,[25] and in dermatomyositis, interferon-inducible genes and proteins are expressed in affected muscle.[26]

Most cells can produce interferon α on activation as part of antiviral defence mechanisms, but plasmacytoid dendritic cells express interferon α constitutively and can produce an amount up to 1000-fold higher than other cells. This high capacity of plasmacytoid dendritic cells, together with their presence in affected organs in systemic autoimmunity, has focused much interest onto these cells. In systemic lupus erythematosus, plasmacytoid dendritic cells can be detected in affected skin,[27] in Sjögren's syndrome they infiltrate salivary glands,[25] and in dermatomyositis these cells are found in affected muscles.[26]

Induction of type 1 interferons in systemic autoimmunity

Typically, type 1 interferon is produced in response to viral infections, but in patients with systemic lupus erythematosus, Sjögren's syndrome, and dermatomyositis, plasmacytoid dendritic cells are also induced to synthesise interferon via toll-like receptor ligation by endogenously derived nucleic acids.[28] Stimulation of toll-like receptor occurs via immune complexes formed by

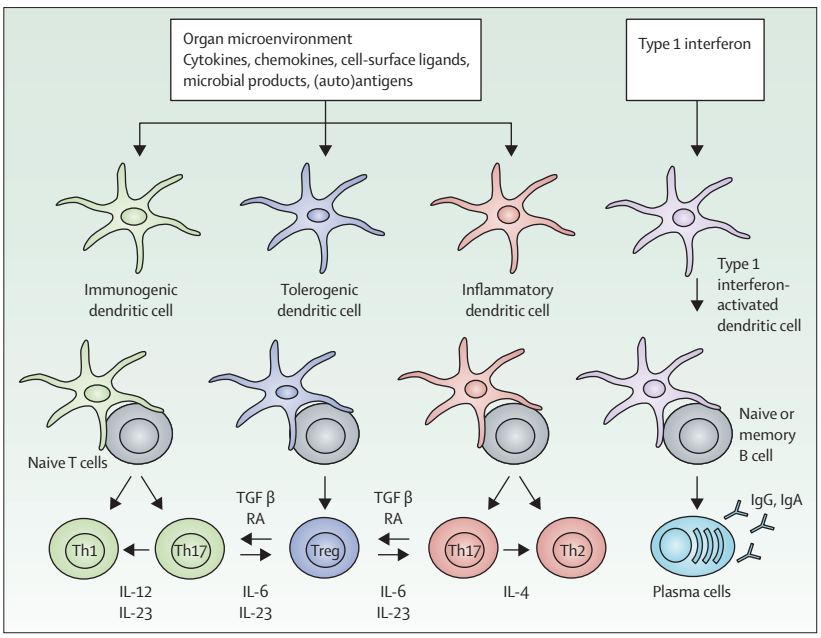

Figure 3: **Effect of local environment in affected organs of systemic autoimmune disease**
Maturation of dendritic cells into immunogenic, inflammatory, or tolerogenic subsets depends on organ microenvironment and is affected by factors such as soluble microbial molecules, cytokines, and ligand interactions. Subsets of dendritic cells support distinct Th-differentiation pathways. The local microenvironment also contributes to plasticity in Th subsets, and transdifferentiation between T-cell subsets can occur depending on the cytokine milieu. Dendritic cells activated by type 1 interferon can interact directly with naive and memory B cells, resulting in plasma-cell differentiation and production of IgG and IgA. IL=interleukin. RA=retinoic acid. TGF β=transforming growth factor β. Th=T-helper cell. Treg=regulatory T cell. Ig=immunoglobulin.

