

Characteristics of Animal Models for Scleroderma

Toshiyuki Yamamoto*

Department of Dermatology, Tokyo Medical and Dental University, Tokyo, Japan

Abstract: Scleroderma is a fibrotic condition characterized by immunologic abnormalities, vascular injury and increased accumulation of matrix proteins in the affected dermis. Although the etiology of scleroderma is not fully elucidated, numerous studies suggest that extracellular matrix overproduction by activated fibroblasts and myofibroblasts results from a complex interactions among endothelial cells, lymphocytes, macrophages, and fibroblasts, *via* a number of mediators. Animal models which exhibit all the aspects of human scleroderma are not currently available, however, several spontaneous or experimental animal models, such as tight skin (Tsk) mouse, Tsk2 mouse, bleomycin-induced scleroderma, sclerodermatous graft-versus-host disease (Scl GvHD) model, UCD chicken, and fibrosis model by exogenous injections of TGF- β /CTGF have been investigated. This review describes different animal models for scleroderma, paying the most attention to the recent progress in bleomycin-induced experimental murine scleroderma. Each model exhibits unique characteristics of dermal fibrosis/sclerosis which can be of great help in exploring the pathogenesis as well as therapeutic strategies of scleroderma.

Keywords: Scleroderma, animal model, bleomycin, Tsk mouse, GvHD, UCD chicken.

INTRODUCTION

Systemic sclerosis (SSc) is a connective tissue disorder characterized by excessive production and deposition of extracellular matrix (ECM) in the affected skin, as well as various internal organs such as lung, kidney, heart, or esophagus [1]. In particular, type I, III collagen, fibronectin, and proteoglycans are abundantly produced by activated fibroblasts in the scleroderma skin. *In vitro*, activated scleroderma fibroblasts continue to synthesize increased amounts of collagen, as compared with control fibroblasts. SSc is also characterized by alterations of microvasculature and abnormal immune functions [2]. Although much work have been performed to investigate the pathogenesis of SSc, its etiology has not been fully elucidated.

The pathogenesis of cutaneous sclerosis may be divided into several stages. Usually, inflammation stage can be recognized prior to the development of sclerosis, and several fibrogenic cytokines released from infiltrated immunocytes are suggested to play a crucial role for initiating and leading to the sequential events of fibrosis/sclerosis [3-6]. Overproduction of ECM proteins from activated fibroblasts and myofibroblasts is supposed to result from complex interactions among endothelial cells, immunocytes, and fibroblasts, *via* a number of mediators.

Animal models are useful for exploring the pathogenesis of cutaneous sclerosis, and also the therapeutic intervention for scleroderma. So far, several spontaneous as well as induced animal models for scleroderma have been investigated. In this review, we summarize the characteristics and recent topics of different animal models of scleroderma.

BLEOMYCIN-INDUCED SCLERODERMA

General Features

Bleomycin was originally isolated from the fungus *Streptomyces verticillus* [7], and is a frequently used antitumor antibiotic for various kinds of malignancies. Due to the lack of the bleomycin-inactivating enzyme, bleomycin hydrolase, in the lungs and the skin [8], bleomycin-induced toxicity occurs predominantly in these organs. Lung fibrosis is a well-known side effect of bleomycin, and bleomycin-induced pulmonary fibrosis is an established rodent model [9-11].

Also, we have established a mouse model for scleroderma by local bleomycin treatment [12-17]. Histological dermal sclerosis was induced by repeated subcutaneous injections of bleomycin in various mice strains, although there is some variation among strains in the intensity and the periods required to induce dermal sclerosis. Administration of bleomycin (usually 10-100 μ g) every day or every other day for 3-4 weeks induced definite dermal sclerosis characterized by thickened collagen bundles, deposition of homogenous materials in the thickened dermis with cellular infiltrates, which mimicked the histologic features of human scleroderma. Masson trichrome stain showed dense deposition of collagen in the thickened dermis. Dermal thickness was gradually increased, and significantly increased up to two-fold when the sclerosis was developed. Furthermore, in some strains, epidermal thickness was also induced as well [14]. Other than skin, lung fibrosis with thickened alveolar walls with cellular infiltrates was also induced earlier. However, kidney, liver and heart were not involved. Cutaneous changes were relatively localized to around the injected site skin, and sclerotic changes were not induced in the remote region, such as fingers or abdominal skin. The induced sclerotic changes remained at least 6 weeks, when left untreated. Thickness of vascular wall in the deep dermis was also observed. C3H/He, DBA/2, B10.D2 and B10.A strains

*Address correspondence to this author at the Department of Dermatology, Tokyo Medical and Dental University, School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan; Tel: +81-3-5803-5286; Fax: +81-3-5803-0143; E-mail: yamamoto.derm@med.tmd.ac.jp

demonstrated intense dermal sclerosis by bleomycin treatment, suggestive of bleomycin-"susceptible" [14]. Whether the susceptibility depends on genetic background has not been examined.

