Precursors to Systemic Sclerosis and Systemic Lupus Erythematosus: From Undifferentiated Connective Tissue Disease to the Development of Identifiable Connective Tissue Diseases

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The pathogenesis of connective tissue diseases (CTDs), such as systemic lupus erythematosus (SLE) and systemic sclerosis (SSc), is characterized by derangements of the innate and adaptive immune system, and inflammatory pathways leading to autoimmunity, chronic cytokine production, and chronic inflammation. The diagnosis of these diseases is based on meeting established criteria with symptoms, signs and autoantibodies. However, there are pre-clinical states where criteria are not fulfilled but biochemical and autoimmune derangements are present. Understanding the underlying processes responsible for disease pathogenesis in pre-clinical states, which place patients at increased risk for the development of established connective tissue diseases, represents an opportunity for early identification and potentially enables timely treatment with the goal of limiting disease progression and improved prognosis. This scoping review describes the role of the innate and adaptive immune responses in the pre-clinical states of undifferentiated CTD at risk for SSc and prescleroderma, the evolution of antibodies from nonspecific to specific antinuclear antibodies prior to SLE development, and the signaling pathways and inflammatory markers of fibroblast, endothelial, and T cell activation underlying immune dysregulation in these pre-clinical states.

Keywords: systemic sclerosis, scleroderma, prescleroderma, pathogenesis, innate immunity, adaptive immunity, systemic lupus erythematosus, autoimmunity

INTRODUCTION

Systemic sclerosis (SSc) is a rare multisystem autoimmune connective tissue disease (CTD) characterized by fibrosis of the skin and internal organs, vasculopathy, and autoimmunity with distinct antibodies. SSc is classified using the American College of Rheumatology/European League of Rheumatism (ACR/EULAR) 2013 criteria (1). However, there are pre-morbid clinical states, including Undifferentiated Connective Tissue Disease at risk for Systemic Sclerosis (UCTD-risk-SSc) and prescleroderma, where autoimmunity and dysregulation of inflammatory pathways occur without the presence of clinical symptoms (2). UCTD-risk-SSc, also known as very early/early SSc, is a label given to patients who do not meet the ACR/EULAR 2013 criteria, but who present with Raynaud's Phenomenon (RP) and either typical SSc capillaroscopic findings (megacapillaries or avascular areas) or serum marker antibodies (anti-centromere, anti-topoisomerase I, anti-RNA polymerase III, anti-Th/To, and anti-Pm-Scl) (3, 4). UCTD-risk-SSc patients have a 35-79% risk of developing definite SSc over time (5–7). Prescleroderma is diagnosed in patients with RP who present with serum marker autoantibodies (anti-centromere or anti-topoisomerase I) and immunofluorescence derived antinuclear antibodies (ANA) at titre >1:320 or serum antibodies and avascular capillaroscopic changes or ANA positivity at 1:320 and avascular areas (7). Moreover, patients with prescleroderma have an even higher risk of developing established SSc than UCTD-risk-SSc (7). Making a diagnosis and intervening early may change the trajectory of disease in these patients.

Another CTD with pre-clinical stages progressing to identifiable disease is systemic lupus erythematous (SLE). SLE which is characterized by features such as arthritis, rash, photosensitivity, serositis, cytopenias, mucositis, glomerulonephritis, fevers and fatigue, may onset insidiously and can be difficult to differentiate from other autoimmune diseases initially (8, 9). Commonly ANA will pre-date SLE diagnosis by years during undifferentiated pre-clinical stages termed "incomplete SLE" or "possible SLE" when ACR criteria for SLE are not met (10, 11). Approximately 55% of patients with incomplete SLE (iSLE) develop SLE (12). Furthermore, as disease progression occurs, more specific antibodies for SLE are produced such as anti-double stranded DNA and anti-Smith antibodies (10, 13).

Ultimately, the changes observed in these pre-clinical stages with varying likelihood of progression to full-blown disease are insidious and driven by derangements in inflammatory signalling and autoimmunity. The purpose of our scoping review was to elucidate the role of the innate and adaptive immune systems and dysregulated signaling pathways in pre-clinical states, and their contribution to the establishment of full-blown disease.