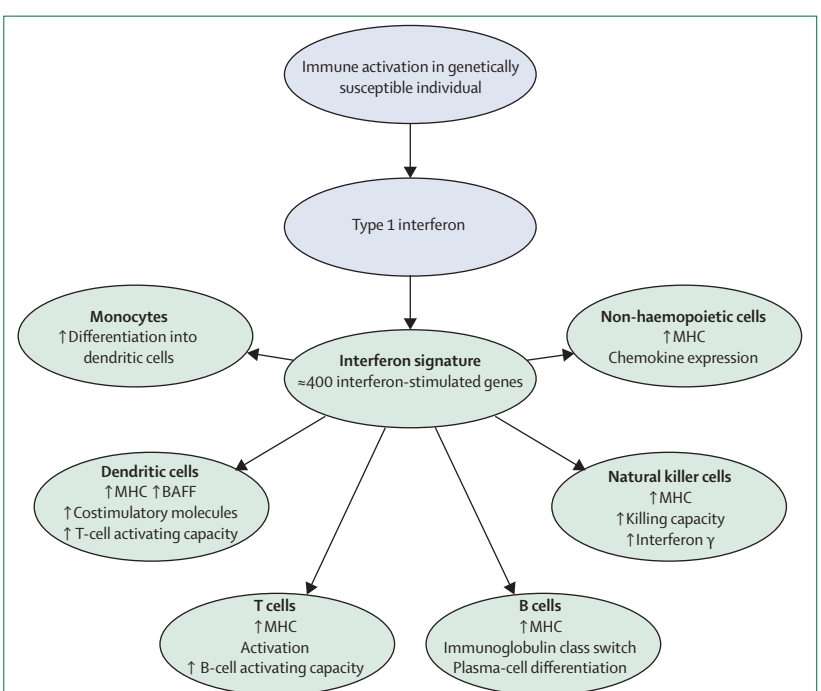

Figure 4: **Effects of type 1 interferon-activated gene expression in different cells**
Immune activation in systemic autoimmunity leads to production of type 1 interferon, which in turn activates about 400 genes—denoted the interferon signature. Depending on cell type, type 1 interferon-activated genes will lead to various outcomes. BAFF=B-cell activating factor (TNFSF13B).

binding of autoantibodies to nucleic acid-associated autoantigens (histones, ribonucleoprotein, Ro/La). Such interferogenic immune complexes,[21] which are internalised via FcγRIIa expressed on plasmacytoid dendritic cells, stimulate the relevant toll-like receptor with subsequent activation of transcription factors and production of interferon α. Enhanced neutrophil extracellular traps containing DNA have been identified as additional triggers of interferon production by plasmacytoid dendritic cells[29] but seem not to be a unique prerequisite for lupus induction.[30]

Associations between genes that play a part in the production and signalling pathways of type 1 interferon and systemic lupus erythematosus and Sjögren's syndrome have been reported. Polymorphisms in *IRF5* are associated with induction of systemic lupus erythematosus, Sjögren's syndrome, and dermatomyositis,[31] and in systemic lupus erythematosus, associations with *IRF7* and *TYK2* have also been noted. The transcription factor *IRF5* is expressed constitutively in plasmacytoid dendritic cells and regulates type 1 interferon gene activation, whereas the Janus kinase TYK2 binds to the type 1 interferon receptor IFNAR and is required for its signalling (figure 1). Variations in *STAT4*—a transcription factor that transmits signals by type 1 interferon—are also associated with systemic lupus erythematosus and Sjögren's syndrome.[32]

Autoimmunity-related effects of interferon α

Interferon α has a range of biological effects related to systemic autoimmunity (figure 4). Usually, immature myeloid dendritic cells capture apoptotic bodies and present their autoantigens without costimulatory molecules to autoreactive lymphocytes, which are deleted or anergised. Type 1 interferon further induces maturation and activation of dendritic cells with increased expression of MHC class I and II molecules, costimulatory molecules, chemokines and chemokine receptors, production of B-cell activating factor (TNFSF13B) and a proliferation-inducing ligand (TNFSF13).[21] The activated dendritic cells subsequently present antigens, including autoantigens, to drive an immune response. Thus, unabated activation of dendritic cells, induced by type 1 interferon, promotes the expansion of autoreactive T cells. Dendritic cells in patients with systemic lupus erythematosus are characterised by their unique in-vitro ability to promote differentiation of CD8+ T lymphocytes into cytotoxic T lymphocytes, which are capable of cell lysis and generation of nucleosomes and granzyme B-dependent autoantigens.[33] Terminally differentiated effector CD8+ cytotoxic T lymphocytes are augmented in the blood of patients with systemic lupus erythematosus. This increase correlates with disease activity[21] and can induce direct tissue damage.

