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Everything you always wanted to know about systemic sclerosis but were afraid to ask: Part 2. Intermediate pathophenotypes: the best pathogenetic background in systemic sclerosis

ABSTRACT

Systemic sclerosis (SSc, scleroderma) is a chronic systemic connective tissue disease with a complex pathogenesis that is still not fully understood, in the course of which attention is increasingly drawn to the dynamic, sequential pathogenetic mechanisms according to disease stage.

An increasing understanding of the diversity of mechanisms underlying this disease, as well as the prevalence of certain pathogenetic elements that depend i.a. on disease stage, will enable more effective therapeutic interventions in the future.

Systemic sclerosis can thus be seen as a complex process, where the main players are immune cells, endothelial cells and fibroblasts, and the focal point is probably impaired function and subsequent damage to endothelial cells.

Systemic sclerosis is also the final stage of a certain continuum of events, starting with a state of susceptibility to the development of the disease (dependent on genetic conditions and environmental influences), followed by disruption of homeostasis and initiation of pathological processes (e.g. as a result of viral infections), progression of pathological responses (inflammation, endothelial damage, fibrosis) and consequently organ damage.

According to most authors, the key event and focal point of the cascade of phenomena is endothelial cell damage, and the mechanisms that lead to this damage are related to the activation of the immune system. There is growing acceptance of the thesis of an autoimmune origin of the disease involving mechanisms of innate and acquired immunity, both cellular and humoral.

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KEY WORDS: systemic sclerosis; pathogenesis; pathophenotypes

THE INNATE IMMUNE SYSTEM — ROLE OF TOLL-LIKE RECEPTORS, "NEW" NON-SPECIFIC IMMUNE CELLS AND THE PHENOMENON OF "INTERFERON SIGNATURE"

Research in recent years has drawn attention to the role of the innate immune system in the pathogenesis of systemic sclerosis (SSc).

Researchers mainly focus on 2 aspects: the role of pattern recognition receptors (PRRs) in the production of interferon I (IFN-I) and other pro-inflammatory cytokines and the role of toll-like receptors (TLRs) in the activation of cells, mainly fibroblasts.

The non-specific immune system responds rapidly to the presence of certain molecular

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structures and patterns, which include structures defined as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) — danger molecules.

Recognition of PAMPs and DAMPs by TLRs results in the production of pro-inflammatory cytokines and chemokines, increased expression of tissue compatibility antigens MHC I, II and costimulatory molecules, enabling the induction of specific mechanisms.

Pattern recognition receptors detect danger and thus play an important role in the development of the disease. These receptors are expressed not only on cells belonging to the innate immune system but also on matrix cells (fibroblasts and endothelial cells) [1, 2]. The detection of tissue damage by cells of the non-specific immune system triggers the initiation and amplification of inflammatory responses, eventually leading to fibrosis [3].

Moreover, the release of pro-inflammatory cytokines (interleukin 1 [IL-1], tumour necrosis factor-α, IL-6) by cells under stress, including oxidative stress, can initiate the production of the fibrosis-enhancing TGF-β by macrophages [4].

The release of alarmin (IL-33, IL-25 also known as IL-17E) and lymphopoietin (thymic stromal lymphopoietin) can activate type 2 innate lymphoid cells (ILCs), which participate in the Th2 response and enhance the production of IL-4 and IL-13, involved in the formation of extracellular matrix proteins.

A large area of research involves the role of TLRs in the pathogenesis of fibrosis. TLR4 was found to have increased expression in scleroderma fibroblasts [5]. TLR8 expression in monocytes may mediate their transformation into fibroblasts, probably in response to EBV infection [6]. Scleroderma monocytes (following TLR8 activation by ssRNA or LPS) produce increased amounts of tissue inhibitor of matrix metalloproteinase, which enhances extracellular matrix accumulation [7]. TLR-8 in pDC cells plays a role in IFN-1 production.

The role of reactive oxygen species (ROS) in the pathogenesis of the disease is also under investigation.

