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INNATE IMMUNITY AND INFLAMMATION IN SYSTEMIC SCLEROSIS

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Abstract

Purpose of review—Recent advances in our understanding of innate immunity and inflammation have direct bearing on how we understand autoimmunity, fibrosis and how innate immune sensors might stimulate both of these key features of systemic sclerosis (SSc)

Recent findings—Nucleic acid containing immune complex (IC) activation of toll-like receptors (TLRs) and induce expression of interferon responsive genes (IRGs) and autoantibodies in systemic lupus erythematosus (SLE). Recent studies indicate that increased SSc expression of IRGs may also be mediated by nucleic acid containing ICs. An expanding array of Non-TLR innate immune pathways have recently been discovered. In particular, nalp3 mediated inflammasome activation of caspase-1 and conversion of pro-IL-1 to IL-1 play a key role in silica- and bleomycin-mediated pulmonary fibrosis. TLR activation stimulates other inflammatory mediators, such as IL-1, IL-6 and TNF α in macrophages and dendritic cells. Activation of these and other inflammatory mediators, through TLR and non-TLR sensors, may cooperate to upregulate fibrotic mediators such as TGF β and IL-13.

Summary—These observations provide a new paradigm for understanding the relationship between immunity/inflammation and fibrosis. New therapeutics, including TLR agonists and antagonists, and IFN inhibitors are currently under investigation. Further understandings of inflammasome mediated fibrosis may provide further insights into SSc pathogenesis.

Keywords

Scleroderma; toll-like receptors; inflammasome; interferon

INTRODUCTION

The complex clinical and pathological features of systemic sclerosis (SSc) complicate understanding the role of the immune system in pathogenesis. Circulating autoantibodies, altered immune mediators and infiltration of mononuclear cells in affected organs argue that immune system dysfunction drives pathogenesis. The clinical overlap with other more clearly defined autoimmune diseases, particularly systemic lupus erythematosus (SLE), further supports immune system activation in the disease process. However, unlike SLE, autoantibodies are not deposited in tissues in SSc and have not been directly implicated in pathology. Thus, the role of autoantibodies and cellular immune system activation in SSc

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appears to be different though related to alterations seen in SLE. Increasingly, innate immune disturbances have become a focus in autoimmune illnesses, as it became clear that such disturbances could precipitate autoantibody production and autoimmune disease. The association of certain chemical exposures with scleroderma-like illnesses further supports the notion that non-antigen specific innate immune responses to inflammatory stimuli might cause SSc.

TOLL-LIKE RECEPTORS AND INNATE IMMUNITY IN SSC

Recent understandings highlight how “first-line” innate immune defenses can promote autoimmunity. In normal, early immune responses against infectious agents, immune cells recognize microbes through pattern recognition receptors (PRPs) (1).

Toll-like receptors in autoimmune disease

PRPs, most prominently toll-like receptors (TLRs), control immune responses by detecting common molecular motifs, including RNA ligands by TLR3, TLR7 and TLR8, DNA ligands by TLR9 and bacterial cell surface proteins such as lipopolysaccharide (LPS) or endotoxin that is a ligand for TLR4 (See Table I). Activation of these or other TLRs on dendritic cells, monocyte/macrophages and B cells stimulate inflammatory cytokines, antigen presentation and development of the adaptive immune response. Mammalian DNA and RNA do not normally engage these receptors, in part because they recognize structural motifs found more commonly on bacterial DNA such as CpG motifs, but also because these receptors are sequestered inside the cell in an endosomal compartment that normally excludes endogenous nucleic acids.