Hydroxyproline contents in the bleomycin-treated skin were significantly increased as compared with control PBS-treated skin in parallel with the development of dermal sclerosis. Increased production of type I collagen as well as upregulation of type I collagen mRNA level were demonstrated in the sclerotic skin after bleomycin treatment [14]. Of interest, autoantibody was detected in the serum after bleomycin treatment [12].

Myofibroblasts have features which intermediate between those of the fibroblasts and smooth muscle cells. The myofibroblastic phenotypes are observed in wound healing or fibrotic process of various tissues including liver, kidney, lung or skin. Alpha-smooth muscle actin (α -SMA) is the most reliable marker of myofibroblastic cells. It is shown that scleroderma skin express α -SMA [18], and myofibroblasts are shown to persist in scleroderma fibroblasts cultures [19]. Transforming growth factor- β (TGF- β) and platelet-derived growth factor (PDGF), both important fibrogenic cytokines in fibrosis, induce the expression of α -SMA by fibroblasts [20, 21]. Following bleomycin treatment, α -SMA-positive myofibroblasts were observed in the dermis, and gradually increased in parallel with the induction of dermal sclerosis [16]. Treatment with anti-TGF- β antibody reduced myofibroblast formation in the lesional skin [16].

Cellular Infiltrates

Mononuclear cell infiltrates in the skin are one of the most characteristic histological features in early scleroderma [4], and suggested to play a role by secreting cytokines which stimulate ECM production. Moreover, infiltrating T cells, predominately CD4+, are also the major lymphocytes seen in the involved skin of scleroderma. As in human scleroderma, a number of mononuclear cells including CD4+ T cells, macrophages and mast cells infiltrates into the bleomycin-treated skin [12]. Our studies confirmed that dermal sclerosis can be induced even in SCID mice comparable to control mice [15], and also dermal sclerosis was inducible in nude mice as well [17]. These findings suggest that bleomycin-induced scleroderma is T cell independent.

Activated macrophages appear to play an important role in fibrosis, because these cells are among the first immune cells found in increased numbers at the early stages of fibrosis, and they release a number of proinflammatory and fibrogenic mediators such as TGF- β and PDGF [22]. Early scleroderma skin includes an increased CD14-positive cells (monocytes/macrophages) compared with normal skin [23]. Ishikawa *et al.* [24] demonstrated that the ratio of infiltrating macrophages to T cells was high, suggesting an important role of cutaneous macrophages in scleroderma. In the bleomycin model, a number of macrophages were detected along with T cells [12], and easily supposed to play an important role as a major source of a number of mediators. Moreover, macrophages are a potent source of reactive oxygen species (ROS), suggested to be one of the mediators

involved in the pathogenesis of bleomycin-induced scleroderma, as discussed later.

Mast cells have been suggested to be one of the important initiators of scleroderma, since mast cells are increased in number in the lesional skin of its early stage [25, 26]. Mast cells produce a number of cytokines, growth factors, and mediators that are capable of activating fibroblasts and endothelial cells. By contrast, pulmonary fibrosis can be induced by bleomycin in genetically mast cell-deficient mice [27]. In the bleomycin model, mast cells were increased in number in parallel with the induction of dermal sclerosis. Also, a marked degranulation was found in particular at the early phase, with elevated plasma histamin levels [12]. On the contrary, bleomycin could induce dermal sclerosis even in genetically mast cell-deficient WBB6F1-W/W^V mice similarly to control littermates [13], with increased levels of type I collagen [28]. We thus speculate that mast cells may be associated but not necessary for the induction of dermal sclerosis. Mast cells may be not the sole pathway to the induction of scleroderma.

