Anti-TGF-β Treatment Prevents Skin and Lung Fibrosis in Murine Sclerodermatous Graft-Versus-Host Disease: A Model for Human Scleroderma

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Scleroderma, a debilitating acquired connective tissue disease, is characterized by fibrosis, particularly of the skin and lungs. Monocyte-produced TGF-\( \beta \)1, a potent stimulus for collagen synthesis, is thought to drive the fibrosis. Here, we thoroughly characterize a murine sclerodermatous graft-vs-host disease (Scl GVHD) model for scleroderma that reproduces important features of scleroderma including skin thickening, lung fibrosis, and up-regulation of cutaneous collagen mRNA, which is preceded by monocyte infiltration and the up-regulation of cutaneous TGF-\( \beta \)1 mRNA. Most importantly, we can prevent fibrosis in both the skin and lungs of mice with Scl GVHD by inhibiting TGF-\( \beta \) with neutralizing Abs. The murine Scl GVHD model provides the unique opportunity to study basic immunologic mechanisms that drive fibrosing diseases and GVHD itself and will be useful for testing new therapies for these diseases. The Journal of Immunology, 1999, 163: 5693–5699.

Materials and Methods

Bone marrow transplantation

Seven- to eight-week-old female B10.D2 (H-2\( ^d \)) and BALB/c (H-2\( ^k \), Jackson Laboratory, Bar Harbor, ME) mice were utilized as donors and recipients, respectively, for BMT to produce Scl GVHD (10–12). Briefly, recipient mice were lethally irradiated with 700 cGy from a Gammacell 137Cs source. Approximately 6 h later they were injected i.v. by tail vein with donor spleen (2 \( \times 10^6 /\) mouse) and bone marrow cells (1 \( \times 10^6 /\) mouse) suspended in RPMI 1640 (BioWhittaker, Frederick, MD) with 10 U/ml heparin (Fisher Scientific, Pittsburgh, PA) (12). A control group of BALB/c recipient mice received BALB/c spleen and bone marrow cells (syngeneic BMT, referred to as control animals). Transplanted animals were maintained in sterile Micro-Isolator cages (Lab Products, Seaford, DE) and supplied with autoclaved food and acidified water. The dose of donor cells used in these experiments was determined from the literature (10–12), and from pilot experiments (data not shown) in which an increasing number of spleen cells produced no additional significant skin thickening.

Inhibition of fibrosis with Abs to TGF-\( \beta \)

A total of 150 \( \mu \)g of anti-pan TGF-\( \beta \) Abs (rabbit polyclonal IgG, Sigma, St. Louis, MO) or 150 \( \mu \)g of control rabbit IgG (Sigma) were administered by tail vein injection on day 1 and again on day 6 post-BMT. The dose was selected as a standard one used for other mouse models (13–15). Mice were sacrificed at day 21 as described below.

Collection of tissue

Three animals per group (experimental or control BMT) per time point were sacrificed via cervical dislocation at days 7, 14, 21, 38, 49, and 75.
post-BMT (36–50 animals per experiment). Back skin was depleted and harvested for RNA extraction (snap-frozen in liquid nitrogen), flow cytometry, immunostaining (frozen on dry ice), and routine histologic staining (fixed in 10% buffered formalin, Surgipath Medical Industries, Richmond, IL). Tongue and lung were also harvested, fixed in formalin, and embedded in paraffin for routine staining.

**Histological and morphometric analysis**

Formalin fixed, paraffin-embedded sections of tissue were stained by hematoxylin and eosin (Surgipath Medical Industries). Frozen skin was embedded in OCT embedding medium (Miles, Elkhart, IN) and sectioned by cryostat (Leica CM1800, Nussloch, Germany) for immunostaining (described below). For morphometric analysis, histological sections of lung or back skin were scanned with a CCD camera (Optronics, Goleta, CA) using an Axiosphot photomicroscope system (C. Zeiss, Oberkochen, Germany), stored as TIFF files, and subjected to image analysis (Optimas 6.1, Bothell, WA). Areas were calculated in arbitrary square units by outlining the dermis on a 10X view for each microscopic image, in which length was fixed and thickness varied, giving an average thickness for a broad area of skin. A minimum of eight measurements were taken from two or more skin sections from each animal. For lung tissue, a minimum of eight measurements were taken from two or more skin sections from each animal. For lung tissue, a minimum of eight measurements were taken from two or more skin sections from each animal.

