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T HELPER CELLS IN THE IMMUNOPATHOGENESIS OF SYSTEMIC SCLEROSIS – CURRENT TRENDS

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Abstract. *Systemic sclerosis (SSc) is a chronic progressive autoimmune disease characterized by skin and multiorgan involvement with alterations in both the innate and adaptive immunities. The hallmark of the disease is widespread fibrosis engaging the skin and multiple internal organs, as well as the musculoskeletal system. There is mounting evidence that T cells are key players in the pathogenesis of scleroderma. The current review discusses the role of the different T helper (Th) lymphocyte subsets in the processes of inflammation and fibrosis, characteristics for the pathogenesis of the disease. Cytokines produced by Th cell populations have a major effect on endothelial cells and fibroblasts in the context of favoring/inhibiting the vasculopathy and the fibrosis spread. The Th2 pro-fibrotic cytokines IL-4 and IL-13 have been shown to induce collagen synthesis by fibroblasts, whereas IFN-γ demonstrates an inhibitory effect. Increased Th17 cells are present in the scleroderma skin infiltrates. The combination of IL-17, IFN-γ and TGF-β levels in CD45RO and CD45RA cells from patients with SSc is useful to distinguish between the limited and the diffuse phenotype of the disease. There are accumulating data for functional and numerical alterations in the Tregs in SSc. High levels of TNF-α which might reduce the suppressive ability of Tregs have been described. According to some studies, the number of Tregs in scleroderma skin biopsies has been decreased against the normal absolute number of Tregs in peripheral blood of the same patients, which suggests suppressed immunomodulatory response. Other studies reported increased frequency of Tregs in peripheral blood of patients with systemic sclerosis and established a correlation with disease activity. The main immunological challenge remains the identification of the trigger of the autoimmune response in SSc, the causes for preferential Th2-type cell responses and the immunological differences between the diffuse and the limited cutaneous form of the disease.*

Key words: *systemic sclerosis, T helper cells, Treg cells*

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INTRODUCTION

Systemic sclerosis (SSc) is a chronic progressive autoimmune disease characterized by skin and multiorgan involvement with alterations in both the innate and adaptive immunities. The hallmark of the disease is widespread fibrosis engaging the skin and multiple internal organs, primarily lungs, heart, gastrointestinal tract, as well as the musculoskeletal system. A unique characteristic of SSc that distinguishes it from other fibrotic disorders is that autoimmunity and vasculopathy typically

precede fibrosis [1]. The fibrotic process destroys the physiological structure of the affected tissues and ultimately leads to organ failure. It is the major cause of morbidity and mortality in SSc patients [1]. Based on the spread of skin fibrosis, physicians divide SSc into two major subsets: limited cutaneous SSc (lcSSc), in which skin fibrosis does not spread beyond the elbows and knees and diffuse cutaneous SSc (dcSSc), characterized by skin thickening affecting the trunk and the proximal extremities. LcSSc is usually associated with mild to moderate and delayed organ fibrosis, whereas in dcSSc earlier and more severe organ damage is present [2]. There is mounting evidence that T cells are key players in the pathogenesis of SSc. This review examines the immunological alterations distinctive for SSc, particularly the role of different T helper (Th) lymphocyte subsets in the mechanisms of inflammation and fibrosis, characteristic for SSc.

WHY SSC IS CONSIDERED AN AUTOIMMUNE DISORDER?

Autoantibodies, immune cells and clinical manifestations

Firstly, more than 95% of the patients with SSc have autoantibodies (autoAbs) directed against nuclear antigens, a hallmark of autoimmunity [3]. Moreover, some of the autoAbs found in SSc patients are specifically associated with particular clinical subsets. For instance, in the majority of cases, anti-centromere autoAbs are associated with lcSSc [4]. On the contrary, autoAbs against DNA topoisomerase-1 (anti-Scl-70) are more often present in patients with dcSSc [5]. Furthermore, there is a correlation between several SSc-distinctive autoAbs and specific clinical manifestations. Patients positive for anti-Th/To autoAbs have a worse prognosis compared to those positive for anti-centromere autoAbs due to the high incidence of associated pulmonary hypertension, interstitial lung fibrosis and renal involvement [6]. Anti-U3-RNP autoAbs are more prevalent in people developing muscular involvement in the course of SSc [7]. The clinical and serological correlations surmise a causal relationship between the specific autoimmune response and the disease phenotype.