The blood of patients shows an increase in oxidative stress markers such as malondialdehyde, a marker of lipid peroxidation, and nitric oxide [8]. ROS can affect monocytes/macrophages, resulting in differentiation towards M2 — macrophages. ROS are involved in the activation of fibroblasts by increasing the production of pro-inflammatory cytokines such as IL-1β [9]. Furthermore, inflammasomes — particularly NLRP3 — are associated with activation of fibroblasts, endothelial cells and macrophages. Increased expression of NLRP3 was described in the skin of patients. It is likely that oxidative stress may be involved in NLRP3 activation [10].

Another element in the inflammatory response sequence, whose role is currently under investigation, is mitochondrial DNA whose increased concentrations are found in the plasma of SSc patients. Increased serum levels are associated with increased production of IFN-I and IL-6 (and, clinically, with progression of SSc-ILD) [11].

Research in recent years has highlighted the role of alternatively activated macrophages (M2) in disease pathogenesis. A marker of these cells is CD163 molecule, the levels of which are increased in the blood of SSc patients and associated with a worse prognosis [12]. M2 macrophages are associated with the production of pro-fibrotic cytokines such as TGF-β, but also IL-4, IL-6 and IL-13. In the FASSCINATE study, blockade of the receptor for IL-6 by tocilizumab resulted in a reduction of macrophage infiltration in the skin [13]. Nintedanib is a tyrosine kinase inhibitor, which blocks the action of receptors for VEGF and PDGF and also reduces macrophage accumulation in tissues [14].

In SSc, circulating monocytes and tissue-resident macrophages — under the influence of IL-4 and IL-13 — increase the expression of CD163 and CD 204 molecules, which initiates fibrosis by enhancing TGF-β production. These cells also produce numerous pro-inflammatory cytokines, chemokines, matrix metalloproteinases and their inhibitors, forming a complex interdependent network and a specific cytokine microenvironment, showing diverse effects according to time and site of action. These mediators are associated with the development of vasculopathy and fibrosis and the exacerbation of pathological phenomena through ineffective repair processes. Experimental studies on the effector functions of macrophages in response to various agents (LPS, BCG) in murine models of scleroderma have shown an impact on the development of fibrotic processes. Therefore, researchers attribute an important role to tissue macrophages in the development of fibrotic processes. In addition to their function as antigen-presenting cells, mDCs may also play an

important role in the development of inflammatory responses [15].

Tissue-resident plasmacytoid DCs (pDCs) respond to platelet-derived factor 4, PF-4, whose levels are significantly elevated in the serum of patients (140) and can form complexes with DNA (136). The interaction of these complexes with TLR 8 and TLR 9 in endosomes enhances the production of IFN-I, whose levels are increased in approximately 50% of patients [16].

More recently described cells belonging to the innate immune system are ILCs. This group includes gamma-delta T lymphocytes — mostly double-negative T cells (CD4– CD8–), B1 lymphocytes and NKT lymphocytes. In humans, gamma delta lymphocytes produce connective tissue growth factor, playing an important role in wound healing and are involved in epithelial repair processes. B1 lymphocytes are a subset of B lymphocytes and possess the CD19 surface antigen typical of all B lymphocytes, as well as CD5 molecules that are specific only to them. Some of the antibodies produced by these lymphocytes appear to be autoantibodies, which play a role in the elimination from the body of autoantigens originating from decaying cells. NKT lymphocytes are a subset of T lymphocytes that express surface markers typical of NK cells. Most of them are double negative (CD4– CD8–) and play a role in the regulation of the immune response.

Innate lymphoid cells are responsible for the rapid and intense production of cytokines following PRR activation. At the current state of knowledge, relatively little is known about the role of these cells in the pathogenesis of SSc, however, there is evidence of an expansion of ILC2 (producing IL-4/IL-13) in the blood and skin. These cells can interact with T helper cells. These cells may also be involved in fibrosis-promoting responses [17]. Some researchers, including Wohlfahrt et al. [17], reported elevated ILC counts in both peripheral blood and skin in SSc patients compared to healthy subjects , and their number correlated with the degree of skin fibrosis. However, not all studies have confirmed these observations [18]. IL-25 and IL-33-stimulated ILC cells are a source of IL-13, which enhances collagen synthesis and induces differentiation of macrophages into the pro-fibrotic subtype. ILC cells are also a source of IL-17A, which plays an important role in lung and skin fibrosis. Much of the current research focuses on the location and role of individual ILCs in the pathogenesis of SSc and the potential opportunities to block their effector functions [19].