Eosinophil infiltration in association with skin fibrosis is occasionally seen [29]. Infiltration of eosinophils precedes and parallels the development of lung fibrosis induced by bleomycin [30]. Eosinophils represent a primary cellular source of TGF- β [31]. *In vitro* studies have shown that eosinophils readily bind to fibroblasts, which leads to the release of mitogens that augment fibroblast proliferation [32] and collagen production [33]. Interleukin-5 (IL-5) and eotaxin are important in the differentiation, proliferation, recruitment, activation and chemotaxis of fibroblasts. Fibroblasts can be a source of eotaxin under appropriate cytokine stimulation [34]. A recent study demonstrates that bleomycin-induced pulmonary fibrosis can be induced both in IL-5 transgenic and deficient mice, suggesting that eosinophils and T cells contribute distinctly to the development of fibrosis *via* their production of different cytokine components [35]. On the contrary, bleomycin-induced pulmonary fibrosis was independent of eosinophils [36]. In the lesional skin of bleomycin model, not only mast cells but also eosinophils were increased in number, both of which were significantly reduced by the administration of anti-TGF- β antibody in parallel with the attenuation of dermal sclerosis [37].

Cytokines

Accumulated studies have delineated a number of cytokines and growth factors that regulate matrix biosynthesis in fibroblasts and their mechanisms of action. Among them, TGF- β is suggested to play a key role in the pathogenesis of SSc [38]. TGF- β , which is found abundantly in platelets and released from activated macrophages or lymphocytes, is a strong chemoattractant for fibroblasts [39]. Multiple biological actions of TGF- β include increase of the synthesis of collagen type I and type III or fibronectin by many cell types, modulation of cell-matrix adhesion protein receptors, and furthermore, regulation of the production of proteins that can modify the ECM by proteolytic action, such as plasminogen activator, an inhibitor of plasminogen, or procollagenase [39]. Also, TGF- β is capable of stimulating its own synthesis by

fibroblasts through autoinduction [40]. *In vivo*, TGF- β induces rapid fibrosis and angiogenesis when injected subcutaneously into newborn mice [41]. Thus, maintenance of increased TGF- β production may lead to the progressive deposition of ECM, resulting in fibrosis.

In the bleomycin-treated skin, TGF- β was detected on the infiltrating cells, which were predominantly composed of macrophages and mast cells, as well as fibroblasts at sclerotic stages. TGF- β 1 and -2 mRNA expression was also detected in the lesional skin. We have recently observed increased expression and synthesis of TGF- β 1 in bleomycin-"susceptible" mice strains [42]. Blockade of TGF- β activity reduced the development of bleomycin-induced scleroderma [37]. Accordingly, TGF- β plays a key role in the bleomycin model.

The Smads are intracellular signal transducers that mediate fibroblast activation and other profibrotic responses elicited by TGF- β . Recent studies raise the possibility that Smads play an important role in the pathogenesis of fibrosis. In bleomycin-treated skin, fibroblasts showed predominantly nuclear localization of Smad3 and intense staining for phospho-Smad2/3 [43]. On the other hand, expression of Smad7, the endogenous inhibitor of TGF- β /Smad signaling, was downregulated, which may account for sustained activation of TGF- β /Smad signaling in the lesional skin. In addition, bleomycin treatment for 4 weeks showed attenuated fibrosis, lower synthesis and accumulation of collagen, and reduced collagen gene transcription in Smad3 null mice, as compared with wild type [44].

Type 2 cytokines are recently regarded to play an important role in fibrosis. IL-13 is a pleiotropic cytokine that is elaborated in significant quantities by appropriately stimulated type 2 cells, and has been implicated in the pathogenesis of fibrotic conditions including SSc [45]. The profibrotic effect of IL-13 is postulated to involve irreversible fibroblast activation, triggered either directly [46] or indirectly through TGF- β [47, 48]. *In vitro* studies demonstrated that IL-13 is a potent stimulator of fibroblast proliferation and collagen production [47, 49]. IL-13 transgenic mice show increased lung fibrosis, as well as increased levels of TGF- β 1 [48]. Both IL-4 and IL-13 were elevated during the pathogenesis of bleomycin-induced pulmonary fibrosis, and neutralization of IL-13, but not IL-4, attenuated the development of lung fibrosis, suggesting that IL-13 has an important role in the development of lung fibrosis [50]. In the bleomycin model, expression of IL-13 was enhanced on the infiltrating mononuclear cells in the lesional skin, in parallel with the induction of dermal sclerosis, and IL-13 mRNA levels and protein production were also significantly increased [51]. IL-13 receptor (IL-13R)-2 expression in the lesional skin was augmented mainly in the infiltrating mononuclear cells and macrophages after bleomycin exposure. IL-13R-2 mRNA level in the whole skin was upregulated, whereas IL-13R-1 mRNA was not significantly enhanced. IL-13 may promote the progression of bleomycin-induced scleroderma.