SEARCH STRATEGY

Our search strategy was developed with an experienced information specialist (Supplementary Material). We searched the databases EMBASE and MEDLINE with restrictions for the English language and included peer-reviewed manuscripts as well as conference abstracts. We sought to include studies which provided information regarding the role of adaptive and innate immune systems and the dysregulation of pathways which contributed to the development of classifiable SSc or SLE. Therefore, we included studies which explicitly studied individuals termed as UCTD-risk-SSc, Very early/early SSc, prescleroderma, pre-SLE, incomplete SLE, or lupus-like. Studies were excluded if they provided information regarding inflammatory pathways where patients with established disease were investigated. The search and inclusion of studies was performed by one reviewer (LMC) with review of included studies performed by both authors (LMC & JEP). Our search yielded 2313 manuscripts after duplicates were removed on August 10, 2021 and pertinent manuscripts have been included (**Figure 1**).

SYSTEMIC SCLEROSIS

Dysregulated Signalling Pathways and Autoimmunity

Progressive inflammation, vasculopathy and fibrosis orchestrated by aberrant cytokine production is a hallmark of SSc. Chemokines involved in extracellular matrix deposition, erroneous activation of fibroblasts, and anomalous immune system activation, including CCL2, MIP-1α/CCL3, CCL4, CCL7/MCP-3, and CXCL8, have been observed to be significantly upregulated in the serum of established SSc patients when compared to healthy controls (14–16). However, the presence of these chemokines is more nuanced in pre-clinical disease. Vettori et. al., compared the serum of UCTD-risk-SSc patients to fibromyalgia and/or osteoarthritis controls without RP, and definite SSc patients for soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular adhesion molecule-1 (sVCAM-1), CCL2, CXCL8, IL-13, IL-33, and transforming growth factor-β (TGF-β) (17). A significant increase was observed in sICAM-1, CCL2, CXCL8, and IL-13 along a disease spectrum gradient from UCTD-risk-SSc to limited cutaneous SSc (lcSSc) to diffuse cutaneous SSc (dcSSc). sICAM-1 is involved in the transmigration of leukocytes from vessels to endothelium and promotes inflammation through T cell activation and cytokine production (18, 19). CXCL8 and CCL2 are pro-fibrotic alter angiogenesis, and affect the migration of monocytes, T cells, and neutrophils (20–22). IL-13 contributes to fibrogenesis through fibroblast activation and TGF-β stimulation (23). Consequently, chemokines increase as disease severity worsens highlighting the progressive derangement of vasculature and autoimmune changes in SSc. Interestingly, higher IL-33 levels were found in UCTD-risk-SSc patients compared to controls and established SSc. IL-33 induces IL-4, IL-5, and IL-13 production leading to arterial vessel media hypertrophy and eosinophilic and mononuclear cell infiltration (24). Therefore, IL-33 functions as a very early mediator in the progression to established SSc, is involved in the fibrotic stage of