Activated dendritic cells can also present autoantigens to B cells, inducing B-cell maturation and differentiation into plasma cells by secretion of interleukin 6, B-cell activating factor (TNFSF13B), and interferon.[34] Interferon α further stimulates CD4+ Th cells to enhance antigen-specific B-cell responses. Together, activated myeloid dendritic cells and autoreactive Th cells will induce autoreactive plasma cells to secrete autoantibodies, which will generate nucleic acid-containing immune complexes. Evidence for alterations in the blood B-cell compartment and altered B-cell tolerance checkpoints in systemic lupus erythematosus comes from analysis of expression of plasma-cell markers and certain B-cell receptor idiotypes. In plasma cells, high expression of HLA-DR marks them as recently activated,[35] whereas in B cells, usage of VH4–34 gene rearrangements,[36,37] which frequently encode autoantibodies of the autoreactive idiotype 9G4, as a surrogate marker of a tolerance breach. In healthy individuals, 9G4 is excluded during the early stages of the peripheral germinal centre reaction,[38] which represents a second B-cell tolerance checkpoint. In systemic lupus erythematosus, 9G4-expressing cells progress through this checkpoint, participate in germinal centre reactions, and are expanded within the post-germinal centre immunoglobulin G memory and plasma-cell compartments.[38]

The genetic polymorphisms found within many components of the innate and adaptive immune systems, and their signalling and effector pathways in patients with systemic autoimmunity, can lead to lowered signalling thresholds and create a feedforward loop that sustains inflammation and disease (figure 5). With activation of dendritic cells, an autoimmune self-amplification loop would produce more interferon, promoting and sustaining

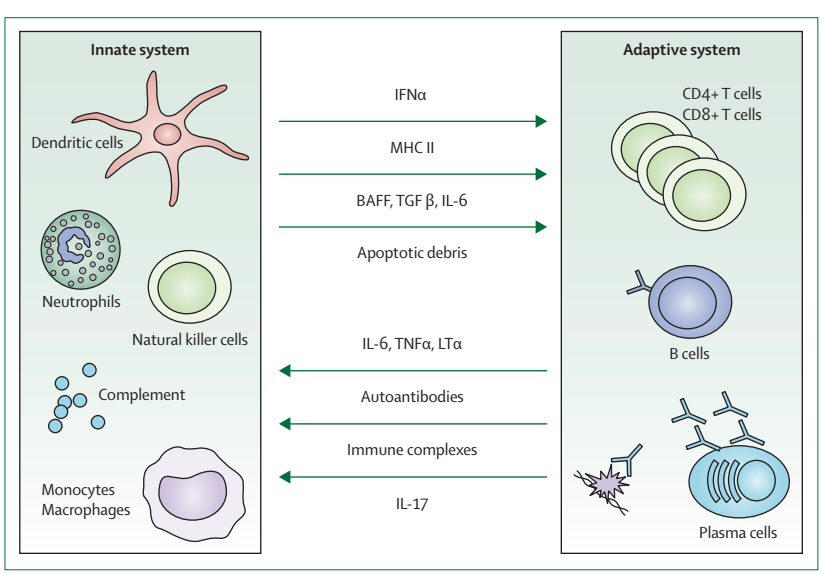

Figure 5: **Feedforward loop between innate and adaptive immune systems in systemic autoimmunity**
Factors of the innate system activate and promote adaptive components, which feedback into the innate arm to promote immune activation. Central components of the innate system that feed into the adaptive system in systemic autoimmune diseases include type 1 interferon and other cytokines, MHC II, and apoptotic debris, while important factors from the adaptive system promoting innate responses encompass proinflammatory cytokines, autoantibodies, and immune complexes. BAFF=B-cell activating factor (TNFSF13B). IFNα=interferon α. IL=interleukin. LTα= lymphotoxin α. TGF β=transforming growth factor β. TNFα=tumour necrosis factor α.

autoreactive responses, keeping activated T cells and B cells in a vicious cycle, and producing autoantibodies.[39] Furthermore, diminished negative regulation—such as that from FcγRIIB—contributes to persistence of the feedforward loop in systemic autoimmunity. The involved subtypes of dendritic cells, in addition to non-haemopoietic cells able to produce type 1 interferon,[40] remain of vital importance.