Secondly, in early skin lesions, there is relevant dermal inflammation characterized by perivascular mononuclear cell infiltrates composed mainly of activated tissue macrophages and T cells, with oedema around the microvessels and a variable extent of fibrosis in the papillary and reticular dermis. These T cells possess an oligoclonal T-cell receptor (TCR) which suggests an (auto)antigen-driven expansion. The cell infiltration correlates with the skin thickening raising the question if there is a relationship between inflammation and fibrosis [8]. The fibroblasts adjacent to the inflammatory infiltrate demonstrate increased synthetic activity, which suggests that mediators released by T cells may enhance collagen deposition by fibroblasts. There is no scientific data concerning the T cellular antigen specificity but a TCR repertoire seen *in vivo* can be extended *in vitro* into T cell/fibroblast co-cultures [9].

Thirdly, there are preliminary data of controlled studies and results from uncontrolled studies, reporting that exhaustive immunosuppressive regimens followed by transplantation of hematopoietic stem cell transplantation, decrease microvascular impairment and prolong overall survival of patients with severe dcSSc [10, 11].

Animal models of SSc

In an animal model of SSc, infusion of immunocompetent cells from tight skin (Tsk) mice into wild-type littermates, leads to induction of skin fibrosis and enhanced transcription of collagen genes, a convincing evidence for the involvement of the immune cells in the fibrotic process [12]. Moreover, genetic ablation of the intercellular adhesion molecule-1 (ICAM-1) in the Tsk mouse model resulted in a reduction of both the number of T cells in skin infiltrate and interleukin (IL)-6 levels, as well as in the attenuation of fibrosis. According to another study, reduction of T cells in a model of SSc improves bleomycin-induced lung injury and fibrosis [13]. The bleomycin model of SSc imitates the inflammatory process typical for the disease, including the influx of T and B cells that induces the expression of pro-fibrotic cytokines.

More recently, another research group reported that bleomycin- and IL-1β – mediated pulmonary fibrosis is highly dependent on IL-17A and simultaneously requires transforming growth factor-beta (TGF-β) in mouse models [14]. A different approach, using two various mouse models of experimental dermal fibrosis, examined the contribution of the signal transducer and activator of transcription 4 (STAT4), associated with autoimmunity, in the development of the fibrotic phenotype in SSc. STAT4-deficient mice turned out protected against bleomycin-mediated fibrotic reaction, thereby suggesting that the inflammation engendered by bleomycin may promote fibrosis through a Th1-like response characterized by STAT4 expression [15]. Nevertheless, it should be noted that the fibrosis induced by bleomycin treatment might occur even in the absence of functioning T cells.

All these direct and indirect evidence prove the autoimmune nature of SSc.

T HELPER CELLS

Th cells originate from T CD4+ lymphocytes precursors (Th0) which can differentiate in several and exclusive phenotypes – Th1, Th2, Th9, Th17, Th22, Treg, depending on the cytokine ambience.

Th1 cells primarily produce cytokines involved in cellular immune response such as tumor necrosis factor alpha (TNF-α), IFN-γ and IL-2. Initial antigen encounter in the presence of IL-12 and/or IFNγ triggers the proliferation of naïve T lymphocytes followed by activation of the STAT3, STAT4, and IL-17 secretion, which subsequently induces the master transcription factor T-bet1.

The Th2 group of cytokines, produced by Th2 cells, comprises of IL-4, IL-13, IL-5 and IL-10, stimulates B cell proliferation and differentiation [16, 17] and is mainly involved in the inflammatory process. In the process of Th2 differentiation, IL-4 activates STAT6 by that means conducting the orchestration of the master regulator GATA3, which results in transcription of IL-4, IL-5, and IL-13. The Th2 prevalence in the immune response might be maintained via autocrine loop with positive feedback of IL-4 (also IL-13) on STAT6 [18, 19].