FIGURE 1 | Flow diagram of scoping review selection process.
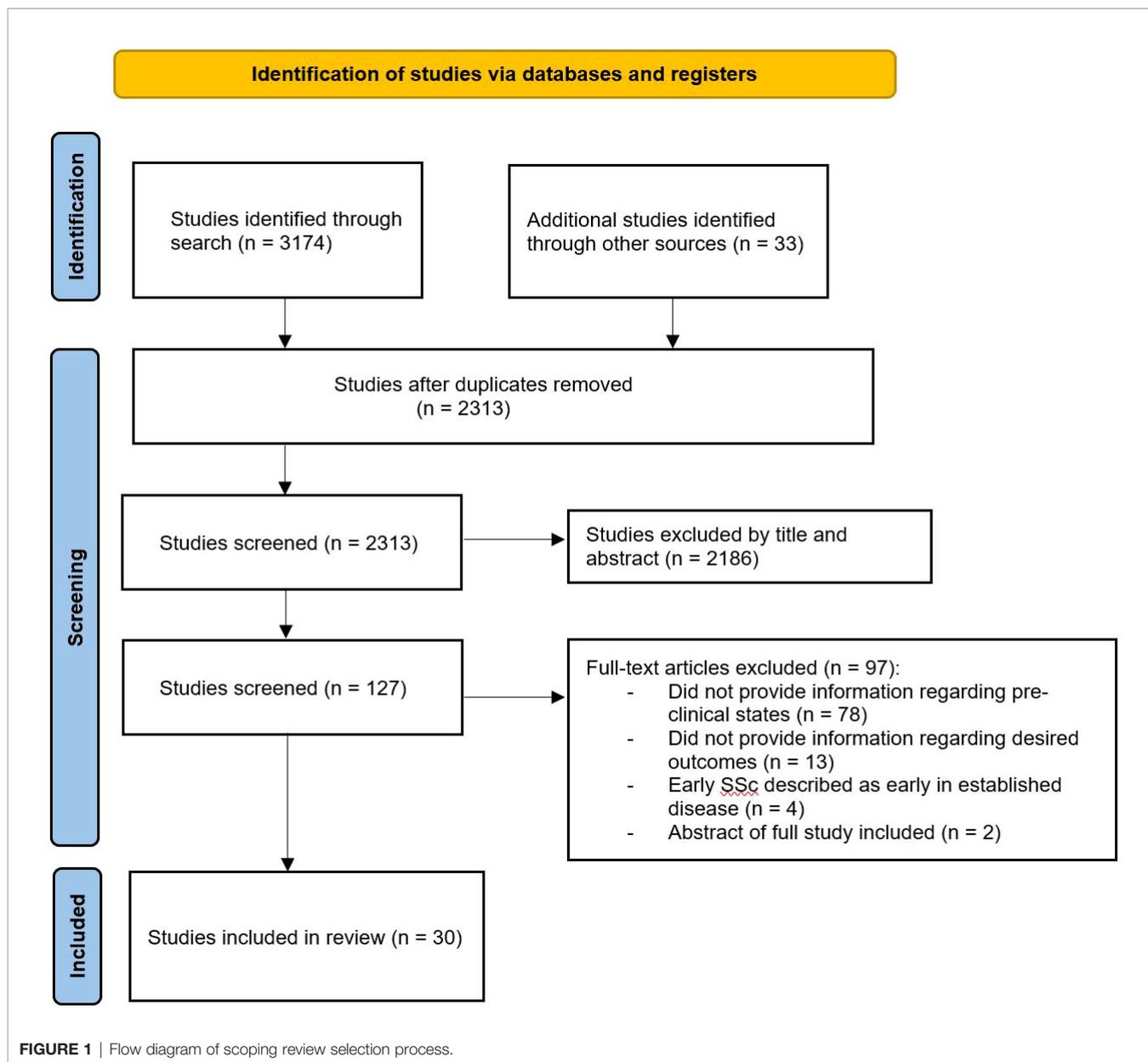
SSc through IL-13 stimulation; and serves as a predictive marker to elucidate which patients will develop established disease (25).

Other cytokines are abnormal in UCTD-risk-SSc including soluble IL-2 receptor alpha (sIL-2Rα), aminoterminal propeptide of type III collagen (PIIINP), and CXCL4 (7, 26, 27). sIL-2Rα functions as a marker of T-cell activation, whereas PIIINP functions as a marker of collagen formation and fibroblast activation (28, 29). CXCL4 functions as a potent anti-angiogenic chemokine and serves to inhibit endothelial cell proliferation and migration (30). Additionally, CXCL4 has pro-fibrotic capabilities through inhibiting interferon-gamma (IFN-γ) expression and stimulating IL-13 and IL-4 production (31). CXCL4 levels, measured from serum, were higher in UCTD-risk-SSc than controls and were associated with anti-Scl 70 antibodies and sICAM-1 (27, 32). Furthermore, CXCL4 levels, drawn from non-platelet poor plasma, were reported to correlate with extent of skin fibrosis and were predictive of pulmonary arterial hypertension and lung and skin fibrosis progression in SSc (33).