Adaptive immune cells

Directing role of cytokines in T-cell differentiation

T-cell abnormalities have been detected in autoimmunity, but whether these aberrations represent primary deviations or reflect a response to exogenous effects by cytokines produced by an impaired immune system is uncertain. Naive CD4+ T cells differentiate into subsets of effector T-helper cells after activation and costimulation, a process controlled by specific cytokines (figure 3). T-helper-1 cell differentiation is promoted by interleukin 12 and interferon γ, T-helper-2 cells depend on interleukin 4, and transforming growth factor β is crucial for development of regulatory T cells. However, transforming growth factor β in the presence of interleukin 6 will lead to differentiation into a highly proinflammatory subset of T-helper-17 cells, initially characterised by interleukin 17 production. Stable differentiation and proliferation of T-helper-17 cells also needs interleukin 21 and interleukin 23.[41] Although differentiation was regarded initially as a terminal process, data suggest plasticity between T-helper cell subsets and that transdifferentiation can take place, dependent on the cytokine milieu[42–44] (figure 3, panel 1); the target tissue is also important in the inflammatory process (panel 1).

T-helper-17 cells in systemic autoimmunity

Increased amounts of interleukin 17, and a high proportion of interleukin 17-producing T cells, have been reported in patients with systemic lupus erythematosus; correlation with disease activity has also been noted.[45,46] T cells that produce interleukin 17 have been detected in the main target organs in systemic lupus erythematosus (ie, skin, kidneys, and lungs), suggesting that interleukin 17 has a role in local inflammation and tissue damage.[47,48] Furthermore, in patients with Sjögren's syndrome, high expression and increased concentrations in plasma of interleukin 17, interleukin 21, and interleukin 23 have been noted.[49] Enhanced expression of interleukin 17 has been identified in muscle specimens from autoimmune myositis patients,[50] together with augmented production of interferon γ and expression of MHC class I.

Several genetic risk factors in systemic lupus erythematosus and Sjögren's syndrome are related to formation or maintenance of T-helper-17 cells. *IRF5* plays a part in type 1 interferon pathways and in expression of genes important for T-helper-17 responses by acting as a transcription factor for interleukin 6 and the p40 subunit of the interleukin 23 cytokine. The potential functional effect of this genetic association is supported by increased amounts of both interleukin 6 and p40 in patients with systemic lupus erythematosus and Sjögren's syndrome,[51,52] and it accords with data from a phase 1 trial blocking interleukin 6-receptor in systemic lupus erythematosus patients, leading to a reduction in disease activity.[53]

IRF8, which acts as a repressor of T-helper-17 cell differentiation, has been identified as a risk locus for systemic lupus erythematosus.[54] Furthermore, SNPs in the *IL21* and *IL21R* genes[55] and a copy-number variation in *IL17F*[56] are associated with systemic lupus erythematosus. Accumulating evidence, therefore, points to a role for T-helper-17 cells in the pathogenesis of systemic autoimmunity; studies of interleukin 17 blockade in systemic lupus erythematosus are awaited.

	Amount of Treg cells, CD4+CD25high, or CD4+CD25+FOXP3+			Treg cell function	Treg cell resistance to Teff cells	Treg cell response to treatment
	Generation	Peripheral blood	Tissue			
Systemic lupus erythematosus	Decreased via diminished TGF β or IL-2 or both; impaired regulation of APC function and increased proinflammatory cytokines	Decreased	ND	Decreased or impaired via fall in γ-chain cytokines; failed cell-contact suppression; decreased IL-10, IL-35, and TGF β	Increased	Increased numbers after glucocorticoid treatment
Sjögren's syndrome	ND	Contradictory data; diminished, normal, and increased amounts reported	Increased and associated with infiltrates; inverse relation of CD4+FOXP3+ cells between blood and tissue	Not impaired	ND	Increased numbers under immunosuppression
Dermatomyositis	ND	Decreased	ND	ND	ND	Increased numbers under immunosuppression

APC=antigen-presenting cell. IL=interleukin. ND=not determined. Teff=effector T cell. TGF=transforming growth factor. Treg=regulatory T cell.

Table 1: **Regulatory T-cell abnormalities in systemic autoimmune diseases**[58-62]

	CD27+ memory B cells	CD27++ plasma cells	Regulatory B cells CD24highCD27+	Formation of germinal centre-like structures or B-cell follicles
Systemic lupus erythematosus	Increased amount in peripheral blood, including a subset of CD95+ memory B cells	Increased amount in peripheral blood, which correlates with disease activity	Functionally impaired	In kidneys
Sjögren's syndrome	Decreased in peripheral blood	Decreased to almost undetectable amounts in peripheral blood	ND	In salivary glands
Dermatomyositis	ND	Increased amount in peripheral blood (in paediatric patients)	ND	In muscle and skin; increased amount of CXCR5+CD4+ T-helper cells

ND=not determined.

