Review Article

Systemic Sclerosis: B Lymphocyte Abnormalities, Diagnosis and Treatment

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Systemic sclerosis (SSc) is a connective tissue disorder characterized by the fibrosis in the skin and internal organs. SSc is characterized by the presence of autoantibodies that are produced by B lymphocytes. Recently, the importance of B lymphocytes in immune response and autoimmunity has been recognized. CD19, which is a critical cell-surface signal transduction molecule of B cells, positively regulates signaling through B cell antigen receptor and controls autoantibody production. B cells from SSc patients exhibit CD19 overexpression that results in disturbed peripheral B cell homeostasis characterized by increased naive B cells and decreased but activated memory B cells. These findings indicate that B cells are potential therapeutic targets in SSc. Since SSc is a heterogeneous disorder, the subset classification of limited cutaneous SSc and diffuse cutaneous SSc (dSSc) is critical for predicting prognosis and selecting appropriate treatment. Although none of drugs have been proved to be effective for SSc by controlled studies, there are some therapeutic choices that may be effective for patients with early dSSc. Oral low-dose steroid is frequently effective for skin sclerosis, when it is used for early dSSc patients with edematous and rapidly progressing skin fibrosis. Many uncontrolled trials have shown that treatment with cyclophosphamide plus steroid may be effective for SSc patients with active alveolitis. Since it is difficult to remove fibrosis once established in SSc, it should be emphasized that early diagnosis and early treatment of dSSc are critical for management of SSc.

Introduction

Systemic sclerosis (SSc) is a connective tissue disorder characterized by fibrosis in the skin and internal organs. In the US, the prevalence of SSc is estimated to be 242 cases per million adults with an annual incidence of 19.3 cases per million adults per year.[1] Absolute survival is 78% at 5 years and 55% at 10 years.[1] SSc occurs significantly more frequently in families with SSc (1.6%) than in the general population (0.026%).[2] Thus, a positive family history is one of the strongest risk factors for SSc; however, the absolute risk for each family member remains quite low (<1%),[2] suggesting the important role of environmental factors in the development of SSc.

Although the pathogenesis of SSc remains unknown, the genetic predisposition and environmental stimuli result in 3 major abnormalities, including collagen accumulation, vascular injury and immune activation.[3] Collagen accumulation mainly results in fibrosis of the skin and lungs. Vascular injury consists of Raynaud's phenomenon, digital ulcers and scars, scleroderma renal crisis and pulmonary hypertension. Immune activation is characterized by autoantibody production, lymphocyte activation and release of various cytokines.

The presence of autoantibodies is a central feature of immune activation associated with SSc; antinuclear antibody has been detected in more than 90% of patients.[4] SSc patients have autoantibodies that react to various intracellular components, such as DNA topoisomerase I, centromere, RNA polymerases, U1RNP, U3RNP, Th/To and histones.[4] The cytokines released by lymphocytes infiltrating the affected tissues may cause vascular injury and collagen production. However, it remains unknown how the 3 major abnormalities can be unified into one hypothesis.

Systemic autoimmunity in SSc

Autoimmune diseases have been classified into organ-specific autoimmune diseases and systemic autoimmune diseases.[5] In organ-specific autoimmune diseases, immune responses are directed toward a limited set of specific autoantigens and the pathogenetic role of autoantibodies is generally clear; for example, pemphigus, an autoimmune skin bullous disease, exhibits autoantibodies to desmogleins that are essential molecules for keratinocyte attachment,

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leading to bullous formation due to inhibition of keratinocyte attachment by autoantibodies. On the other hand, systemic autoimmune diseases, including SSc and systemic lupus erythematosus (SLE), are thought to emerge from more global immunoregulation abnormalities.[5] Furthermore, it is basically unknown how autoantibodies are involved in disease expression of systemic autoimmune diseases.