The serologic level of tumor necrosis factor (TNF) increases with the clinical severity and biologic activity of the disease [52]. TNFRp55-deficient mice developed severe sclerotic changes of the dermis following bleomycin

exposure at extremely earlier time points, as compared with wild type [53]. Induction of matrix metalloproteinase-1 (MMP-1) expression was significantly inhibited in the bleomycin-treated skin of TNFRp55-null mice. The authors suggest that signaling mediated by TNFRp55 plays an essential role in MMP-1 expression and a key role in the collagen degradation process in the bleomycin-induced scleroderma.

Chemokines

The biological actions of chemokines are mediated through a family of seven transmembrane G-protein-coupled receptors present on the surface of target cells. CCL2/monocyte chemoattractant protein-1 (MCP-1) upregulates type I collagen mRNA expression in fibroblasts [54]. Current studies have demonstrated increased expression of CCL2 in patients with SSc [55-59]. Distler *et al.* [57] reported that stimulation with PDGF resulted in a significant increase in CCL2 mRNA and protein. Furthermore, we demonstrated the autoinduction of CCL2 in scleroderma fibroblasts, but this effect was not observed in normal fibroblasts [55]. These findings suggest an important involvement of CCL2 in the pathogenesis of scleroderma. CCL2 acts indirectly *via* IL-1 [60]. IL-1 as well as IL-1 receptor levels, in turn, were shown to be significantly increased in scleroderma [61]. In addition, IL-1 as well as TNF- α , are potent inducers of CCL2. Thus, in addition to a direct autocrine stimulatory loop, a mutual induction between IL-1 and CCL2 might lead to a self-perpetuating activation of scleroderma fibroblasts.

In the bleomycin model, expression of CCL2 and CCR-2, a major receptor of CCL2, was elevated at both protein and mRNA levels in the lesional skin. CCL2 as well as CCR-2 were detected on the infiltrating mononuclear cells at early stages following bleomycin treatment, and also detected on the fibroblasts at later stages in the sclerotic skin [62]. Continuous application of neutralizing antibody against CCL2 reduced the development of scleroderma in this model [62]. These results suggest that CCL2/CCR-2 signaling plays an important role in the pathogenesis of bleomycin-induced scleroderma. A recent report demonstrates that CCR-2-deficient mice are protected from pulmonary fibrosis [63], suggesting that CCR-2 signaling promotes a profibrotic cascade. IL-4 has been shown to induce significant levels of CCL2 production in stromal cells [64, 65]. On the contrary, CCL2 upregulates IL-4 mRNA expression and protein production [66]. Thus, mutual induction of CCL2 and IL-4 has greatly been speculated.

Reactive Oxygen Species (ROS)

Excessive oxidative stress has been implicated in the pathogenesis of SSc [67, 68]. Bleomycin is known to generate ROS, such as superoxide and hydroxyl radicals. ROS can cause endothelial cell damage, stimulate skin fibroblast proliferation, and increase collagen production. We previously demonstrated the inhibitory effect of lecithinized superoxide dismutase (SOD) on bleomycin-induced scleroderma [69], suggesting the involvement of ROS.

Oxidative stress transiently induces CCL2 mRNA and protein expression in cultured skin fibroblasts [70]. Therefore, elevated levels of CCL2 in this model might be induced, in part, *via* ROS by bleomycin.

Apoptosis

Apoptosis occurs in a well-choreographed sequence of morphological events. Recent studies suggest the involvement of apoptosis in several autoimmune disorders, including SSc [71, 72]. In the bleomycin model, excessive apoptosis was observed mainly on infiltrating cells, in parallel with the induction of dermal sclerosis [73]. Increased expression of Fas and Fas Ligand (FasL) was observed in the lesional skin, and caspase-3 expression as well as activity was also found. Furthermore, neutralization with FasL antibody attenuated the dermal sclerosis. These results suggest that excessive apoptosis is involved in the pathogenesis of bleomycin-induced scleroderma.