Type I IFN represents another significant contributor to the pathogenesis of SSc through the upregulation of genes involved in the activation of the innate and adaptive immune systems. The increased expression of these type I IFN regulated genes, termed the type I IFN signature, has been previously observed in SLE and other autoimmune diseases (34, 35). Brkic et al., investigated the whole-blood samples of healthy controls without RP, patients with primary RP, UCTD-risk-SSc, and definite SSc patients to determine the expression of 11 type I IFN inducible genes (36).

Authors report increased type I IFN related gene expression in UCTD-risk-SSc patients compared to healthy controls, but not in primary RP compared to controls. This finding eludes to the early contribution of the type I IFN pathway in the pathogenesis of SSc. Furthermore, the presence of polymorphisms of IFN regulated genes have been found to confer increased risk of SSc (37).

Vasculopathy and Fibrogenesis

Cossu et al. investigated angiogenetic and endothelial dysfunction markers involved in vasculopathy (38). Authors sampled the serum of healthy controls without RP, UCTD-risk-SSc, lcSSc, and dcSSc patients for angiopoietin-2 (ang-2), CXCL16, e-selectin, sICAM-1, CXCL8, sVCAM-1, and VEGF. There was a significant trend along a disease spectrum from controls to UCTD-risk-SSc to lcSSc and to dcSSc for ang-2, CXCL16, e-selectin, and sICAM-1. Authors also observed a significant difference in ang-2 between controls and UCTD-risk-SSc. Ang-2's functioning is contextual as it facilitates angiogenesis if VEGF is present, but causes blood vessel regression if pro-angiogenic stimuli are absent (35). Clinically, ang-2 correlates with the extent of skin involvement in SSc as measured by the modified Rodnan skin score (mRSS), disease activity, and C-reactive protein (39). Tabata et. al., found that IGF-1, VEGF, and RANTES levels are significantly higher in mild established SSc compared to pre-clinical SSc (40).

Fibrogenic inflammatory pathways resulting from chronic inflammation and orchestrated through fibroblast dysfunction lead to excessive accumulation of extracellular matrix components, including hyaluronic acid, fibronectin, and proteoglycans, in SSc (41). Sera of healthy controls without RP, UCTD-risk-SSc, and non-fibrotic SSc patients were analyzed whereby elevated markers (CXCL10/IP-10, CXCL11/I-TAC, tumor necrosis factor receptor type II (TNFRII), and chitinase 3-like protein 1) were higher in UCTD-risk-SSc patients compared to controls (42). CXCL10 and CXCL11 are angiostatic and migration chemokines which drive smooth muscle cell proliferation, and recruit T cells, monocytes, and natural killer cells (43–45). Importantly, CXCL10 and CXCL11 levels are associated with UCTD-risk-SSc patients most at risk for developing established SSc (25, 46). Furthermore, CXCL10 and CXCL11 are observed to be correlated with type I IFN signature and decrease with type I IFN receptor blockade with anifrolumab (47).TNFRII has a role in the proliferation and activation of regulatory T cells (48). Additionally, TNFRII co-stimulated lymphocytes secrete pro-fibrotic cytokines in patients with SSc (49). Chitinase 3-like protein 1 has been implicated in regulating and stimulating angiogenesis and fibrogenesis through activation of Syndencan-1 and focal adhesion kinase (50). Furthermore, in SSc patients, chitinase 3-like protein 1 has been correlated with articular involvement and T cell activation (51). These findings highlight the interplay between the adaptive and innate immune systems alongside fibrogenesis.

Alterations of natural killer (CD 56+) and natural killer T cells (CD56+ CD3+) in early SSc compared to controls, primary RP, and established SSc were found and thought to be related to differential Toll-like receptor (TLR) 1/2 stimulation (52). Early