Nonetheless, autoantibodies are produced by autoreactive, memory B cells and recent studies have clearly shown that B cells play a critical role in the development of SSc. Recent assessment of the role of B cells in the immune system has indicated that B cells are more than just the precursors of antibody-secreting cells.[5] B cells have more essential functions in regulating immune responses than had previously been appreciated. B cell functions include antigen-presenting cells, production of various cytokines, lymphoid organogenesis, differentiation of T effector cells, and influence of antigen-presenting dendritic cell function.[5] Abnormalities of these B cell functions could contribute to the induction or development of clinical manifestations as well as autoimmunity in systemic autoimmune disorders, such as SSc.

Response regulators of B cell signaling

B cells respond to numerous stimuli that regulate negative selection in the bone marrow, the generation of humoral immune responses in the periphery, and the establishment and maintenance of tolerance and memory. The outcome of these B cell responses is determined by signaling thresholds through the B cell antigen receptor (BCR) complex (Figure 1).[6] The signaling thresholds are regulated by an array of cell surface molecules or cytoplasmic signal transduction molecules ("response regulators") that augment or
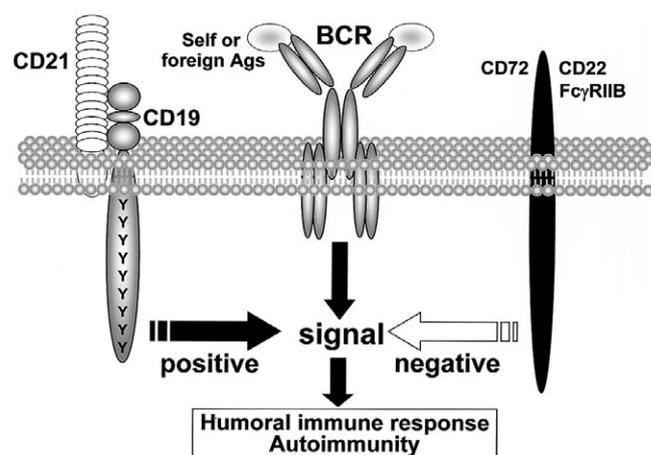

Figure 1. Response regulators of B cell signaling. B cell responses to foreign or self antigens (Ags) are controlled in part by interaction between positive and negative response regulators. CD19 and CD21 are positive response regulators that augment BCR signaling, while CD22, CD72, and FcγRIIB are negative response regulators that reduce BCR signals. Abnormal regulation of the response regulator function and expression may result in autoantibody.

diminish BCR signals during B cell development, as well as during responses to self and foreign antigens.[6-8] Thus, "response regulators" could be a useful concept for understanding how autoimmunity is induced, as well as how normal humoral immune responses are regulated.

CD19 expression is restricted to B lineage cells and follicular dendritic cells that are antigen-presenting cells located in the murine spleen.[9] CD19 is expressed by early pre-B cells from the time of immunoglobulin (Ig) heavy chain rearrangement until plasma cell differentiation. CD19 is a 95,000 Mr glycoprotein member of the Ig superfamily with an extracellular region containing two C2-type Ig-like domains separated by a smaller potentially disulfide-linked domain. On B cells, CD19 is associated with CD21, a receptor for complement C3 cleavage fragments and Epstein-Bar virus. The CD19 cytoplasmic region of 240 amino acids contains 9 conserved tyrosine residues. Lyn, a Src-family protein tyrosine kinase member, is the dominant protein tyrosine kinase that phosphorylates CD19.[10]