In 1990, using immunohistochemical techniques, it was revealed that fibroblasts located in the skin and lungs of SSc patients contain smooth muscle actin filaments and are myofibroblasts [20]. They produce more collagen than in healthy individuals and are an important source of endothelin 1, which is an important vasoconstrictor and thus contributes to the development of pulmonary arterial hypertension. Myofibroblasts are also a source of VEGF and angiopoietin 1 and 2, which stimulate new vessel formation. Excessive production of these cytokines by myofibroblasts may lead to abnormal vascular remodelling and cause vasculopathy [21]. These cells also produce type I collagen — a component of the extracellular matrix.

The effect of immunocompetent cells on myofibroblast formation and function is illustrated in Figure 1.

Research in recent years has also focused on the role of interferons in the pathogenesis of autoimmune diseases.

In the case of acute viral infection, IFN-I production increases rapidly, which usually leads to elimination of the virus. In certain situations, however, persistent IFN-I synthesis occurs after the pathogen has been ineffectively eliminated in the acute phase. This results in impaired production of cytotoxic CD8 T lymphocytes, increased differentiation of B lymphocytes into antibody-producing cells and synthesis of pro-inflammatory cytokines, which enhances the inflammatory response of tissues. Other mechanisms considered to be involved in the mechanism of autoimmunity include impaired degradation of viral particles and endogenous nucleic acids, activation of endosomal TLRs. Inteferons increase TLR expression, which enhances the recognition of DAMPs molecules released from damaged tissues. Interferons are important anti-angiogenic factors, which is of particular importance in SSc in which angiogenesis is impaired. They also activate the synthesis of the nuclear factor NF-kappa-B, which significantly enhances fibrosis and increases the synthesis of cytokines involved in fibrosis.

Approximately half of SSc patients show increased expression of IFN-induced genes in peripheral blood cells (defined as an “interferon signature”) [22].

There is growing evidence that interferons may play an important role in the pathogenesis