**Antibodies**

**Immunostaining.** Anti-CD11b (anti-Mac-1, mAb M1/70, rat IgG2b, PharMingen, San Diego, CA) was used to identify monocyte/macrophages. Goat anti-rat IgG-horseradish peroxidase (Vector Laboratories, Burlingame, CA), followed by Streptavidin alkaline phosphatase (Kirkegaard & Perry Laboratories, Gaithersburg, MD) and diaminobenzidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD) were used for detection. Isotype control Abs were rat anti-IgG2b (R35-38, PharMingen).

**Flow cytometry.** PE-labeled mAb M1/70 (PharMingen) was used to detect mononuclear CD11b-positive cells in skin. FITC-labeled mAb to CD3e (145-2C11, Armenian Hamster IgG, PharMingen) was used to detect T cells in skin.

**Immunostaining**

Staining on frozen sections of skin was performed at least three times on each specimen by standard methods (16).

**Isolation of RNA from skin**

Total RNA was isolated from snap-frozen skin by guanidinium HCl method (17) and stored at −80°C until use in RT-PCR analysis and RNase protection assays.

**RT-PCR for TGF-β1**

Specific oligonucleotide primers for TGF-β1 or G3PDH (both from Clontech, Palo Alto, CA) were employed in RT-PCR reactions with total RNA from skin as previously described for analysis of other cutaneous cytokine mRNAs using Gene Amp 9600 PCR System (Perkin-Elmer, Norwalk, CT) (18). The cycle number of 30 was chosen so that the TGF-β1 and G3PDH signals were in the linear range on ethidium bromide-stained gels, which were photographed and acquired via GelDoc (Bio-Rad, Hercules, CA). The bands were then analyzed by image analysis using Optimas 6.1 software and the results expressed as the relative density for TGF-β1 following normalization for the RNA loading amount based on the G3PDH band.

**RNase protection assays to detect proα(I) collagen mRNA**

We prepared riboprobes from cDNAs internally labeled with digoxigenin UTP according to the manufacturer’s instructions (Genius II kit, Boehringer Mannheim, Indianapolis, IN). RNase protection assays with a riboprobe for proα(I) collagen (cDNA, a kind gift from E. Vuorio, University of Turku, Turku, Finland), (19) were performed according to the manufacturer’s instructions (RPAII kit, Ambion, Austin, TX) to assay and quantify collagen mRNA expression in mouse skin. Briefly, the gel-purified riboprobes were hybridized to mRNA prepared from skin and digested with RNase to remove nonhybridized sequences. The hybridization products were separated on a 5% nondenaturing polyacrylamide gel, electrophotochemically transferred to HybondN+ nylon membrane (Amersharm, Arlington Heights, IL), and detected with peroxidase-conjugated Abs to digoxigenin (Boehringer Mannheim) by chemiluminescence (Supersignal, Pierce, Rockford, IL). Images were obtained by exposure to x-ray film and the results analyzed by image analysis using Optimas 6.1 software. Collagen mRNA expression was normalized to a β actin or 28S RNA control (Ambion).

**Preparation of dermal cell (DC) suspensions for flow cytometry**

Small pieces of depleted skin were digested in RPMI containing 10 mM HEPES (Irvine Scientific, Santa Anna, CA), 0.01% DNase (Sigma), 0.27% collagenase (Sigma), and 1000 U of hyaluronidase (Sigma) at 37°C for 2 h (20). The digested dermis was filtered through nylon mesh to generate a single cell suspension of DCs containing resident cells (fibroblasts, endothelial cells, and perivascular cells) and infiltrating cells (lymphocytes and monocytes). The cells were stained with PE-labeled mAb for CD11b (PharMingen). After fixation with 1% paraformaldehyde in PBS, flow cytometry was performed with an Epics Elite Cytometer (Couler, Hialeah, CA) and the data were analyzed with Couler Elite software. Analysis of CD11b+ cells was performed with and without gating to exclude polymorphonuclear cells based on light scatter.