Previously, in vivo CD19 function was assessed using CD19-deficient mice and CD19-transgenic mice, named TG-1, that overexpress CD19 by 3-fold.[11-16] Splenic B cells from CD19-deficient mice exhibit reduced proliferation to various transmembrane signals compared with wild type B cells, while B cells from TG-1 mice show augmented proliferation.[11,14] Serum Ig levels are decreased in CD19-deficient mice, whereas they are generally increased in TG-1 mice.[11,14] Furthermore, serum levels of autoantibodies, such as anti-DNA antibody and rheumatoid factor, are decreased in CD19-deficient mice, but are increased in TG-1 mice.[13] CD19 expression on B cells correlates positively and closely with the production of autoantibodies.[13] Analysis using a transgenic mouse model of autoreactive B cells and peripheral tolerance has revealed that CD19 overexpression disrupts peripheral tolerance in B cells and thereby induces autoantibody production.[17] These results indicate that CD19 expression levels regulate autoantibody production by augmenting B cell signaling. Importantly, splenic B cells stimulated with anti-IgM antibody, lipopolysaccharide, or interleukin-4 up-regulate I-A, a major histocompatibility complex class II molecule that is a marker for B cell activation, while CD19 expression is not affected by B cell activation.[13] Thus, CD19 expression is tightly regulated during the B cell activation process, suggesting that intrinsic CD19 expression levels may determine a genetic predisposition to autoimmunity.

CD19 expression levels on B cells from SSc

To assess whether there is CD19 overexpression in human autoimmune disorders, CD19 expression was investigated on B cells from autoimmune disorders. B cells from SSc patients exhibit CD19 overexpression.[18] Flow cytometric analysis of the cell surface CD19 density on blood B cells has revealed that CD19 expression levels in SSc patients are significantly 20% higher than normal controls.[18] Furthermore, the CD19 overexpression is detected in both naive B cells and memory B cells from SSc patients.[19] Expression of CD21, which is associated with CD19 on B cells, is also higher in SSc

patients.[18] In contrast, CD40 and CD20 levels are normal in SSc patients. CD19 overexpression appears to be specific for SSc, since it is not detected in other autoimmune disorders, such as SLE, dermatomyositis or autoimmune bullous disorders.

Intrinsic B cell abnormalities in SSc

Cell-surface CD27 is a useful marker of human memory B cells,[20] since essentially all circulating $CD27^+$ B cells display hypermutated rearranged V_H genes, while no mutations are identified in $CD27^-$ B cells. According to CD27 expression, $CD19^+$ blood B cells were grouped into $CD27^-$ naive B cells, memory B cells expressing medium levels of CD27 ($CD27^{med}$), and plasmablasts/early plasma cells with high levels of CD27 ($CD27^{hi}$).[21,22]

Recent studies using CD27 as a marker of memory B cells have revealed a disturbance in peripheral B cell compartments and homeostasis in systemic autoimmune disorders. Patients with SLE exhibit an expanded population of $CD27^{hi}$ plasmablasts that correlates with disease activity, while the number of $CD27^-$ naive B cells and $CD27^+$ memory B cells is reduced due to marked B lymphocytopenia.[21,23,24] On the other hand, patients with Sjögren's syndrome show a normal number of naive B cells, but a significantly reduced number of $CD27^+$ memory B cells without expansion of plasmablasts.[22,25] Thus, these findings indicate distinct types of abnormal B cell homeostasis in some systemic autoimmune disorders.