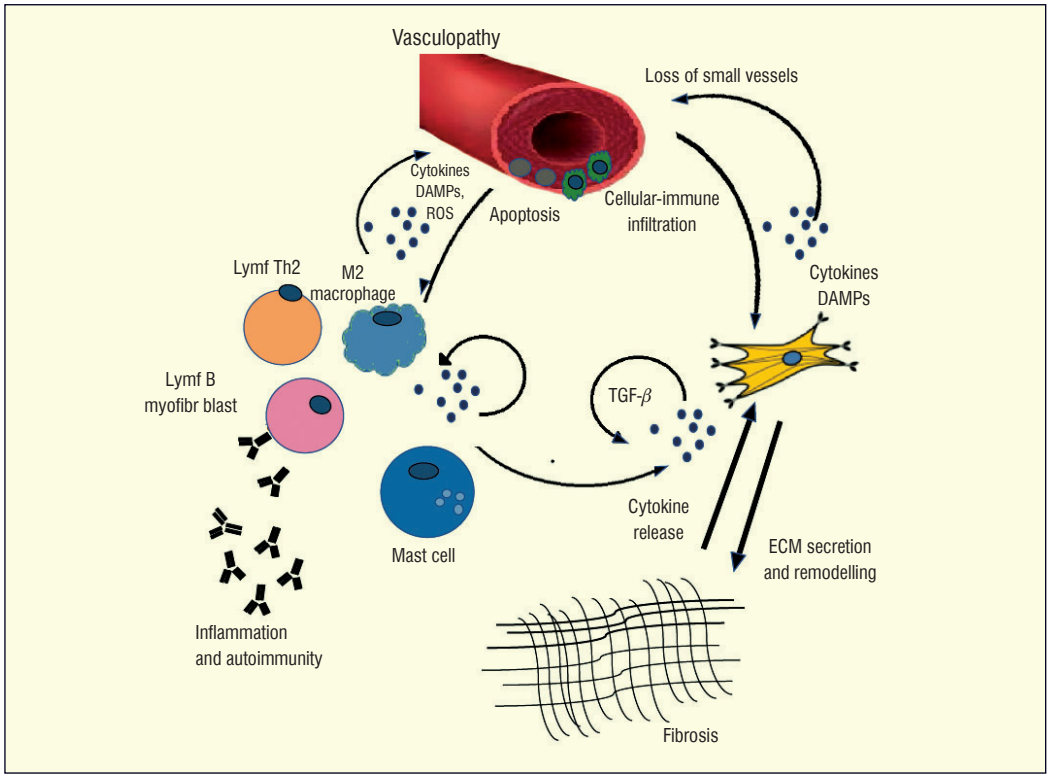

Figure 1. Effects of immunocompetent cells on myofibroblast formation and function

of SSc and serve as a bridge between tissue damage and mechanisms of abnormal tissue regeneration, resulting in uncontrolled fibrosis [23].

AUTOIMMUNITY

THE ROLE OF B LYMPHOCYTES IN THE PATHOGENESIS OF SSC

Autoimmune disorders also play an important role in the pathogenesis of the disease. This thesis is supported by the presence of autoantibodies in patients. Antinuclear antibodies are common (in more than 95% of patients) and are specific antibodies which are not found in the healthy population.

The presence of autoantibodies directed against various autoantigens is often associated with a specific clinical manifestation — however, there is no experimental evidence of such an association and the presence of autoantibodies is a phenomenon and not a clinically useful biomarker.

B lymphocytes are thus an important part of disease pathogenesis. The role of B lymphocytes is manifold — they are not only a source of antibodies but can also be involved in fibrotic processes through the production of cytokines (IL-6, TGF-β) and through interaction with dendritic cells, which enhances the Th2 response. B cells in SSc are hyperactive, as reflected by hypergammaglobulinemia, production of specific autoantibodies, increased serum levels of free immunoglobulin light chains. The increased proliferation and survival of B lymphocytes in SSc is affected, in addition to BCR activation, by numerous co-stimulatory molecules (CD19, CD40) [24]. In SSc, CD19 — a molecule that is crucial for B cell function — is overexpressed. B-cell activating factor levels were also found to be increased in SSc, and the CD22 molecule that inhibits B cell function is neutralised by autoantibodies directed against it.

A pathogenetic role for autoantibodies such as anti-endothelin-1 type A receptor antibodies or anti-angiotensin II type 1 receptor antibodies — which are involved in collagen production by fibroblasts and in angiogenesis — is also under consideration. Increased fibrosis — induced by ongoing inflammation — can also result from auotibodies directed against ICAM--1, which causes an increase in the production of ROS that damage tissue and induce apoptosis, thereby increasing the expression of ligands for TLRs, which induces the activation of the inflammatory response. Autoantibodies directed against MMP1 and MMP3 block the enzymatic activity of these proteins, which increases the accumulation of matrix proteins [24].

Regulatory B cells (Bregs) are another mechanism whose importance has been strongly emphasised in recent research into the pathogenesis of the disease. They play an important role in maintaining immune tolerance. Bregs are induced under conditions of ongoing inflammation and are capable of reducing it. The main property of this population is the ability to secrete IL-10 and probably also IL-35. Studies indicate that the number of IL-10-producing Bregs is reduced in SSc. A reduction in the number of Bregs occurs particularly in SSc that is associated with pulmonary fibrosis [25].