Increasingly, data from both murine and human studies have implicated TLR activation in the pathogenesis of SLE (2). SLE patient sera contain endogenous ligands for TLRs, particularly the nucleic acid sensing TLRs, TLR7, TLR8 and TLR9 (3). The ligands for these receptors in SLE sera are immune complexes (ICs) formed by autoantibodies to nucleic acids or nucleic acid binding proteins. Autoantibodies in such ICs bind nucleic acid directly (anti-DNA antibodies), or indirectly by binding to nucleic acid binding proteins, such as Sm proteins. Dendritic and B cells can internalize these nucleic acid-containing ICs through Fc and surface immunoglobulin receptors, respectively (4–7). Such internalization targets the bound nucleic acid to the proper endosomal compartment, activating TLR7 (by RNA) or TLR9 (by DNA). TLR activation leads to dendritic cell production of interferon (IFN) and B cell maturation. These observations provide new pathogenic functions for anti-nuclear autoantibodies in SLE, discussed further below in the context of SSc, and indicate that innate immunity regulates key aspects of autoimmunity.

Although the role of TLRs or other PRPs in SSc is less clear, several parallels can be drawn that suggest mechanisms of innate immune dysfunction operating in SLE may also be important in SSc. In particular, both diseases are associated with autoantibodies to nucleic acid-binding proteins and both diseases are associated with increased expression of interferon-responsive genes by peripheral blood mononuclear cells.

Interferon-responsive genes and innate immunity in SSc

Several years ago we and others showed that SSc patients, like SLE patients, show increased expression of interferon-responsive genes (IRGs), known as the interferon “signature” (8,9). IFNs include type I, type II and more recently identified type III IFNs. The type-I IFNs include 13, mostly co-regulated, IFN α subtypes and IFN β , signaling through a common receptor. Although these IFNs are difficult to measure directly in the blood, serum IFN α can be detected through biological assay in SLE patients, where it correlates with the IFN signature (10).

Dendritic cells, particularly plasmacytoid dendritic cells (pDCs), are the major source of IFN α and these cells secrete high amounts of IFN upon TLR 7 or TLR9 activation.

In contrast to SLE, the IFN responsible for stimulating IRGs in SSc is not known. As the type I IFNs, IFN α and IFN β , stimulate the same receptor, and type I and type II IFNs stimulate a similar set of genes, it is not possible to tell clearly from the pattern of PBMC gene expression which IFN is responsible for the IFN signature in SSc patients. If similar mechanisms as those in SLE stimulate the IFN signature in SSc patients, then we would anticipate primarily IFN α . This would implicate dendritic cells, the major source of IFN α . On the other hand, non-immune cells including fibroblasts produce IFN β , and TH1 and NK cells are the primary sources of IFN γ . Thus, determining the type(s) of IFN driving IFN gene expression in SSc will also provide insight into the cell types activated in the immune response.

Sera or purified Immunoglobulin from SSc patients can stimulate IFN α by peripheral blood mononuclear cells (PBMCs) (11). This activity is mainly found in sera containing autoantibodies to topoisomerase. IFN α secretion stimulated by SSc sera was blocked by the addition of an antibody to BCDA2, a receptor that inhibits IFN α production by pDCs. Activity was also blocked by antibody to Fc γ R2, the surface receptor that has been shown to be important for IC uptake, and bafilomycin, an inhibitor of endosomal acidification. Together these results are most consistent with the notion that ICs in SSc stimulate endosomal TLRs after Fc-mediated internalization by pDCs, i.e., the same mechanisms implicated in SLE (5).

Type I IFNs have been implicated in B cell maturation (12,13), but have not generally been considered pathogenic factors in SSc. IFNs block the effects of TGF β on fibrosis, suggesting that they might actually ameliorate this aspect of SSc pathogenesis. However, TLR activation of dendritic cells and macrophages also stimulates IL-1, TNF and IL-6 production, and these or other undefined mediators might drive inflammation and fibrosis in SSc.