In addition to significant autoantibody production, hyper-γ-globulinemia and polyclonal B cell hyperactivity are detected in SSc patients.[26,27] Furthermore, recent analysis of gene expression using DNA microarrays has revealed up-regulation of genes related to B cells in the SSc lesional skin.[28] These observations suggest the presence of intrinsic B cell abnormalities in SSc. To determine intrinsic B cell abnormalities in SSc, phenotypic and functional abnormalities of blood B cell subsets were assessed.[19] In patients with SSc, total blood B cells are expanded. Remarkably, peripheral B cell homeostasis and subsets are disturbed in SSc: naive B cells expand, while memory B cells and plasmablasts/early plasma cells diminish. Furthermore, memory B cells from SSc patients show increased expression of CD80 and CD86. Since it has generally been accepted that B cell activation is required to up-regulate expression of both CD80 and CD86, critical co-stimulatory molecules of B cells, this finding indicates that memory SSc B cells are chronically activated in vivo, possibly due to CD19 overexpression. Furthermore, CD95 expression, which is up-regulated following B cell activation, is also increased on SSc memory B cells. The increased CD95 expression coincides with the acquisition of marked sensitivity to CD95-mediated apoptosis.[29] Consistently, the sensitivity to spontaneous apoptosis is augmented in SSc memory B cells. This enhanced spontaneous apoptosis of SSc memory B cells may result in a diminished number of these cells in the blood. Furthermore, it is possible that the continuous loss of memory B cells and plasmablasts/early plasma cells increases the production of naive B cells in bone marrow to maintain B cell homeostasis. Remarkably, although the memory B cell number is decreased in SSc patients, stimulated SSc memory B cells have enhanced ability to produce IgG, resulting in hyper-γ-globulinemia and possibly autoantibody production. Thus, SSc patients have distinct abnormalities of blood homeostasis and B cell compartments characterized by expanded naive B cells, and activated but diminished memory B cells. Furthermore, CD19 overexpression in memory SSc B cells may be related to their hyper-reactivity, since memory B cells as well as naive B cells from SSc patients overexpress CD19. Collectively, B cells or B cell-specific signaling molecules, such as CD19, may be potential therapeutic targets for SSc.

Disease classification

SSc is a heterogeneous disorder, since it includes a very broad spectrum of manifestations from patients with only Raynaud's phenomenon to patients with diffuse skin and internal organ involvement. Therefore, the classification of disease subsets is critical for evaluating clinical manifestations, predicting prognosis, and selecting appropriate treatment in each patient. The most widely accepted disease classification is diffuse cutaneous SSc (dSSc) and limited cutaneous SSc (lSSc).[30] The major distinction between dSSc and lSSc is the extent of skin sclerosis; for example, a patient with skin sclerosis proximal to the elbow is considered to have dSSc, while a patient with skin sclerosis distal to the elbow has lSSc. However, this subset distinction also depends on other important clinical findings. For example, dSSc patients have significant and early incidence of internal organ involvement, such as lung fibrosis and scleroderma renal crisis. By contrast, lSSc patients have low and late incidence of pulmonary hypertension. The most important finding for the subset distinction is the specificity of autoantibodies. Anti-topoisomerase I antibody and recently identified anti-RNA polymerase antibody are associated with dSSc, whereas anticentromere antibody is linked to lSSc.[4]

Natural course of SSc and target for treatment with disease modifying drugs

Recent analyses demonstrate the natural course of each SSc subset (Figure 2).[31] In dSSc, skin sclerosis is getting worse within the first 5 to 6 years from the onset. After the peak, skin sclerosis is gradually improving, which is called the atrophic stage. Within first 5 to 6 years, internal organ involvement frequently occurs. In the later atrophic stage, significant complications are almost secondary to the established and remaining fibrosis. For example, secondary pulmonary hypertension and right heart failure result from preceding lung fibrosis. By contrast, in lSSc, the development of skin sclerosis is very slow for several decades, or skin sclerosis is even lacking. In the later stage, severe pulmonary hypertension occurs in 20% of Caucasian patients, while it occurs in only less than 2% of
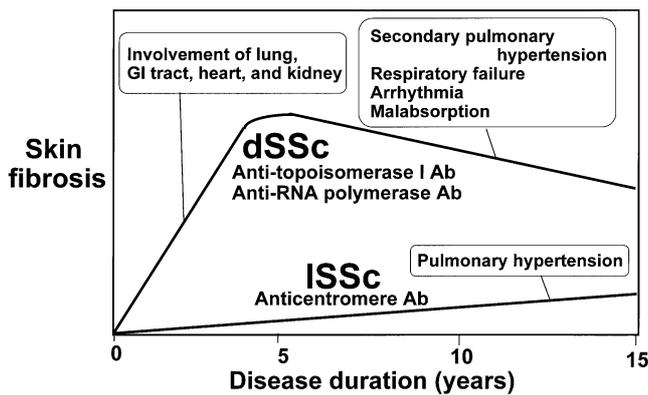
Figure 2. The natural course of SSc. GI tract and Ab represents gastrointestinal tract and antibody, respectively.