SSc demonstrated an intermediate activation pattern regarding CD56+ secretion of IL-6, TNF-a, and MIP-1α/CCL3 compared to controls with significant differences of IL-6 secretion. An increasing trend in CD56+ activation for TNF-α and CCL3 occurred between early SSc and controls. This pattern of elevated IL-6, TNF-α, and CCL3 alludes to the role of underlying innate immune mechanisms in prescleroderma or early SSc; which, may eventually lead to established SSc. The development of SSc is shown over time (**Figure 2**).
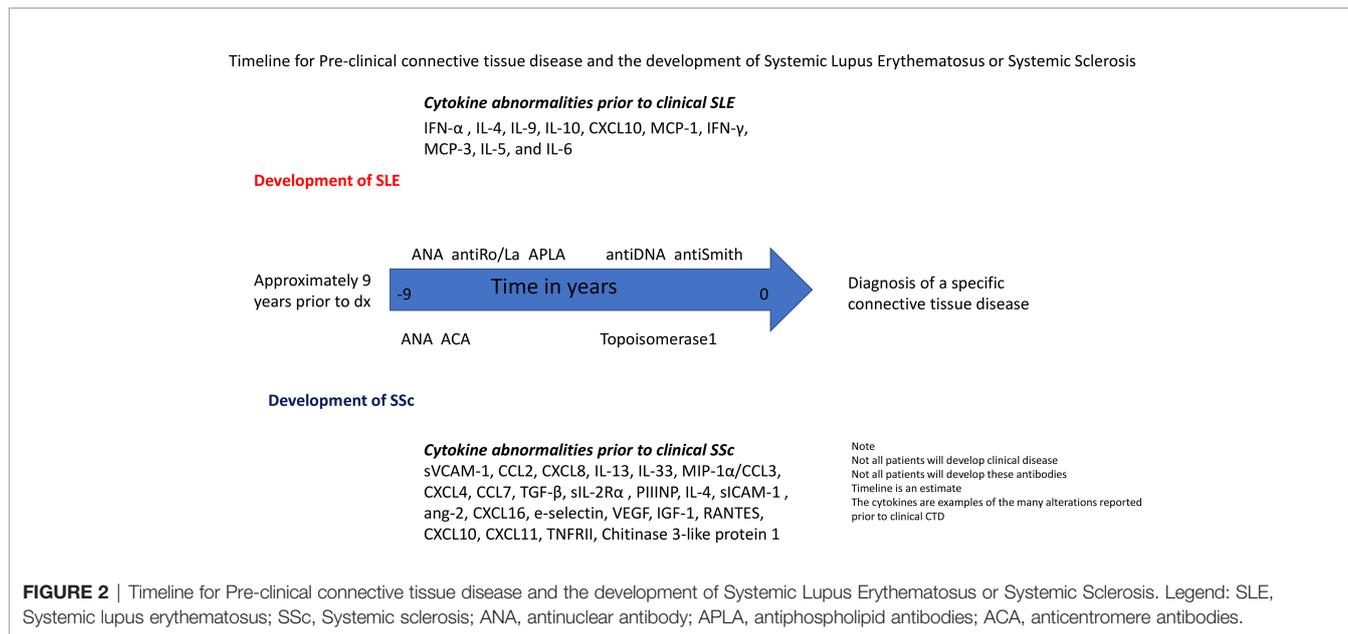
SYSTEMIC ERYTHEMATOSUS LUPUS

Autoimmunity and Dysregulated Pathways

Antibodies predate the diagnosis of SLE by multiple years in a characteristic pattern evolving from non-specific ANA to more specific SLE antibodies prior to diagnosis. In a large serology study, a cohort of 130 military personnel who ultimately developed SLE were followed from first detection of ANA to diagnosis of SLE a median of 9.2 years later (11). Furthermore, anti-Ro, anti-La, anti-phospholipid, anti-double stranded DNA, anti-Smith, and anti-nuclear ribonucleoprotein (anti-RNP) antibodies were reported to have a time of first detection to diagnosis of 9.4 years, 8.1 years, 7.6 years, 9.3 years, 8.1 years, and 7.2 years, respectively. This observed pattern, corroborated by further studies, reflects progressive antibody evolution towards more specific SLE antibodies over time in patients ultimately diagnosed with SLE as ANA, anti-double stranded DNA, and anti-Smith antibodies have 86%, 94.7%, and 99% specificity, respectively (53–56). The presence and development of SLE specific antibodies can also serve as predictive makers of developing established disease. Munroe et. al., investigated unaffected blood relatives of SLE patients to identify risk factors of disease establishment (57). Relatives who developed SLE had elevated ANA and anti-Ro titers, and were likely to be anti-dsDNA and anti-RNP positive at baseline and follow up compared to those who did not transition. Anti-cardiolipin antibody positive patients also had more risk of developing SLE (58–60).