Table 2: **B-cell abnormalities in systemic autoimmune diseases**[35,36,66-69]

***Panel 2:* Models of B-cell abnormalities in systemic lupus erythematosus and generation of autoantibodies**[70,71]

Abnormal preimmune repertoire due to defective negative central selection

- Defective central B-cell tolerance checkpoints, and emergence of autoreactive B cells (lack of counterselection)

Defects in peripheral selection

- Pathogenic autoantibodies encoded by highly mutated immunoglobulin genes, and a shorter complementarity determining region 3 than polyreactive immunoglobulin
- Loss of autoantigen binding when reverted back to germline configuration

Enhanced germinal centre activity and preferential selection by autoantigens

- B-cell hyper-responsiveness
- Exaggerated T-cell responses generating enhanced somatic hypermutation
- Accumulation of apoptotic material within the germinal centre, and positive selection of autoreactive B cells
  - Establishment of germinal centre structures
  - Expansion of post-germinal centre B-cell subsets and generation of B-cell effectors
  - Antigen presentation by B cells based on HLA associations

Positive selection for autoreactivity by follicular dendritic cells presenting apoptotic or necrotic debris permitting emergence of autoreactive B cells

- Emergence of positively selected autoreactive memory B cells, and production of autoantibodies in the periphery

Activation of B cells by dendritic cells as part of T-cell independent extrafollicular responses

- Occurrence of autoantibodies and autoreactive B cells without censoring by germinal centres, thereby escaping negative selection

Skewed numbers and suppressive activity of T regulatory cells

Abnormalities in the number and function of regulatory T cells, and resistance of effector T cells to suppression, support the idea that aberrant T-cell activity contributes to autoimmunity. By contrast with rheumatoid arthritis, for which little support has been reported for a defective regulatory T cell compartment, findings of several studies have identified reduced numbers and frequencies of CD4+CD25high regulatory T cells in systemic lupus erythematosus (table 1).[57] These regulatory T cells were identified as a unique population of T-helper cells able to control activated effector lymphocytes and maintain T-cell and B-cell tolerance. Phenotypically different subtypes of regulatory T cells (natural and adaptive)[58] differ in induction pathways, stability, and persistence but share a suppressive capacity. In systemic lupus erythematosus, regulatory T cells correlated inversely with disease activity in several studies, although in a few reports no correlation or increase in regulatory T cells was noted.[58] Defective suppressive activity of regulatory T cells on effector T-cell proliferation and interferon γ production has also been shown variably.[58] Notably, the defect in suppression often correlated with disease activity.[58] Effector T cells also seem to be resistant to regulatory T cell-mediated suppression, but report findings are less consistent.[58,63] The balance of regulatory T cells and T-helper-17 cells in systemic lupus erythematosus patients may be of greater importance than focussing on individual subsets.[62] In Sjögren's syndrome, data are conflicting: both raised and reduced amounts of regulatory T cells have been recorded in peripheral blood.[63] Foxp3+ lymphocytes circulating in the blood correlate inversely with those infiltrating salivary glands.[64] Fewer regulatory T cells have been recorded in advanced versus moderate salivary-gland infiltrates, supporting the view that T-cell differentiation could shift from the default regulatory T cell to a T-helper-17 differentiation pathway in an inflammatory environment. Robust studies on regulatory T cells in dermatomyositis are needed. Follicular helper T cells are an important CD4 subset and are crucial for full germinal centre formation and B-cell and plasma-cell maturation and, therefore, are considered as therapeutic targets. Their role in systemic autoimmunity requires further study.[65]

B cells in autoimmune systemic disease

Table 2 presents B-cell disturbances in systemic autoimmunity; hypergammaglobulinaemia and autoantibodies are judged disease hallmarks. Although anti-double-stand DNA, anti-Ro/SSA and La/SSB, and anti-cardiolipin have pathogenic relevance, the immuno-

pathogenic role of other antibodies (eg, anti-Sm, anti-RNP) is still debated. Findings suggest how autoreactive clones form and persist and how B-cell differentiation and activation thresholds are disturbed, leading to skewing of the B-cell pool and immunoglobulin production (table 2, panel 2). Panel 2 presents several possibilities for how B cells can escape tolerance.