Figure 3. NFB on the finger cuticle of a SSc patient.

Figure 4. Disease distribution of NFB in various connective tissue disorders and normal persons.

Japanese patients. Importantly, the autoantibody specificity is useful for the subset distinction. Therefore, if the autoantibody specificity is known in each patient at the very early time point, natural course of the patient is generally predicted and the patient is informed of what will happen in the future.

The natural course is critical for selecting patients who can respond to treatment with disease modifying drugs. The treatment targets are early dSSc patients whose disease duration is less than 6 years, because their fibrosis still can be reversible at this stage. In the later stage, it is difficult to improve the already established fibrosis. In contrast to dSSc, lSSc is not a target for treatment with disease modifying drugs, since its prognosis is fairly well.

Early diagnosis by nailfold bleeding (NFB)

To start treatment as early as possible, early diagnosis is important. NFB is an easy, practical, useful, and cheep finding for early detection of SSc.[32] NFB is punctate or linear bleeding spots on the cuticle observed by naked eyes and is often accompanied by the enlarged capillary loops in the nailfold (Figure 3). The nailfold is the most preferred site to visualize systemic vascular injury associated with SSc.

The disease distribution of NFB was examined in large numbers of various connective tissue disorders (Figure 4). The scleroderma spectrum disorder is disease concept that unifies SSc-related disorders, including SSc, mixed connective tissue disease (MCTD), and secondary Raynaud's phenomenon with specific autoantibodies, mainly anticentromere antibody.[33] NFB is detected in 70% of the scleroderma spectrum disorder. Among the scleroderma spectrum disorder, NFB is observed in 60 to 70% of SSc or MCTD. Remarkably, in patients with secondary Raynaud's phenomenon who do not develop skin sclerosis yet, NFB is detected in 70%, which is similar to the frequency in SSc. Most of these patients with secondary Raynaud's phenomenon are positive for anticentromere antibody, a marker for lSSc. Therefore, NFB can identify early lSSc patients before the progression of skin sclerosis. By contrast, NFB
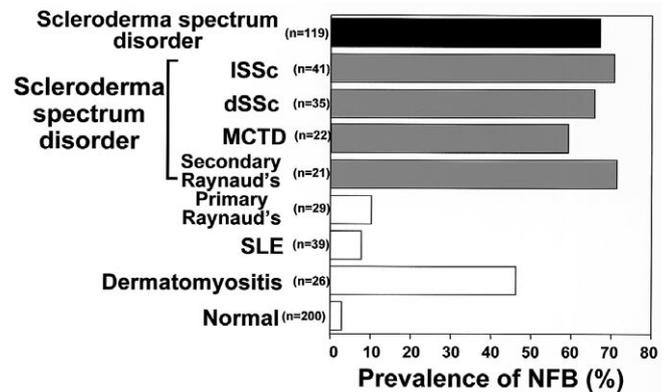
is observed in only 10% of patients with primary Raynaud's phenomenon, 8% of SLE patients, and 3% of normal persons. However, it should be noted that about 50% of dermatomyositis patients show NFB. We further assessed whether NFB was detected in early dSSc patients with anti-topoisomerase I antibody. In these patients, the presence of NFB correlates significantly with shorter disease duration of less than 5 years. Therefore, NFB is also useful for early detection of dSSc with anti-topoisomerase I antibody.