Th17 differentiation and production of IL-17 are driven by antigen encounter in the presence of IL-1 and IL-6 proinflammatory cytokines in combination with TGF-β and IL-23 [20].

Tregs are immunosuppressive subpopulation induced by IL-2 in cooperation with TGF-β which converts naïve $CD4^{+}CD25^{-}$ cells to $CD25^{+}Foxp3^{+}$ Treg and which is responsible for their expansion [21]. IL-2 and TGF-β as well are both essential for the induction and maintenance of immunologic tolerance as they enhance forkhead box protein P3 (Foxp3) transcription factor expression by natural CD4+ CD25+ regulatory T cells [22].

Further, Th subsets have been identified and baptized Th9 and Th22 since they produce preferentially IL-9 and IL-22.

All these T helper subsets have distinct functions, their typical transcription factors and cytokines, critical for their differentiation, thus, making them "players" in various pathophysiological processes. It is still unknown if these T cell subsets express transient states or they are terminally differentiated, particularly in the case of Th9, Th17, Th22 and Tregs (Fig. 1).

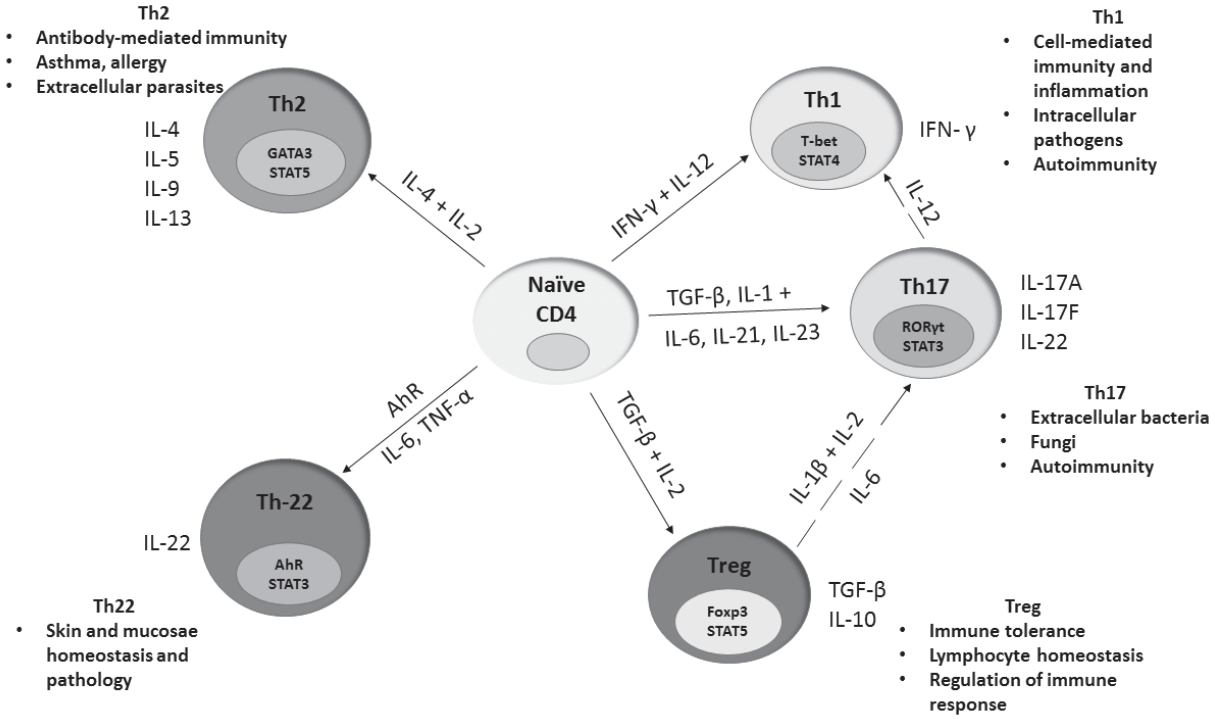

Fig. 1. The figure illustrates the four major classic T helper subsets, as well as the recently described Th22 cells; their key intracellular signaling molecules, master transcription factors and the cytokines needed for their terminal differentiation from naïve CD4+ T cells. The main functions of the particular Th subsets are marked along with their involvement in autoimmunity. The dashed lines designate the cell communication pathways between the particular Th subsets. The model aims to illustrate the idea that the T cell subsets represent transient states, rather than terminally differentiated cells, and that under the impact of microenvironmental cytokines the Th subsets might modify their own cytokine pattern and thereby their function.