Mechanisms controlling autoreactive B cells

Two main ideas prevail for how autoreactive B cells emerge. First, disturbances of central checkpoints could permit preimmune autoreactive B cells to arise. Second, peripheral barriers might fail after T-cell dependent responses have been initiated.[70] Defects in central selection of autoreactive B cells have been suggested, because patients with systemic lupus erythematosus cannot remove naive B cells expressing self-reactive B-cell receptors,[72] including impaired selection from immature to mature naive B cells.[73] However, the preimmune B-cell receptor generated initially by $V_H(D)J_H$ recombination seems similar to that seen in controls.[74] Although it is tempting to conclude that systemic lupus erythematosus includes defects in checkpoints against autoimmunity, these barriers could still be functional but overridden by chronic polyclonal B-cell activation.[70] In support of this idea, autologous stem-cell transplantation in patients with systemic lupus erythematosus showed that the disease can undergo long-term remission in individuals repopulated predominantly by naive peripheral lymphocytes.[75] This result indicates clearly that autoreactivity in systemic lupus erythematosus depends on acquired abnormalities after antigenic stimulation, differentiation, and selection of memory lymphocytes and is less likely to be related to disturbances within the naive repertoire.

The expanded peripheral memory B-cell repertoire in systemic lupus erythematosus[37,53,76] is characterised by exaggerated somatic hypermutation and increased receptor editing after extensive activation (unspecific or by autoantigen).[74] One idea is that autoimmunity could be driven by selection, using apoptotic material displayed on follicular dendritic cells, causing the emergence of pathogenic autoantibodies by impaired selection,[70] and thus germinal centre formation might not be essential. Therefore, apoptotic material bound on the surface of follicular dendritic cells might select autoreactive B cells positively, which arise from non-autoreactive B-cell precursors as a result of somatic hypermutation.[70] The modest effect of costimulation blockade (abatacept)[77] and recurrence of disease after anti-CD20 treatment (rituximab)[78] accords with the idea that targeting adaptive immunity alone could be insufficient to control systemic lupus erythematosus.

B-cell differentiation and imbalances

Skewing of B-cell populations in systemic lupus erythematosus includes increased frequencies of preimmune B cells, memory B cells, and plasma cells (table 2). Of preimmune B cells, augmented proportions of transitional, prenaive, and naive B cells can be detected.[79] These cell types associate with peripheral B-cell lymphopenia, indicating a population shift within the preimmune B-cell compartment in people with systemic lupus erythematosus towards more immature B cells, a process that is independent of disease activity.

Phenotyping of peripheral blood B cells in systemic lupus erythematosus shows a substantial increase in antigen-experienced post-switched memory B cells that are less susceptible to immunosuppressive treatment and easy to activate because of reduced suppression via FcγRIIb. These memory cells can be activated rapidly—independent of antigens and T cells—by combination of toll-like receptor agonists and TNFSF13 or TNFSF13B and by cytokine combinations such as interleukin 21 and TNFSF13B.[80] Patients with Sjögren's syndrome have dominating naive B cells,[66] but no abnormality was detected in juvenile dermatomyositis.[59] Evidence in mice suggests the three receptors of TNFSF13B (BCMA, BR3, and TACI) may have different roles, because knockout did not affect emergence of systemic lupus erythematosus,[81] whereas knockout of BCMA[82] could lead to exacerbation of systemic lupus erythematosus.

Another abnormality in systemic lupus erythematosus is a significant increase of CD27high plasmablasts,[36] which correlates with disease activity.[37,53] Polymorphisms in the *PRDM1* gene encoding a transcription factor (BLIMP1) essential for plasma-cell differentiation have also been associated with systemic lupus erythematosus.[83] Plasmacytosis in patients with systemic lupus erythematosus[36,37,53,67] represents a signature of the overactive immune system, which diminishes after successful treatment.[36,53,84,85]

Disturbed B-cell signalling

Several candidate genes in systemic lupus erythematosus have roles in B-cell-related signalling pathways (figures 1 and 6).[86] These include molecules downstream of the B-cell receptor signalling complex, such as LYN and LYP (encoded by *PTPN22*), together with BANK1 and BLK that have a role in modification of B-cell receptor responses. Genetic polymorphisms related to systemic lupus erythematosus have also been identified within NFκB-dependent activation pathways—ie, *TNFAIP3* (*A20*), *TNIP1*, and *PDRM1*—in which CD40 and endosomal toll-like receptor activation synergise with B-cell receptor signalling resulting in B-cell proliferation, cytokine production, plasma-cell differentiation, isotype switching, and antibody secretion. Thus, activation that is both dependent on and independent of the B-cell receptor might be disturbed because of genetic variants, and even small changes of intracellular enzyme activity could allow a break in tolerance or lead to B-cell overactivity.