Summary of previously reported efficacy of disease modifying drugs for SSc

Steroid treatment is promising therapy for skin sclerosis in SSc.[34] By contrast, a recent controlled trial has shown that D-penicillamine is not effective for skin fibrosis, the frequency of scleroderma renal crisis and mortality.[35] Many uncontrolled trials have shown the efficacy of cyclophosphamide for lung fibrosis.[36] These 3 major drugs

are discussed below in detail. Methotrexate is now proven ineffective by a recent controlled trial.[37] Cyclosporin seems to be effective for skin fibrosis, but not for lung involvement.[38] The problem is that cyclosporin was reported to induce scleroderma renal crisis because of its renal toxicity. This is also true of tacrolimus. The efficacy of interferon-γ is extremely variable from good response to no effect. A controlled trial has shown that interferon-α is not effective; rather, lung function gets worse. Regarding stem cell transplantation, strong immunosuppression before stem cell infusion often results in death.[39] At present, this therapy remains experimental. Regarding photopheresis, one controlled trial showed promising results, but another showed no effect. Relaxin is a hormone secreted during pregnancy and has antifibrotic effects.[40] The Phase II controlled trial showed improvement in skin fibrosis,[40] but the Phase III controlled trial demonstrated no effect. Therefore, there have been no drugs that were proven to be effective for SSc by controlled studies.

Steroid treatment

For skin sclerosis, oral low-dose steroid is usually used in our scleroderma clinic.[34] Treatment with oral low-dose steroid significantly reduces skin fibrosis in early dSSc patients (Figure 5). It is crucial to select patients who could respond to steroid treatment. First, a treatment target is an early dSSc patient whose disease duration is less than 6 years from onset of skin sclerosis, which is the same criterion for a treatment target by disease modifying drugs. Second, skin sclerosis consists predominantly of edematous change. Third, skin sclerosis rapidly progresses within several months to 1 year. We usually use initial prednisolone dose of 20 to 30 mg/day.

However, there are several problems of steroid treatment. First, prednisolone of more than 15 mg/day was reported to be a risk factor for scleroderma renal crisis in Caucasian SSc patients.[41] However,
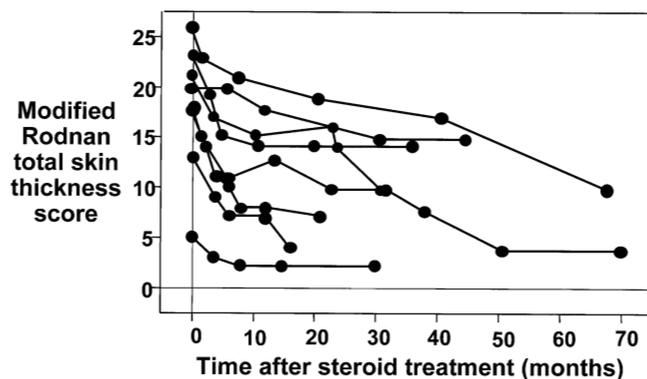

Figure 5. The efficacy of low-dose steroid for skin fibrosis in SSc. All SSc patients exhibited dSSc and were treated with low-dose oral steroid (prednisolone, 20-30 mg/day). Skin score was measured by scoring technique of the modified Rodnan total skin thickness score. The 17 anatomic areas were rated as 0 (normal skin thickness), 1+ (mild but definite skin thickening), 2+ (moderate skin thickening) and 3+ (severe skin thickening), and the modified Rodnan total skin thickness score was derived by summation of the scores from all 17 areas (range 0-51).

the frequency of renal crisis in Japanese SSc patients is less than 5%, which is much lower than 20% in Caucasian SSc patients. Furthermore, we have not experienced scleroderma renal crisis induced by low-dose steroid treatment in any Japanese SSc patients. Nonetheless, caution must be used when prescribing steroids. Second, steroid treatment is not effective for lung fibrosis that is a major cause for death in SSc. Third, long-term use of prednisolone may result in the deterioration of vascular damage. Finally, a controlled study has not been performed yet for the efficacy of steroid treatment. Therefore, a clinical controlled study will be needed to confirm its efficacy for skin sclerosis and effect on prognosis.