THE ROLE OF T CELLS IN THE PATHOGENESIS OF SYSTEMIC SCLEROSIS

The demonstration of perivascular cellular infiltrates (from T cells, dominated by CD4+ cells, macrophages, mast cells) in skin sections from patients in the early phase of the disease, prior to histological features of fibrosis, was one of the first pieces of evidence implying that immune cells and chronic inflammation are relevant in the pathogenesis of the disease. As fibrosis increases, the activity of the inflammatory process at the cellular level decreases.

It has been found that the Th2 response is predominant in SSc and polarisation in this direction occurs in the presence of IL-4, whose secretion is probably initiated by activated monocytes — perhaps the first cell lineage responsible for the development of the immune response [26].

When interacting with ILC2, Th2 cells (CD4+ cells producing IL-4 and IL-13) and Tc2 cells (CD8+ cells producing IL-13) are involved in increased deposition of extracellular matrix proteins. Both IL-4 and IL-13 directly enhance collagen production by fibroblasts [27].

Interleukin 13 induces fibrosis directly, in a manner independent of the classical TGF-β-related pathway [28]. Attention is drawn to the involvement of this cytokine in the development of scleroderma-associated interstitial lung disease (SSc-ILD) [29]. Il-13 also affects extracellular matrix remodelling by increasing tissue inhibitor of metalloproteinase-1 activity, which leads to increased extracellular matrix deposition.

Interleukin 13 also enhances TGF-β production by macrophages and thus increases extracellular matrix deposition [30].

When exposed to IL-33, T reg cells (Tregs) present in the skin of patients can transform into effector Th2 cells which secrete cytokines that enhance fibrosis. Th1, Th17, Th22 cells are also present in the skin biopsy specimens of patients, and these cells are involved in the development of the inflammatory phase when the fibrosis process is not yet intense.

Another aspect of CD4+ and CD8+ T cell activity is their cytolytic activity, participating in endothelial cell apoptosis, which consequently leads to vasculopathy [31].

Another interesting T cell subtype is follicular T cells (Tfh cells), whose increased numbers are found both in the blood of patients and in skin biopsy specimens. These cells produce IL-21 that stimulates the secretion of Ig G and Ig M by B (CD19+ CD27+ CD38hi) cells. In experimental models, IL-21 blockade resulted in reduced skin fibrosis, indicating a link between Tfh cells and immunologically mediated fibrotic processes. Studies based on RNA sequencing revealed the presence of a type of T cells (CD4+) that is unique to SSc and is marked by the expression of CXCL13 and IL-21. These cells activate the B lymphocyte response at early stages of inflammation [32].

Recently, a particular subtype of T cells (CD4+ CD8+) — double-positive T cells — has been identified in the skin of SSc patients. These cells secrete very high IL-4 counts [33]. Their exact role in the pathogenesis of SSc is not yet well understood.

In SSc patients, CD8+ cells also secrete large Il-4 counts (increased amounts of this cytokine were found both in the skin of patients and in material obtained from BAL), implying the involvement of this cytokine in the pathogenesis of SSc-ILD.

Research into the role of T cells in disease pathogenesis shows that their action profile is highly diverse, depending both on the disease stage and the modifying cytokine microenvironmental influence. This allows for the "matching" of the functions of individual cells to the tasks assigned to them and more efficient use of the capabilities of a given cell subset.

However, attention is drawn to the predominance of Th responses in the early stages of the disease (inflammatory phase and fibrotic phases) and type 1 responses (involving IFN-gamma and IL-17) in the later stages of the disease, when fibrosis is reduced, at least in the skin.

The involvement of innate and acquired immunity mechanisms in the pathogenesis of SSc is shown in Figure 2.