Therapeutic Intervention

Interferon- γ (IFN- γ) causes potent inhibition of collagen production in cultured skin fibroblasts [74-76]. *In vitro* studies showed that IFN- γ decreased TGF- β -induced α -SMA expression in palatal fibroblasts, as well as alteration of morphology [77]. In the bleomycin model, systemic administration of IFN- γ together with bleomycin reduced dermal sclerosis, even after the onset of scleroderma [78]. IFN- γ is a powerful type 1 inducer of cellular immunity, which may indirectly contribute to the improvement of the imbalance of the type 2 shift. A recent report has shown that IFN- γ inhibits the TGF- β -induced phosphorylation of Smad3 and the accumulation of Smad3 in the nucleus, whereas induces the expression of Smad7 which prevents the interaction of Smad3 with the TGF- β receptor [79].

Halofuginone has an inhibitory effect on collagen synthesis, and shows anti-fibrotic effects in a few animal models of scleroderma, as discussed later. By contrast, bleomycin-induced scleroderma was not attenuated by the treatment with halofuginone [80].

Hepatocyte growth factor (HGF) was originally identified and cloned as a potent mitogen for hepatocytes. Recent findings demonstrate that HGF prevented the progression of liver cirrhosis, renal fibrosis, and pulmonary fibrosis. Wu *et al.* [81] demonstrated that gene transfer of HGF not only prevented the ongoing dermal sclerosis induced by simultaneous local injections of bleomycin, but also ameliorated the previously induced dermal sclerosis. This effect was mediated by suppressing TGF- β levels.

TIGHT SKIN MOUSE

General Features

Tight skin (Tsk) mice are heterozygous for a mutation in the fibrillin-1 gene (Tsk1/+). The most striking feature is the presence of thickened and tight skin that is firmly bound to the subcutaneous and deep muscular tissues [82]. Additionally, Tsk1/+ mice display certain visceral changes

in the lungs and hearts; however, vascular involvement has not been reported. The lung abnormalities, characterized by greatly distended lungs, are present at birth and histologically they resemble human emphysema, with little fibrosis. Numerous biochemical and molecular abnormalities that closely resemble those present in patients with SSc. The histologic evidence suggesting an increase in collagen content in the Tsk1/+ mice has been confirmed by extensive biochemical studies. Pablos *et al.* [83] found that mRNA expression of TGF- β , type I, III and VI collagen were under temporal and spatial regulation during postnatal growth and development in the Tsk1/+ mice. Collagen 1(I) and 1(III) gene-expressing fibroblasts were increased in Tsk1/+ fibrotic lesions. Transient transfections of a series of human 1(I) procollagen promoter constructs into Tsk1/+ fibroblast cultures showed increased transcription rates caused by the lack of the strong inhibitory influence of the regulatory sequence contained in the promoter between -675 and -804 bp [84]. Additionally, Tsk1/+ nuclear extracts displayed decreased binding to a consensus AP-1 sequence. Transcriptional activation of collagen genes was demonstrated in Tsk1/+ mice *in vivo* employing reporter transgenes harboring upstream fragments of the 5' flanking region of the mouse 2(I) collagen genes.

Cellular Infiltrates

In Tsk1/+ mice, mast cells are abundant in the thickened dermis and exhibit prominent degranulation [85]. A decrease in fibrosis associated with inhibition of mast cell degranulation by cromolyn and ketotifen was also reported in the Tsk1/+ mice [86]. Further analysis using Tsk1/+ and mast cell-deficient mice showed that maximal mast cell infiltration and degranulation was observed long after the onset of fibrosis [87]. Mast cells did not initiate fibrosis and mast cell number is similar as compared with control littermates, however, mast cells are suggested to contribute to fibrosis at later stages.

CD4+ T cells have been shown to be required for the excessive accumulation of dermal collagen in Tsk1/+ mice [88]. By contrast, the Tsk phenotype fully develops in SCID mice [89, 90].

Cytokines/Chemokines

TGF- β and IL-4 are suggested to play important roles in the pathogenesis of fibrosis in Tsk1/+ mice. TGF- β was expressed only during the rapid postnatal growth of the skin in parallel with high expression of 1(I), 1(III), and 2(VI) collagen genes. Fibroblasts from Tsk1/+ mice are hyperresponsive to IL-4 and TGF- β , displaying increased synthesis of 1(I) collagen mRNA, collagen protein, and activity of a luciferase reporter construct containing 2(I) collagen promoter [91]. Targeted mutations in either the signaling chain of the IL-4 receptor or STAT6 prevents the cutaneous hyperplasia in Tsk1/+ mice, suggesting the importance of IL-4 [91, 92]. After IL-4 stimulation, JAK-1 and JAK2 were phosphorylated to a greater degree in Tsk1/+ fibroblasts than in C57BL/6 fibroblasts [93].