Anti-nuclear autoantibodies in SSc

Autoantibodies found in SSc patients are mostly distinct from those seen in SLE. However, many of the proteins targeted by SSc-associated autoantibodies, strikingly, share the common feature of binding, directly or indirectly, to nucleic acids. Although topoisomerase-I is known as a DNA-nicking enzyme, it also associates with a variety of proteins that bind RNA (14). Thus, ICs in SSc sera containing anti-topoisomerase antibodies might bind DNA or RNA. As RNase blocks innate immune activation by topoisomerase-containing ICs more efficiently than DNAase (11), TLR7/8, the single stranded RNA sensors, are most strongly implicated in topoisomerase-1 associated innate immune activation. However, this study did not exclude the possibility that other autoantibodies present in the sera were responsible for the observed activity.

Anti-centromere autoantibodies target a variety of centromere proteins, but most consistently CENP-B (15). CENP-B binds to highly repetitive α -satellite DNA through a highly conserved 17 base pair sequence known as the CENP-B box (16). Thus, ICs formed from autoantibodies binding centromere proteins might be associated with α -satellite DNA. One might anticipate that such ICs could have similar effects on innate immune activation as anti-DNA antibodies in SLE, although the specific sequences associated with centromere DNA could potentially lead to different immune responses, as has been seen for responses to synthetic DNA sequences (17).

Anti-nucleolar antibodies are seen in ~30% of SSc patients and a smaller proportion of SLE patients. These antibodies target a subclass of small nucleolar ribonuclear proteins (snoRNPs) that methylate rRNA, including U3 snoRNP and associated proteins: fibrillarin and Mpp10 (18). Fibrillarin, the most common target of anti-nucleolar antibodies, binds directly to

snoRNAs through the C/D box, two highly conserved sequences found in both the 3' and 5' ends of C/D snoRNAs (19,20). Thus, antibodies to fibrillarin might deliver C/D snoRNA associated with fibrillarin to TLR7/8 receptors. As for DNA activation of TLR9, the sequence of RNA affects TLR7/8 activation (21). Thus, RNA sequences specific for snoRNAs may affect innate immune responses. In particular, RNA sequence can affect the degree of IFN α , IL-6 and TNF α production. One might speculate that TLR7 activation by RNA in anti-Sm ICs found in SLE patients might elicit different responses from RNA in anti-nucleolar antibody ICs, but there is currently no data to directly support this possibility.

TLR activation of autoreactive B cells

In addition to stimulating dendritic cells, TLR activation can be a key step in the maturation of autoreactive B cells. Anti-chromatin antibodies found commonly in SLE patients can stimulate B cell proliferation that depends on uptake of the IC through surface immunoglobulin and subsequent interaction of DNA in the IC with TLR9 (7). Autoantibodies to RNA binding proteins can in the same manner stimulate TLR7-mediated B cells proliferation (4). Anti-Sm autoantibodies markedly stimulate B cell proliferation in the presence of purified snRNPs. This stimulation depends on RNA in the snRNP IC, and requires Myd88, a principal signaling component for most of the TLRs, including TLR7. These data suggest that ICs or simply circulating cell debris containing centromere DNA and/or snoRNPs might bind directly to B cells expressing surface autoantibody specific for, respectively, CENP-B or fibrillarin. Internalization of the DNA or RNA containing material might activate, respectively, TLR9 or TLR7 and expansion of autoantibody producing B cells.

Notably in relationship to SSc-associated autoantibodies, TLR7 has been strongly implicated in formation of anti-nucleolar antibodies in mice harboring the Y-linked autoimmune accelerator (Yaa) gene. Mice harboring a deletion of the inhibitory Fc γ RIIb receptor spontaneously develop autoimmune disease and antinuclear autoantibodies. In Fc γ RIIb deficient mice, the Yaa gene accelerates disease severity, but also shifts the autoantibody profile toward anti-nucleolar antibodies. Recent studies have shown that the Yaa includes a duplication of TLR7 (22). These results emphasize a strong dosage effect of TLR7 on autoantibody production and suggest that TLR7 mediates activation of autoreactive B cells that have taken up nucleolar/RNA-containing cellular debris through surface immunoglobulin receptors.