AhR, aryl hydrocarbon receptor; Foxp3, forkhead box P3; GATA3, GATA-binding protein 3; IFNγ, interferon-γ; IL, interleukin; RORγt, retinoic acid receptor-related orphan receptor-γt; STAT, signal transducer and activator of transcription; T-bet, T-box transcription factor; TGF-β, transforming growth factor-beta; Th, T helper; Treg, regulatory T cell.

T HELPER CELLS INVOLVEMENT IN SSC IMUNOPATHOGENESIS

It is thought that the cytokine production by T cells influences the function of fibroblasts and endothelial cells, thereby playing a central role in vascular disease and fibrosis development. Therefore, lots of efforts have been made to identify the cytokine patterns in SSc. The majority of the studies performed in SSc patients have examined the characteristics of T cells isolated from peripheral blood mainly because of the easier access. However, it is worth keeping in mind that there can be a discrepancy between the T cell responses in the peripheral compartment and the ongoing inflammatory process in the target organs. In addition, the pathological mechanisms involved in the fibrosis of various tissues and organs are not identical, as well as they differ in the distinct SSc subsets (i.e., lcSSc, dcSSc). Eventually, the T cell alterations vary substantially according to the disease progression [16]. There is a functional heterogeneity between the T lymphocytes in the peripheral blood of patients with SSc and the corresponding T cell subsets in skin lesions or internal organs.

According to some studies, increased levels of IL-12 in patients` sera may relate to the activation of Th1 cells in SSc and that IL-12 overproduction could be associated with renal vascular damage [23]. Other authors reported Th2 predominance and CD30 expression by high numbers of CD4+ T cells in skin infiltrates of patients with SSc [24], as well as elevated serum levels of the Th2 cytokines – IL-4, IL-10, and IL-13 in SSc patients [25].

Th1/Th2 polarization in SSc

In SSc, Th1 cytokines are noted as “inflammatory”, whereas Th2 cytokines are “pro-fibrotic”.

In many autoimmune diseases, Th1-type predominance has been observed, quite the opposite, SSc demonstrates preponderant Th2 polarization. Although the reason remains unknown, an assumption has been made that predominantly Th2-type response has evolved as an attempt of fast tissue repair to destructive pathogens [26]. Indeed, Th2 cells can themselves direct monocytes to differentiate into a peculiar dendritic cell subset that demonstrates high IL-10 secretion and CD275 expression [27]. CD275 by itself might promote IL-4 responses as IL-10 has been involved in the suppression of Th1 polarization. Several studies reported high levels of Th2 pro-fibrotic cytokines in SSc, such as IL-4 and IL-13 [28, 29]. IL-13 is an inductor of TGF-β gene expression via interleukin-13 receptor subunit alpha-2 (IL-13Rα2) on macrophages. Thereby, IL-13 and IL-13Rα2 gene polymorphisms have been strongly associated with the dcSSc, surmising that immunomodulation of the IL-13/IL-13R pathway could be considered in target therapy [29]. Another cytokine engaged in the Th1/Th2 axis is IL-21. IL-21 treatment reduces the ability of Th cells to develop in respond to IL-12 by decreasing the total amount of cellular STAT4 resulting in diminished IFN-γ production by Th1 cells. These findings suggest that IL-21 is a cytokine produced by previously derived Th2 effector cells that serve to modulate the development of IFN-γ–producing Th1 cells and consequently amplify a Th2 response [30].