Recent studies show that CCL7/MCP-3 was highly overexpressed by neonatal Tsk1/+ fibroblasts [94]. Increased

MCP-3 protein secretion by Tsk1/+ fibroblasts was observed. Immunohistochemistry revealed that MCP-3 was detected abundantly in the dermis of Tsk1/+ mice at 10 days and 3 weeks old.

Defects of the Immune Systems

Aged (8 months and older) Tsk1/+ mice produce autoantibodies such as topoisomerase-I (Topo-I) [95]. Treatment with mercury enhanced the serum levels of anti-Topo-I antibodies [96]. B cell functional defects caused by the loss of CD19 significantly decreased skin fibrosis in Tsk1/+ mice, suggesting that B cells play an important role [97]. In Tsk1/+ mice overexpressing CD19, anti-Topo-I antibody levels were significantly increased, although skin thickness was not enhanced [98]. B cell antigen receptor crosslinking augmented activation of extracellular signal-regulated kinase in Tsk1/+ B cells [98]. Thus, disrupted B cell signaling may contribute to immunologic abnormalities in Tsk1/+ mice.

Therapeutic Intervention

Administration of anti-IL-4 antibody to neonatal Tsk1/+ mice prevented skin fibrosis and dermal collagen content [99]. Intravenous immunoglobulin therapy decreased splenocyte secretion of IL-4 and TGF- β , resulting in aggradation of fibrogenesis in Tsk1/+ mice [100].

Halofuginone attenuates collagen synthesis, as well as collagen gene expression in avian and murine skin fibroblasts [101]. Halofuginone specifically inhibits 1(I) collagen gene expression without affecting the synthesis of other types of collagen such as type II and III [102, 103]. Halofuginone inhibited TGF- β -induced upregulation of collagen protein and activity of 2(I) promoter, as well as phosphorylation and subsequent activation of Smad3 after TGF- β stimulation [104]. Dermal application of halofuginone on Tsk1/+ mice for 60 days reduced dermal fibrosis as well as collagen 1(I) gene expression [105]. Intraperitoneally administered halofuginone also prevented the thickening of the dermis and eliminated the increase in skin collagen in both Tsk1/+ and chronic graft-versus-host disease (cGvHD) models [103].

TSK 2 MOUSE

The second murine tight skin mutation was a result of administration of the mutagenic agent, ethylnitrosourea [106]. This mutation has been localized to mouse chromosome 1, is inherited as an autosomal dominant trait, and only heterozygous (Tsk2/+) animals survive. Tsk2/+ mice develop a tight skin phenotype that becomes apparent at 3-4 weeks of age. Histologic examination of skin revealed marked accumulation of collagen similar to that observed in Tsk1/+ mice. However, in contrast to Tsk1/+ mice, a prominent mononuclear cell infiltration is present in the dermis and adipose tissue of Tsk2/+ mice. Biochemical analysis showed that Tsk2/+ skin had 50% more collagen than the normal mouse skin. Collagen synthesis in Tsk2/+ cultured dermal fibroblasts was 100% higher compared with normal fibroblasts. Transient transfection experiments with

1(I) collagen promoter constructs demonstrated increased transcriptional activity of the gene, and Sp1 and NF-1 transcriptional factors were involved in the upregulated transcriptional activity of 1(I) collagen promoter in Tsk2/+ fibroblasts [107]. Also, sequences from -96 to +16 bp of the 1(III) collagen promoter play an important role in the upregulated expression in Tsk2/+ fibroblasts [108].

SCLERODERMATOUS GRAFT-VERSUS-HOST DISEASE (SCL GVHD) MODEL

Patients who have hematologic malignancy and severe combined immunodeficiency treated with ionizing irradiation and heterologous bone marrow transplantation (typically from siblings or other donors matched for major HLA markers but not minor loci) sometimes develop chronic GvHD with skin and visceral fibrosis that resembles scleroderma, so-called Scl GvHD. Stastny *et al.* [109] generated chronic GvHD in rats and pointed out the similarities between homologous disease and scleroderma. Jaffee and Claman [110] studied murine chronic GvHD models, suggesting that they would be good models for human scleroderma. Only a few strain pairs can be used to generate Scl GvHD (donor LPJ and recipient C57BL; donor B10.D2 and recipient BALB/c). By contrast, most animals transplanted across minor histocompatibility loci develop cytotoxic GvHD with alopecia and injury to epithelia (skin, lung, gut, liver), leading to death. Therefore, murine GvHD resembles human disease in which most patients develop classic GvHD with dermatitis, diarrhea, and hepatitis rather than Scl GvHD with chronic fibrosis of the skin and lungs.