**Results**

**Scl GVHD in the model**

Lethally irradiated BALB/c mice transplanted via tail vein injection with bone marrow and spleen cells from B10.D2 mice develop skin thickening in the setting of mild histological and clinical changes of cutaneous GVHD (Scl GVHD). Animals that do not engraft die, and control BALB/c animals receiving syngeneic transplants do not develop GVHD or skin thickening. All engrafted animals show colony formation in the spleen, while the nonengrafted animals that died lacked spleen colony formation at autopsy. GVHD is clearly present in experimental animals, seen histologically as satellitosis and apoptotic keratinocytes in tongue epithelium and skin (data not shown). However, the numbers of dyskeratotic keratinocytes in epithelium, indicating severity of GVHD, are small (21). The form of GVHD is fibrotic, rather than cytotoxic. The experimental animals show no alopecia or diarrhea, but fail to gain weight, as expected, from 8 to 18 wk following BMT.

**Sclerodermatous cutaneous disease occurs in experimental animals by day 21**

Sclerodermatous thickening of skin is detectable by day 21 post-BMT by image analysis of routine histopathological sections (Fig. 2A), with an increase in total dermal area in animals with Scl GVHD of ~40% over the syngeneic BMT control animals (Fig. 2B). We have noted skin thickening as early as day 14, however 21 days is usually required to observe significant skin thickening in all transplanted animals. The variability in the earliest detectable skin thickening is most likely due to the success of the BMT and the quality of the spleen cell and bone marrow preparations. The skin thickening remains constant as late as 76 days post-BMT.
Cutaneous TGF-β1 mRNA is increased during early Scl GVHD

Increased TGF-β1 mRNA was observed by semiquantitative RT/PCR analysis of total RNA prepared from whole mouse skin (Fig. 3). Approximately 3-fold more TGF-β1 mRNA is seen in the skin of experimental compared with control animals as early as 6 days post-BMT. On day 21 post-BMT, TGF-β1 is at least 2-fold higher in animals with Scl GVHD than in syngeneic BMT control animals. Each lane represents data from one animal and is representative of four experiments with two or three animals per group for each experiment. Therefore, increased TGF-β1 in skin precedes skin thickening, as predicted by the hypothesis.

Collagen mRNA is up-regulated in skin in early Scl GVHD

We used an RNase protection assay to quantify collagen mRNA production in the skin of mice with Scl GVHD. Up to 15-fold increased collagen mRNA synthesis is seen in the skin of experimental animals with Scl GVHD (E) compared with control (C) animals on day 38 post-BMT (Fig. 4). The increased message is detectable on day 21 and returns to near control levels by day 75. Therefore, increased collagen mRNA synthesis correlates with increased dermal fibrosis. A digoxigenin-labeled β-actin riboprobe added to each hybridization solution served as an internal standard for the amount of RNA in the hybridization reaction and the amount loaded on the gel. All collagen densities were normalized to the β-actin signal. For the representative day 38 ribonuclease protection assay (RPA) gel lanes shown, the β-actin signals could not be equalized due to the extreme up-regulation of the collagen mRNA signal. The calculation of 15-fold up-regulation takes into account the differing β-actin signals. Similar results were obtained using a 28S ribosomal RNA probe.

Because others have shown that up-regulation of collagen synthesis and skin fibrosis in scleroderma is accompanied by mononuclear cell infiltrates (22), we hypothesized that monocytes infiltrating skin at early time points could be the effector cells driving cutaneous fibrosis in our model. Activated by donor T cells, these
monocytes could be the source of increased fibrogenic TGF-β1 in skin (Fig. 1).