The role of Th17 cells in SSc

Th17 cells provide a massive inflammatory response against pathogens. The Th17 cells secrete IL-17, IL-21, IL-22. The Th17 cytokines have been reported by several studies as “allies” involved in the pathogenesis of autoimmune diseases such as asthma, rheumatoid arthritis, multiple sclerosis, psoriasis, inflammatory bowel disease, and graft versus host disease (GVHD). IL-17 induces stromal cells to produce inflammatory cytokines such as IL-1, IL-6, IL-8 and TNF-α which proves his pro-inflammatory nature [31]. IL-17 is an inductor of ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) surface expression by the endothelial cells and enhances the proliferation of fibroblasts. One study reported overproduction of IL-17 by T cells isolated from the peripheral blood, fibrotic skin and lung lesions in SSc patients [32]. It is believed that IL-17 oversecretion plays an important role in the pathogenesis of SSc, especially in the early stages of the disease by enhancing the production of IL-1 and fibroblast proliferation as well as the expression of adhesion molecules on endothelial cells. IL-21, mainly produced by Th17, Th2 and NK cells, increases the Th17 inflammatory response via amplification of IL-23 receptor expression and inhibition of Tregs. Differentiating Th cells exposed to IL-21 specifically inhibits the secretion of IFN-γ but other Th1 cytokines, such as IL-2 or TNF-α, are not affected.

Th17 differentiation is favored by IL-23 in cooperation with IL-6 and TGF-β, via increased IL-22 production. The IL-23 receptor (IL-23R) encoding gene has been recently identified as a susceptibility gene for SSc development. IL-23R polymorphisms have been

reported as associated with anti-Scl-70 positivity and lower frequency of pulmonary arterial hypertension [33]. LcSS and dcSSc phenotypes can be distinguished using combined analyses of Th17 profiles, TGF-β and IFN-γ in CD45RO and CD45RA T helper cells from SSc patients [34]. However, a recent study demonstrated IL-22 secreting population of human skin-homing memory T cells, which produces reduced amount of IL-17 [35].

Tregs changes in SSc

There are accumulating data for functional and numerical alterations of Tregs in SSc. The $CD4^+$ $CD25^+$ $Foxp3^+$ T cells produce anti-inflammatory cytokines including TGF-β and IL-10. Tregs are mandatory to establish immune tolerance. Depletion of Tregs not only promotes autoimmunity but also intensifies responses to foreign antigens. A number of alterations of Tregs have been reported in rheumatoid arthritis and systemic lupus erythematosus [36]. In SSc, high levels of TNF-α have been described, thus elevated TNF-α might reduce the suppressive ability of Tregs. TNF-α downmodulates the function of Tregs, mediated through ligation of TNF-R2, and inhibition of TNF-α with Infliximab restores their suppressive activity [37]. According to another study, even though the absolute number of Tregs in peripheral blood of SSc patients did not differ from healthy controls, a smaller number of Tregs in SSc skin biopsies was found, which suggests suppressed immunomodulatory response [38]. Another study described an increased frequency of Tregs in peripheral blood of SSc patients and a correlation with disease activity was established [39]. Nonetheless, the Tregs expansion in the peripheral compartment was not associated with functional decline in the Treg population. Radstake et al. also found an increased overall number of Tregs in SSc patients but in this study, it was combined with compromised functional ability of Tregs, related to a diminished CD69 and TGF-β expression [40].

CD4+ $CD8^+$ T cells and $CD8^+$ cells

It is worth remembering that in SSc most of the data available on polarized patterns of cytokine production have been obtained in $CD4^+$ T cells. Recently, an outlandish subtype of $CD4^+CD8^+$ double-positive T cells, which secrete very large quantities of IL-4, has been determined in the skin of SSc patients [41]. According to the same study, not only $CD4^+CD8^+$ double-positive T cells but also $CD8^+$ single-positive T cells demonstrated increased levels of IL-4 compared with $CD4^+$ single-positive T cell populations in SSc patients. A previous study revealed an identical increase in IL-4 produced by $CD8^+$ single-positive T cells, in lung bronchoalveolar lavage fluid. The high IL-4 levels correlated with a considerable decline in lung function over time, indicating its pathogenic role [42].

A recent study reported a positive correlation between $CD8^+$ T cells secreting increased levels of IL-13 after *in vitro* T-cell activation and the extent of skin thickening in SSc patients [43]. Moreover, this $CD8^+$ T cell population was dependent on a transcription factor GATA-3, as silencing of GATA-3 with small interfering RNA significantly reduced IL-13 production by $CD8^+$ T cells, demonstrating a causal relationship between GATA-3 and IL-13 [44]. The data suggest that GATA-3 could be a novel therapeutic target in SSc, as inhibition of the central transcription factor would inhibit differentiation of naïve T cells to Th2-polarized T cells and thus diminished production of pro-fibrotic mediators and inhibit already differentiated Th2 cells from secreting IL-13 directly after differentiation.