D-penicillamine treatment

D-penicillamine was proposed as therapy for SSc in 1966, because it interferes with the molecular cross-linking of collagen and has immunomodulatory effects. Since then, numerous uncontrolled studies reported the clinical usefulness of D-penicillamine in SSc treatment.[42] However, the results of a well-designed controlled trial for D-penicillamine efficacy published in 1999[35] indicated that the course of the skin score and frequencies of scleroderma renal crisis and mortality in the high-dose D-penicillamine group were not different from those in the very low-dose D-penicillamine group. This study, however, cannot answer whether low-dose D-penicillamine is effective or not. Nonetheless, almost all scleroderma investigators now think that D-penicillamine is not effective for SSc.

Cyclophosphamide treatment

It has been widely accepted that steroid treatment alone is not effective for lung fibrosis. Many uncontrolled trials have shown that cyclophosphamide is effective for early, active lung fibrosis showing alveolitis.[36,43] Cyclophosphamide can stabilize or improve dyspnea, %vital capacity (%VC), %diffusion capacity for carbon monoxide (%DLco), and chest computed tomography (CT). Regarding dose used in the previous reports, 1 to 2.5 mg/kg/day for oral administration and 500 to 1400 mg/day for intravenous pulse administration (once per month for 6 to 9 months) are used. Intravenous pulse is generally preferred because of its low incidence of side effects. Cyclophosphamide is usually used in combination of various dose of prednisolone, such as 10 mg/day to 1 mg/kg/day. The effect for skin sclerosis is unknown because of the effect of steroid used in combination.

Cyclophosphamide has side effects, including bone marrow suppression and carcinogenesis. Therefore, it is critical to identify patients who have active lung fibrosis and could respond to this therapy. In our scleroderma clinic, we assess the activity of lung fibrosis by following findings. First, ground glass or reticular appearance of chest CT is the most important sign for active lung fibrosis. Second, serial decrease in %DLco during the disease course is the most sensitive finding suggestive for the activity. Third, serum levels of KL-6

or surfactant protein-D (SP-D) are useful, serological markers for lung fibrosis and dramatic increase during the disease course may indicate the activity.[44,45] Fourth, bronchoalveolar lavage (BAL) analysis with more than 3% of eosinophils, 5% of neutrophils, or 15% of lymphocytes indicates the activity. Usually, increase in lymphocytes is the earliest event, followed by increase in eosinophils, and then neutrophils. If the activity cannot be determined based upon these 4 laboratory tests, lung biopsy may be performed. The earliest change is inflammatory infiltration including macrophages, neutrophils, lymphocytes, and eosionphils, which indicates the activity.

Summary of criteria for treatment in SSc

First, the treatment target is early dSSc with disease duration of less than 6 years (Figure 6). Regarding treatment for skin sclerosis, if a patient fulfills one of the two criteria that include edematous skin change and rapid progression of skin sclerosis, treatment with prednisolone of 20 to 30 mg/day is considered. Regarding treatment for lung fibrosis, if a patient shows positive findings of chest CT plus 2 of the 3 criteria that consist of serial decrease in %DLco, serial increase in KL-6 or SP-D, and positive findings of BAL, treatment with intravenous cyclophosphamide pulse therapy of 500 to 1000 mg/day (once a month for 6 months) in combination with prednisolone of 20 to 30 mg/day is considered. This treatment is a private opinion based on our experience, but not on evidence. However, it is often difficult to perform clinical controlled trials because of the rarity of SSc. Therefore, in rare disorders such as SSc, the experienced expert opinion would be also important.

1. Target: early dSSc with <6 years from the onset

2. Treatment of skin sclerosis
One of the following 2 criteria: ➡ **Prednisolone 20-30 mg/day**
  1. Edematous skin change
  2. Rapid progression of skin sclerosis

3. Treatment of lung fibrosis
Chest CT (grand glass or reticular)
+ 2 of the following 3 criteria: ➡ **Prednisolone 20-30 mg/day + Cyclophosphamide Pulse intravenous 500-1000 mg/day (once a month, 6 months)**
  1. Serial decrease in %DLco
  2. Serial increase in KL-6/SP-D
  3. Positive findings of BAL

Figure 6. Summary of criteria for treatment in SSc.

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