B cells in patients with systemic autoimmunity might

also be activated by dendritic cells (figure 3). Direct and efficient stimulation of naive and memory B cells into plasma cells that produce immunoglobulin G and immunoglobulin A is obtained with the help of type 1 interferon.[87] Thus, direct interaction of dendritic cells and B cells effected by cytokines can drive autoimmunity, emphasising the important role of dendritic cells.

Role of target tissue in autoimmune disease development

Interactions between organ tissue and the immune system are important to establish the degree of autoreactivity (panel 1). Organs serve as direct autoimmune targets and provide the microenvironment for induction and maintenance of ectopic immune responses, which further propagate tissue damage and dysfunction.

Ectopic lymphoid follicles

Lymphocytic infiltrates develop within affected organs in systemic autoimmune diseases.[88] A clear role in disease manifestations is not established, because the degree of infiltration does not always correspond with severity of symptoms. However, infiltrates are likely to contribute to tissue destruction and loss of function. In Sjögren's syndrome, autoimmune salivary gland tissue seems active itself in the inflammatory process, and expression of adhesion molecules (crucial in lymphocyte recruitment) is noted in intraglandular vessels. Furthermore, glandular epithelia express lymphotactic chemokines, MHC class II, and costimulatory molecules[89,90] and, thus, might participate in both attraction and activation of lymphocytes.

Within target-organ infiltrates, structures that closely resemble organised lymphoid tissue with germinal centres can develop.[91,92] These structures contain aggregates of proliferating B cells and T cells in close proximity to networks of follicular dendritic cells. A central role for interleukin 17 and T-helper-17 cells in formation of ectopic germinal centre-like structures has been noted, particularly relating to initial formation of ectopic germinal centre-like structures in target tissues.[93] Polymorphisms mapping to *LTA*, a lymphotoxin that is also central to formation of germinal centres, have been associated with Sjögren's syndrome, although a distinction from the HLA signal remains to be established because *LTA* resides within the MHC locus.[94] Disease-related autoantibodies (Ro/SSA, La/SSB) can be produced within lymphocytic infiltrates, and presence of ectopic germinal centres is associated with raised autoantibody concentrations.[95] Signs of lymphoid neogenesis, immunoglobulin class switch, and affinity maturation have been recorded in the kidneys of patients with systemic lupus erythematosus[96] and in muscle tissue of those with autoimmune myositis,[97] indicating that this process is common to systemic autoimmune disorders.