**CD11b<sup>+</sup> mononuclear cells in cutaneous infiltrates in Scl GVHD**

We performed immunostaining of frozen sections of skin, and showed prominent infiltration of CD11b<sup>+</sup> mononuclear cells (brown-staining) by day 21 in the skin of animals with Scl GVHD compared with syngeneic BMT controls (Fig. 5A). There are a few infiltrating CD11b<sup>+</sup> cells by day 7, they are markedly increased by day 14, and prominent by day 21. Isotype control Ab immunostaining is negative. The examples shown are representative of three experiments. The infiltrates are especially dense in deep dermis, where fibrosis typically occurs first in human scleroderma.

To quantify the cells infiltrating skin in early Scl GVHD, we performed flow cytometry on DC suspensions isolated from the skin of animals with Scl GVHD and syngeneic BMT control animals on day 21 post-BMT (Fig. 5B). The results correlate with immunostaining, showing increased CD11b<sup>+</sup> cells infiltrating the skin of experimental animals with Scl GVHD (E) but not control animals (C) on day 21. The scatter plots shown are representative of three experiments. The CD11b<sup>+</sup> cells present were mononuclear cells, not polymorphonuclear neutrophils (PMN), which also stain for CD11b. Histological analysis has also confirmed that there are very few PMN infiltrating the skin of Scl GVHD mice.

The remainder of cells in the dermal suspensions used for flow cytometry studies are T cells, resident dendritic cells, fibroblasts, endothelial cells, follicular and sebaceous epithelial cells. To evaluate the T cell component in Scl GVHD, we stained the DC suspensions isolated from the skin of animals with Scl GVHD at days 7, 14, and 21 post-BMT. The scatter plots shown are representative of three experiments. CD11b<sup>+</sup> cells present were mononuclear cells, not polymorphonuclear neutrophils (PMN), which also stain for CD11b. Histological analysis has also confirmed that there are very few PMN infiltrating the skin of Scl GVHD mice.

**Inhibition of skin fibrosis with anti-TGF-β Abs**

Because TGF-β1, a fibrogenic cytokine (23), appears to play a central role in the development of scleroderma and Scl GVHD, we next asked if Abs to TGF-β could prevent skin fibrosis. Anti-pan-TGF-β Ab (polyclonal rabbit IgG) was administered to animals at days 1 and 6 post-BMT followed by sacrifice on day 21. Day 21 post-BMT was chosen for sacrifice because it is the time point by which all parameters of Scl GVHD are demonstrable, including skin thickening and up-regulated TGF-β1 and collagen mRNA. This dose is also within the range of normal for Ab inhibition of TGF-β in other mouse models (13–15). The anti-TGF-β1 Ab treatment did not prevent successful BMT, as evidenced by colonization of the spleen in both control and experimental animals, however, the blocking Ab prevented the skin thickening seen in murine Scl GVHD (Fig. 6, A–D). Untreated syngeneic BMT control animal skin is shown in Fig. 6A. Skin thickening accompanied by prominent mononuclear cell infiltrates in untreated animals with Scl GVHD is shown in Fig. 6B. Anti-TGF-β Ab treatment prevented skin thickening in these experimental animals (Fig. 6C and Fig. 7). Anti-TGF-β treatment of syngeneic BMT control animals had no effect on skin thickness (Fig. 6D). Therefore, the administration of an antagonist to the fibrogenic cytokine TGF-β can prevent the cutaneous fibrosing process in early Scl GVHD, presumably by blocking TGF-β.

**Pulmonary fibrosis in Scl GVHD is inhibited by anti-TGF-β Abs**

Because pulmonary fibrosis is a major cause of morbidity and mortality in scleroderma, we next asked if our model would be useful for the study of lung as well as skin fibrosis. Animals with Scl GVHD (Fig. 6F), but not control animals (Fig. 6E), show loss of the normal lacy alveolar pattern of lungs by day 21 post-BMT, with the decrease in alveolar space in Scl GVHD animals being approximately 30% (Fig. 8). Lung fibrosis has not been previously reported for this murine model. In the inhibition experiment, Abs

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**Table 1. Time course of events in Scl GVHD**

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<th>Analysis</th>
<th>Days After Bone Marrow Transplantation</th>
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<tr>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt; cells in skin</td>
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<td>TGF-β1 mRNA</td>
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<td>Collagen mRNA</td>
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* –, Low or absent; +, increased; ++++, markedly increased.