Linking TLRs to fibrosis

How TLR stimulation leads to fibrosis is not certain, but in most cases is likely mediated by TGF β (23). In addition, recent studies have suggested that TLR activation might directly stimulate fibroblast conversion to profibrotic myofibroblasts through TLR3 (24) or TLR9 (25). Sera from SSc patients, but not healthy controls or patients with primary Raynaud's phenomenon, show evidence of a circulating TLR4 agonist {van Lieshout, 2009 #588). The nature of this putative TLR4 ligand remains unclear. However, other studies of anti-fibroblast antibodies (AFA) have also implicated TLR4 agonist activity in SSc serum. AFA-induced expression of CCL2 by fibroblasts is diminished in TLR4-deleted fibroblasts (26). These latter studies are similar to studies suggesting that SSc sera contain TLR7 agonist activity discussed above (11). Further studies should clarify the composition and TLR specificity of these activities.

Matrix derived TLR ligands

Several TLR ligands are generated from matrix molecules during tissue injury. Hyaluronan generated during acute lung injury can activate TLR2 and TLR4, contributing to macrophage activation (27). Biglycan, a small leucine-rich proteoglycan, can also act as a TLR2 or TLR4 ligand (28) and the extra domain A (EDA), found in one of the alternatively spliced forms of

fibronectin, is a ligand of TLR4. Stimulation of TLRs by these matrix molecules provides another source for innate immune activation during inflammation and might act to initiate or perpetuate inflammation and fibrosis in SSc.

TLR-INDEPENDENT INNATE IMMUNITY AND FIBROSIS

Although TLRs link adaptive to immune responses and are required for appropriate immune sensing they do not account for all adjuvant-like innate immune activation (29). TLR-independent innate immune sensors include mediators in the NOD-like receptor (NLR) family. NLRs include NOD1 and NOD2 that recognize bacterial peptidoglycan fragments (30), inducing NF- κ B, and cryopyrin/Nalp3 that recognizes a wide array of ligands and induces the inflammasome (see Table II). Other innate immune sensors are the helicases: retinoid acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene-5 (mda5). These receptors sense cytoplasmic double stranded RNA with binding and downstream activation of IFN β , thus sharing these features with TLR3, an endosomal dsRNA sensor (31,32). Recently, another innate immune receptor for cytosolic DNA, AIM2, has been described that activates caspase-1/inflammasome (33).

Silica, bleomycin and the inflammasome

Environmental or occupational exposure to silica dust leads to fibrosis (34) and has been associated with SSc (35). Recently, several groups have shown in murine models that silica dust activates inflammation and fibrosis through the inflammasome (36,37). Activation of the inflammasome also contributes to bleomycin-induced lung injury (38). The inflammasome is an intracellular protein complex that includes cryopyrin/Nalp3, originally identified by gain-of-function mutations causing the autoinflammatory diseases, Muckle-Wells syndrome, familial cold autoinflammatory syndrome, and neonatal-onset multisystem inflammatory disease (39). It is triggered by “danger signals”, including monosodium urate and calcium pyrophosphate dihydrate crystals. Nalp3 activates caspase-1 converting the inflammatory cytokines, pro-IL-1 β , pro-IL-18 and pro-IL-33 into their active forms. Interleukin-1 plays a key role in both lung fibrosis stimulated by both silica and bleomycin. The mechanism linking IL-1 to fibrosis is uncertain, but involves TGF β /smad3 dependant stimulation (40). Bleomycin induced pulmonary fibrosis also depends on TLR signals and has recently been described as a TLR2 ligand (41). Inflammasome- and TLR-mediated signals provide complementary activities with TLR agonists stimulating pro-IL-1 production and caspase-1 converting it to its active form (39). Despite these links to fibrosis, a role for the inflammasome has not been shown in SSc.