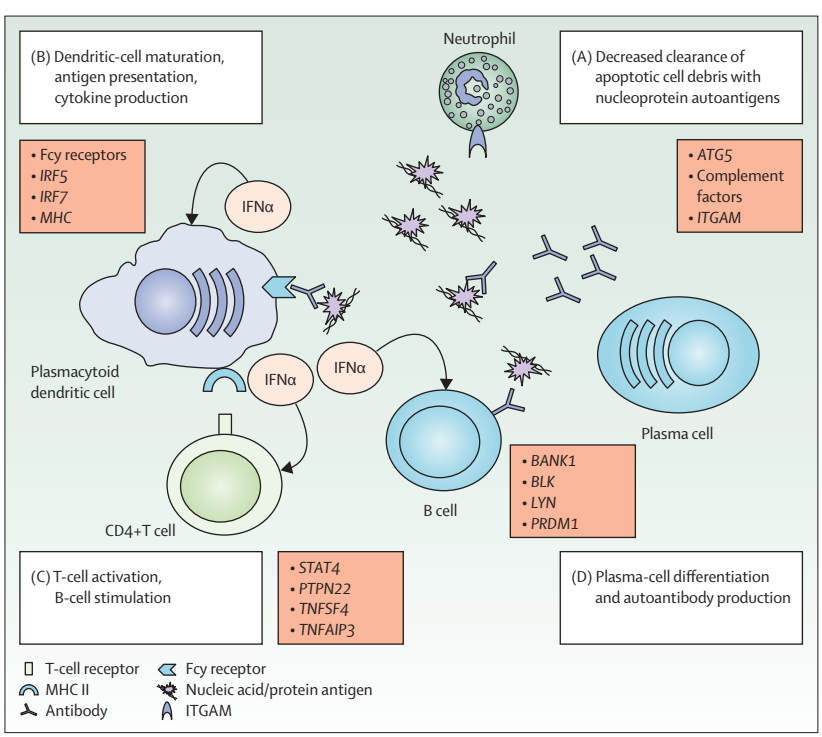

Figure 6: **Disease-related immune events and associated polymorphisms**
(A) In systemic autoimmune diseases, autoreactivity is often directed towards nucleic acids (DNA, RNA) or associated proteins. Such autoreactive specificities might be fuelled by generation and defective uptake of apoptotic cell debris by phagocytes (neutrophils, macrophages). (B) Activation of plasmacytoid dendritic cells increases production of MHC class II for antigen presentation and augments release of IFNα, leading to (C) T-cell activation and (D) differentiation of B cells into antibody-producing plasma cells. Polymorphisms associated with systemic autoimmunity that might lower cellular thresholds for activation or maturity progression are indicated in orange boxes at the different steps, although they could be operational in several processes and cells. IFN=interferon.

Defective clearance of potential autoantigens

Differences in the microenvironment and structure of ectopic germinal centres were reported in chronically inflamed tonsils compared with traditional germinal centres.[98] Such differences could possibly contribute to a disturbed selection process, allowing autoreactive clones to emerge. Autoantigens could be provided readily within the target organ via several different processes. Usual immune homoeostasis within tissues is balanced between responses directed at tissue-specific antigens (cell debris from apoptotic and necrotic processes) and the ability of the tissue to inhibit and control these responses. In systemic lupus erythematosus the balance is disrupted, and defects in clearance of cell debris might be central to induction of autoimmunity (figure 6).[99] Increasing evidence also points to involvement of neutrophils in the tissue-destructive process. Microarray analysis of blood from patients with systemic lupus erythematosus reveals a striking signature of neutrophil-specific transcripts, the expression of which correlates with disease activity and with the presence of nephritis and vasculitis.[100] Neutrophils could contribute to end-organ damage through release of proteases, possibly after triggering of the immune complex. Immature neutrophils might then die outside their natural microenvironment

and could represent a major source of nuclear autoantigens—an autoantigenic load further increased by the clearance defect in systemic lupus erythematosus.[99]

Considerations for therapeutic development and future directions

Targeting central molecules in upstream regulatory checkpoints in initiation of the inflammatory cascade, before all downstream pathways have been activated, has been suggested as a potentially effective strategy for treatment of systemic autoimmune diseases. However, the effectiveness of targeted treatments is hard to predict. Although not the primary factor in regulation of inflammation, tumour necrosis factor α has proved a target for amelioration of joint-related autoimmune diseases. When inhibitors of tumour necrosis factor α were tried in patients with systemic lupus erythematosus[101] and autoimmune myositis,[102] results were somewhat disappointing, as were trials in Sjögren's syndrome.[103] However, the notion of cotargeting upstream and downstream mechanisms is attractive because of their interference with regulatory and effector mechanisms. Unfortunately, key targets have not been delineated clearly.

Here, we have highlighted several emerging hypotheses about systemic autoimmunity (panel 1). However, we can still learn important lessons from assessment of immunotherapies that interrupt chronic disease. The heterogeneity of systemic lupus erythematosus, Sjögren's syndrome, and dermatomyositis means that either several targets or an essential one could be important. One candidate is type 1 interferon, but other immune networks regulated by distinct dendritic cells also play a part and may need to be inhibited safely. However, biological drugs aimed at specific cells or molecules are emerging and will help to break the vicious cycle spurring organ damage and failure. Nonetheless, further improvements in our understanding of immunopathogenesis and better study designs are needed before we can truly re-establish immunological homoeostasis and start thinking in terms of disease cure.

Contributors

TD produced the tables and MW-H prepared the figures. Both authors did the literature search and wrote the report.

Conflicts of interest

TD has received support for clinical and preclinical studies as principal investigator from Immunomedics, UCB, and Sanofi; and honoraria as speaker and adviser for Roche, UCB, Takeda, Sanofi, GlaxoSmithKline, and Medimmune. MW-H declares she has no conflicts of interest.

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