Cytokine changes in pre-clinical SLE have been studied (61). Interferon-α, IL-4, IL-9, IL-10, CXCL10 and monocyte chemotactic protein-1 (MCP-1/CCL2) were studied in sera of 35 patients prior to established SLE. CXCL10 was significantly higher in pre-clinical sera compared to controls and was correlated with interferon-α. One of the drivers of innate and adaptive immune dysregulation occurs through an up-regulation of interferon regulated genes, which is also known as the IFN signature of SLE (62). IFN-α, a type I IFN, stimulation leads to increased dendritic cell maturation, increased Th1 cell development and response, and enhanced NK, B, and T cell proliferation and survival (62). IFN-α correlates positively with IgG, and negatively with IgM autoantibodies (63). CXCL10 and IFN-α concentrations are higher in pre-clinical patients who are positive for any antibody compared to antibody negative patients.

Type II IFN (IFN-γ) is additionally implicated in SLE development (64). IFN-γ leads to production of IFN-α and the B-lymphocyte stimulator (BLyS) (65, 66). BLyS, otherwise

FIGURE 2 | Timeline for Pre-clinical connective tissue disease and the development of Systemic Lupus Erythematosus or Systemic Sclerosis. Legend: SLE, Systemic lupus erythematosus; SSc, Systemic sclerosis; ANA, antinuclear antibody; APLA, antiphospholipid antibodies; ACA, anticentromere antibodies.

known as B-cell activating factor of the TNF family (BAFF), is produced by innate immune cells and serves as a mediator of B cell proliferation and survival (67–69). BLyS induces a Th1 cellular response which coordinates both innate and cytotoxic immunity (65). Munroe et al., studied the timing and role of type I and II IFN, IFN-associated mediators, and antibody formation in pre-clinical patients who would later develop established SLE (70). Elevated IFN-γ, CXCL10, and MCP-3 levels occurred prior to IFN-α activity and antibodies. IFN-γ and MCP-3 are abnormal more than 4 years prior to the development of SLE. Therefore, though type I IFN is observed to be elevated in association with antibody positivity prior to SLE diagnosis, type II IFN and IFN-associated mediators seem to represent the pathogenetic intermediaries altering innate and adaptive immune system derangements through elevation of IFN-α and autoantibody formation. These findings agree with a finding that IFN-γ, IL-5, and IL-6 were elevated at least 3.5 years prior to classification (71). Importantly, these observed temporal differences may be secondary to the measurement techniques used in these studies and further investigations with direct measurements tools, such as single-molecule arrays, may further elucidate the temporal relationship between type I and II IFN. **Figure 2** shows a timeline for the development of SLE and SSc.

DISCUSSION

Understanding the immunological and inflammatory perturbations involved in the development of CTDs such as SSc and SLE provides clinicians with an opportunity to recognize pre-clinical patients that may benefit from close monitoring, investigations, and potentially early intervention to limit disease progression. Pre-clinical disease states, such as UCTD-risk-SSc,

prescleroderma, and incomplete SLE, present with underlying aberrations, often years before clinical disease is present, of the innate and adaptive immune systems, and inflammatory pathways which drive pathogenesis and increase risk of developing established disease.

The pathogenic mechanisms present in UCTD-risk-SSc and prescleroderma include immune signal dysregulations, erroneous immune system recruitment, aberrant angiogenesis leading to vasculopathy, and inappropriate fibroblast activation leading to tissue fibrosis. Multiple cytokines are observed to increase along a disease spectrum from UCTD-risk-SSc to classified SSc and include sICAM-1, CCL2, CXCL8, ang-2, CXCL16, e-selectin, and IL-13 (**Table 1**). The mechanism of action of these cytokines includes transmigration of lymphocytes endothelium, innate immune cell activation and signal propagation, and extracellular matrix deposition. Furthermore, there are disease markers which are observed to be predictive of SSc and include sIL-2Rα, PIIINP, CXCL4, CXCL10, and CXCL11 (**Table 2**). Patients with SSc who have the limited cutaneous SSc subset frequently develop RP and anti-centromere antibody 8 years before other manifestations of SSc often followed by dilated nailfold capillaries, then puffy fingers or

TABLE 1 | Elevated chemokines observed in UCTD-risk-SSC orchestrating SSc pathogenesis.