Scl GvHD was produced by transplanting B10.D2 bone marrow and spleen cells into BALB/c mice after lethal gamma irradiation of recipients [111]. Scl GvHD mice exhibited remarkable skin thickening and pulmonary fibrosis by 21 day after bone marrow transplantation, with significant increase of type I collagen mRNA expression and protein synthesis. In the lesional skin, a number of CD11b+2F8+ monocytes/macrophages and CD3+ T cells were seen. This form of GvHD may model early scleroderma. The immune cells infiltrating in the skin of Scl GvHD mice were of donor origin because Y chromosome-specific sequences from donor male mice were detected by PCR analysis of Y chromosome sequences in female recipients when female mice were transplanted with male cells. TGF- β 1 mRNA levels were upregulated in the lesional skin of Scl GvHD mice at early phases. Neutralization of TGF- β prevented fibrosis in the skin as well as in the lung [111]. In this model, expression of C-C chemokines including CCL2, CCL3/macrophage inflammatory protein-1 (MIP-1) and CCL5/RANTES was increased in the lesional skin before skin thickening and infiltration of CD45+ cells [112]. The same group recently showed that latency-associated peptide (LAP) prevented skin fibrosis as well as skin thickening in this model [113]. LAP is released from latent TGF- β . LAP treatment also abrogated the upregulation of mRNA levels of TGF- β and connective tissue growth factor (CTGF). LAP showed no suppressive effects on immune cell activation or recruitment into the skin, suggesting a more specific targeting of TGF- β .

Additionally, Scl GvHD can be produced by transplanting allogeneic (C57BL/6J) bone marrow and

spleen cells into lethally irradiated recipients (LP/J). Onset of GvHD starts at 7 days after transplantation, with epidermal injury and round cells infiltrating in the dermis and subcutis with mononuclear cell exocytosis. By day 14 after bone marrow transplantation, the dermis and subcutis of GvHD mice become sclerotic with compressed and atrophic pilosebaceous units. Extracutaneous involvement includes periportal mononuclear cell infiltrating liver and interstitial round cell influx to lungs and kidneys.

Very recently, a modified model of GvH-induced SSc has been developed [114]. Injection of spleen cells from B10.D2 mice into RAG-2 knockout mice on the BALB/c background induced dermal thickening, progressive fibrosis of internal organs and autoantibody generation. However, lung fibrosis was absent.

UCD CHICKEN

UCD-200/206 chickens spontaneously develop an inherited scleroderma-like disease showing the entire spectrum of SSc, such as vascular occlusion, severe perivascular lymphocytic infiltration in the skin and viscera, fibrosis of skin and internal organs, antinuclear antibodies, anti-cardiolipin antibodies, anti-endothelial cell antibodies, rheumatoid factor, and distal polyarthritis [115]. In hereditary avian scleroderma, lymphocytes in the deep dermis and subcutaneous tissues are enriched for γ , CD4+ T cells. The early acute stage proceeds to a chronic stage characterized by fibrosis with excessive accumulation of collagen type I, III and VI.

Of interest, endothelial cells are shown to be the primary target of the autoimmune attack, subsequently undergoing apoptosis in UCD-200/206 chickens [116, 117].

SKIN FIBROSIS BY EXOGENOUS INJECTION OF TGF- AND CTGF

TGF- injection into newborn mice caused granulation tissue formation and skin fibrosis [41]. Takehara's group showed that TGF- induced subcutaneous fibrosis and subsequent CTGF or basic fibroblast growth factor (bFGF) application caused persistent fibrosis [118, 119]. They suggest that TGF- plays an important role in inducing granulation and fibrotic tissue formation, and CTGF and bFGF are important in maintaining fibrosis [120].

CONCLUSION

Animal models are useful tools for the better understanding and exploring effective therapies for sclerotic conditions such as scleroderma. Further approaches including cellular and molecular investigations will be expected.

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