THE CYTOKINE PROFILE IN SSC PATIENTS

The circulating cytokines in the peripheral compartment

Correlations between serum/blood cytokine levels in patients with SSc and the disease severity have been reported. The circulating cytokines both of Th1-type (IFN-γ, TNF-α, IL-1α, IL-2, CXCL10) and of Th2-type (IL-4, IL-10, IL-13, MCP-1) are elevated in SSc patients comparing to healthy controls [23, 44-46]. Correlations between the serum levels of IL-6, IL-8, IL-10, B- cell activating factor (BAFF), monocyte chemoattractant chemokine (MCP)-1, sCD30 and the spread of skin fibrosis have also been reported, as higher levels were measured in dcSSc compared to lcSSc patients [47]. According to another study, increased levels of IL-2, IL-6, IL-7, IL-8, IL-10, IFN-α cytokines in exhaled breath condensate or bronchoalveolar lavage fluid are associated with lung fibrosis and its severity. In early SSc, a TGF-β serum level is raised and its co-expression with IL-17 and IFNγ was related to SSc progression [34]. The manifestation of pulmonary fibrosis has been suggested to correlate with the increased IL-6, MCP-1, IL-15, ET-1, a proliferation-inducing ligand (APRIL) [48] and blood TNF-like weak inducer of apoptosis (TWEAK) in peripheral blood of SSc patients [49].

The cytokine pattern in situ

The TGF-β expression in situ is an important marker of skin and lung impairment during the early stage of SSc and consequently of the fibrosis spread. In situ expression of connective tissue growth factor (CTGF) and platelet-derived growth factor (PDGF) in the skin infiltrate might contribute to the induction of collagen production. The local hypoxia in SSc skin

measured by a polarographic oxygen microelectrode system is linked with raised levels of vascular endothelial growth factor (VEGF). In situ IL-4, IL-17 and MCP-1 expression were closely related to the extent of fibrosis in SSc skin and lungs [44]. Other studies reported increased in situ mRNA expression of IL-17, IL-21, IL-33 and IFN-α in SSc skin compared to healthy individuals. Furthermore, BAFF is also upregulated in the fibrotic skin of SSc patients with early dcSSc, which supports the B cell involvement in SSc. BAFF has been shown to bring off some self-reactive B cells from apoptosis and to upregulate Bcl-2 expression as well as nuclear factor kappa-B (NF-κB) activation, thereby advantaging B cell survival [1].

CONCLUSION

Major headway has been achieved in the attempts of revealing the immunopathogenic mechanisms of SSc. Especially during the past decade, integrated cognition generated through animal models of fibrosis and in vitro has emphasized how the dysregulated interactions between immune cells and mediators might induce inappropriate adaptive responses and support the inflammation which ultimately results in fibrosis and vasculopathy. T cells play a critical role in the pathogenic processes characteristic for SSc. Cytokines produced by Th cell populations have a major effect on endothelial cells and fibroblasts, in the context of favoring/inhibiting vasculopathy and fibrosis spread. A predominant Th2 polarization affected by the secretion of IL-4 and IL-13 has been shown to induce collagen synthesis by fibroblasts, whereas IFN-γ demonstrates inhibitor effect. Increased number of Th17 cells has been detected in SSc skin infiltrates; many alterations have been reported in Tregs in both skin and peripheral blood. However, important issues remain unresolved, among them, identification of the trigger of the autoimmune response in SSc, the causes for preferential Th2-type cell responses and the immunological differences between the dcSSc and lcSSc. Another unanswered riddle is the reason(s) for gradual attenuation of the inflammation, which takes place over time and is characteristic for the atrophic phase of SSc. The available modern technologies enable fast acquisition of a huge amount of information based on the study of thousands of patient samples. It will be a challenge to extract and analyze properly the relevant data in order to provide every single SSc patient with a personalized treatment approach.

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