Cytkine	Function
sICAM-1	Transmigration of leukocytes, T cells activation
CCL2	Chemotaxis of monocytes, T cells, neutrophils
CXCL8	Angiogenesis induction, immune cell proliferation
IL-13	Fibroblast activation, TGF-β secretion stimulation
Ang-2	Angiogenesis induction, monocyte activation
TNFRII	Regulatory T cell proliferation, profibrotic cytokine secretion
CHI3L1/YKL-40	Angiogenesis and fibrogenesis regulation

TABLE 2 | Taxa Cytokines observed to be predictors of SSc development.

Cytkine	Function
sIL-2Rα	Marker of T cell activation and proliferation
PIIINP	Marker of collagen formation and fibroblast activation
CXCL4	IL-13 and IL-4 stimulation
CXCL10	Smooth muscle cell proliferation, immune cell chemotaxis
CXCL11	T cell, monocyte, natural killer recruitment

sclerodactyly and other features of SSc (2–4). The presence or absence of these features is significant in risk stratification where patients with RP but without antibodies or nailfold capillary changes are at 1.8% risk of definite SSc compared to 79.5% in those with RP and positive antibodies and nailfold capillary changes (5). At this point in time, other than treating RP to try to prevent ischemic changes, there is no specific treatment to change the natural history of future development of SSc. Also, 1/3 may develop SSc over the next 5 years (so 2/3 won't) and this can lead to over-diagnosis, and patient anxiety. Interventions such as smoking cessation and reducing RP attacks and encouraging a healthy lifestyle including a diet high in omega3 fatty acids may be appropriate but this is speculation. Patients with diffuse cutaneous SSc do not develop RP until close to their diagnosis (often 1 to 2 years before or at the time of other signs and symptoms of SSc), so finding prescleroderma clinical features in the majority of these patients has not been possible.

Likewise, SLE development is rooted in aberrations of the innate and adaptive immune systems. Pre-clinical SLE is characterized by an evolving IFN signature and progressive SLE-specific antibody formation prior to disease classification. IFN-γ and IFN associated mediators can predate diagnoses by 3.5 years, and are present prior to and alongside antibody positivity. Throughout pre-clinical SLE, antibody formation occurs in a pattern that evolves from non-specific ANA to more specific SLE antibodies. Namely, ANA and anti-Ro formation can predate diagnosis by 9 years or more but are considered less specific. Whereas, the more specific anti-Smith and anti-dsDNA develop closer to disease onset. The development of SLE specific antibodies can function as predictive markers of transformation to clinical SLE.

Clinically, it is difficult to ascertain what to do with the findings. Other than close monitoring of patients at risk, it is not feasible to check cytokine panels (with high variability) and redoing antibodies is likely not cost effective. However, the changes in immune regulation that predate clinical CTD help in the understanding of pathogenesis and may in future provide

targeted treatment for patients with a high probability of converting to chronic debilitating disease. It has been suggested that treating patients at risk for SLE with hydroxychloroquine may change the disease trajectory but large controlled studies are needed to determine if there is benefit in this approach (72); one such multicenter, randomized, placebo-controlled, double-blind clinical trial is currently underway (NCT0303118). Interestingly, there are already drug targets in clinically active SLE targeting signalling that has been shown to be abnormal prior to disease onset such as BlyS (belimumab) and type I interferon with anifrolumab. Intervening prior to clinical disease would not be appropriate with the knowledge we have but in future, personalized medicine may help to give a more robust prediction of who will develop chronic autoimmune CTD.

CONCLUSION

Ultimately, the coordinated dysregulation of the innate and adaptive immune systems, and inflammatory signalling pathways leads to the pathogenesis of connective tissue disease. Our improved understanding of these underlying aberrations in pre-clinical stages of disease will serve to better identify patients at increased risk.

AUTHOR CONTRIBUTIONS

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.869172/full#supplementary-material

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