Toxic oil syndrome and innate immunity

The 1980's outbreak of “toxic oil syndrome” (TOS) in Spain following ingestion of contaminated rapeseed oil that had been intended for industrial use caused pulmonary hypertension a scleroderma-like illness with autoantibody production and skin thickening. Although, the contaminant leading to disease was not definitively established, two molecules have been identified as probable culprits: 1,2-di-oleyl ester (DEPAP) and oleic anilide (42, 43). Evidence for a role of the innate immune system is supported by increased secretion of IL-1 and IL-6, and activation of NF- κ B in mice treated with oleic anilide (44), and development of high titer anti-nuclear antibodies in genetically predisposed MRL/lpr mice exposed to these oils (45). Although such activity has not been directly related to innate immune activation, fatty acid esters have known adjuvant activity (46).

Gadolinium and macrophage activation

Gadolinium exposure leading to nephrogenic systemic fibrosis appears to be mediated by macrophage activation of profibrotic cytokine secretion (47). The receptor for this is unknown

but gadolinium is known to block a variety of ion receptors and activate the vallinoid receptor (48).

MONOCYTE/MACROPHAGES AND IL-13 IN SSc AND FIBROSIS

Macrophages are a prominent cell type in the dermis of scleroderma patients (49) and become profibrotic through alternative activation by IL-13.

Monocyte/macrophage activation in fibrosis

Both circulating monocytes and tissue macrophages in SSc patients highly express Siglec-1/sialoadhesin, a marker type-I IFN (9). Subsequent studies have found that monocyte Siglec-1 expression correlates with disease severity in SLE, but whether this marker of IFN-induced macrophage activation is associated with disease severity or progression in SSc has not been reported. Dermal macrophages in SSc patients also show increased expression of the IFN γ -inducible gene, allograft inflammatory factor-1 (AIF1) (50,51). Although upregulation of these genes implicates different IFNs, it is important to recognize that type I and type II IFNs stimulate similar sets of genes and both Siglec-1 and AIF1 might be stimulated by either type I or type II IFN (IFN γ). Macrophages can secrete a variety of profibrotic mediators, including PDGF and TGF β . A paradigm for macrophage activation has been defined on the basis of cytokines released by the two Th subtypes (52). "Classically activated" monocytes are activated by the Th1 cytokine IFN γ , whereas alternatively activated macrophages are activated by Th2 cytokines IL4 and IL13 (52). Alternatively activated macrophages are proposed to be profibrotic, possibly by activating TGF β . This paradigm has not been fully explored yet in SSc.

IL-13 and fibrosis

Bleomycin-induced skin fibrosis is partially dependent on skewing toward TH2 cytokines particularly IL-13, as more severe fibrosis is seen in mice deleted of the TH1-skewing transcription factor, t-bet (53,54). Despite these studies suggesting that T cells are important in bleomycin-induced fibrosis, dermal fibrosis can also be induced by bleomycin in nude and rag mice (53,55) and lung fibrosis by bleomycin can be induced in scid mice (56). Thus, T cells are not required for bleomycin to induce fibrosis, suggesting that monocyte/macrophages or dendritic cells may be the key cells producing IL-13. Lung alveolar macrophages from patients with pulmonary fibrosis have been shown to produce IL-13 (57), consistent with the notion that this profibrotic cytokine may be a key mediator of innate immune induced fibrosis. IL-13 is elevated in the serum of SS patients, suggesting that it plays a role in fibrotic disease in SSc (58).

CONCLUSIONS

Multiple independent studies show that innate immune sensors can lead to autoantibody production and fibrosis. The common feature of autoantigens in SSc and SLE patients as nucleic acid-binding proteins implicates nucleic acid sensing TLRs, TLR7, TLR8 and TLR9 in pathogenesis. Matrix molecules and circulating innate immune activators in SSc patients suggest possible roles for TLR2 and TLR4. Environmentally induced scleroderma-like illnesses highlight the potential importance of inflammasome activation and IL-1 in SSc pathogenesis

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