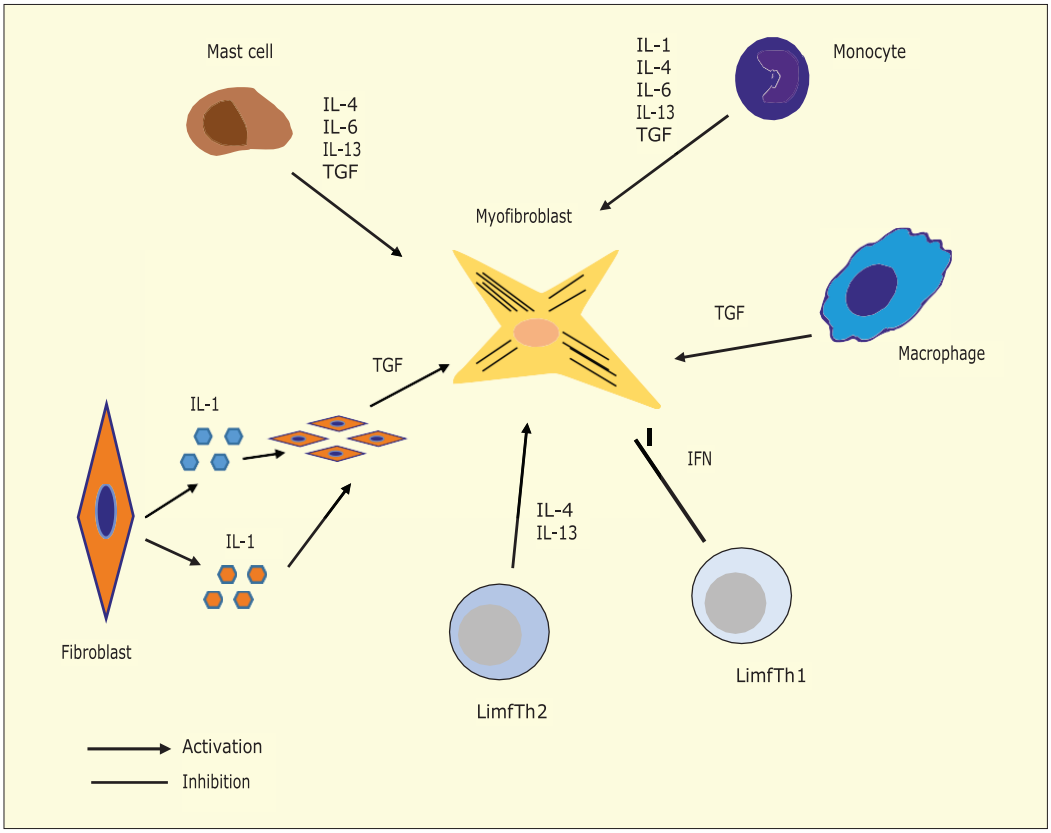

Figure 2. Involvement of innate and acquired immunity mechanisms in the pathogenesis of systemic sclerosis

THE ROLE OF THE MICROBIOME IN THE PATHOGENESIS OF SYSTEMIC SCLEROSIS

There is no doubt that the microbiome affects the host's immune response, both by presenting antigenic challenges that the immune system has to face, mainly by establishing immune tolerance, but also by responding to microbiota metabolism products. Dysbiosis is a condition of microbiota modification with multiple consequences (both immunological and metabolic).

Systemic sclerosis shows changes both in the skin flora, with an increase in the proportion of G(–) species, and the gut flora with an increase in pro-inflammatory species, mainly *Desulfovibrio* and opportunistic strains of *Clostridium* and *Streptococcus*, and a decrease in protective butyrate-producing bacteria. It is thus highly likely that the dysbiosis status may be important in the initiation and development of the disease process, however, this requires further research [34].

CONCLUSIONS

Systemic sclerosis is a chronic, systemic connective tissue disease of still incompletely understood aetiology, marked by progressive fibrosis of the skin and internal organs, which is caused by excessive collagen synthesis and maturation.

Currently, SSc is mainly seen as an autoimmune disorder, however, the mechanisms that initiate the disease and determine its specific course are not sufficiently understood.

However, research in recent years has drawn attention to the role of components of the innate (non-specific) response system in the pathogenesis of the disease.

The mechanisms of innate immunity are perceived here as connecting links between the phenomena of autoantibody synthesis, cytokine production and T- and B-lymphocyte activation.

An increasing understanding of the diversity of mechanisms underlying this disease will enable more effective therapeutic interventions in the future.

CONFLICT OF INTEREST

None declared.

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