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**FIGURE 5.** Infiltration of skin of animals with Scl GVHD by CD11b<sup>+</sup> mononuclear cells (brown-staining cells) by day 14 after BMT. The upper panel of micrographs (A) shows immunostaining results on acetone-fixed frozen skin sections from animals with Scl GVHD at days 7, 14, and 21 post-BMT. The low panel shows immunostaining results on skin from control animals (all at 40× magnification; scale bar = 43 μm). B. Flow cytometric analysis of single cell preparations from whole back skin demonstrates the influx of cutaneous CD11b<sup>+</sup> mononuclear cells in Scl GVHD on day 21 post-BMT. Scatter plots show CD11b<sup>+</sup> cells as a percentage of total DCs in each experimental condition. C, control syngeneic BMT animals; and E, animals with Scl GVHD.
to TGF-β prevented lung fibrosis as well (Fig. 6G, plotted in Fig. 8). Anti-TGF-β treatment had no effect on the percentage of alveolar space in syngeneic BMT control animals (Fig. 6H). The treatment of Scl GVHD and control animals with nonspecific rabbit IgG also had no effect on skin or lung fibrosis (data not shown).

**Discussion**

**Validity of the murine Scl GVHD model for scleroderma**

We have demonstrated that the Scl GVHD model faithfully reproduces the skin fibrosis as well as the up-regulation of TGF-β1 and collagen mRNA synthesis that occurs in human scleroderma. Furthermore, we have established the following temporal sequence of these events: TGF-β1 mRNA up-regulation and CD11b+ mononuclear cell infiltration are seen by day 7 post-BMT and precede detectable increases in collagen mRNA and skin thickening (summarized in Table I, a composite of data from several experiments).

Scl GVHD was first presented as a model for scleroderma by Jaffe and Claman (10), Claman et al. (11), and Korngold and Sprent (12), but until now, the cellular changes and the molecular events occurring in the model have not been thoroughly characterized. We have established a time course for skin fibrosis in this murine Scl GVHD model and characterized molecular events that make this model extremely useful for testing therapies for autoimmune fibrosing disorders such as scleroderma and GVHD. Until the present, our understanding of the pathophysiology of scleroderma and consequently our ability to treat this chronic progressive disease have been limited by the lack of a suitable animal model. Scleroderma-like disease occurs in the University of California at Davis autoimmune chicken (reviewed by Van De Water et al., Ref. 3), and the existing murine models (Tight Skin Mouse, Tsk; and Tsk2 mice) show some, but not all features of human scleroderma (3, 24–26). Tsk animals have mutations in the regulation of DNA-binding proteins for collagen and matrix protein expression (27–29), and do not exhibit the vascular or pulmonary changes of human scleroderma, although they do exhibit skin thickening (3) and autoantibodies (30). The lung pathology is described as emphysematous, rather than fibrotic. Tsk2 animals display cutaneous fibrosis and a mononuclear cell infiltration in skin; however, TGF-β expression is not related to fibrosis as it is in scleroderma (26, 31). These models are useful for studying fibrosis, but their value for analyzing the complex immunologic dysregulation in scleroderma is limited.

In contrast, the murine Scl GVHD model (B10.D2 > BALB/c) that we have characterized in the present study faithfully reproduces the most important features of human scleroderma, including skin thickening, mononuclear cell infiltrates, lung fibrosis, and the percentage of alveolar space was determined by image analysis of hematoxylin and eosin-stained tissue sections using Optimas software as described in Materials and Methods.

**FIGURE 6.** Routine histology of skin (A–D) and lung (E–H) on day 21 post-BMT showing inhibition of fibrosis by Abs to TGF-β in animals with Scl GVHD. Anti-TGF-β was administered on days 1 and 6 post-BMT at a concentration of 150 μg/mouse. A and E, control BALB/c animals (transplanted with syngeneic BALB/c bone marrow and spleen cells). B and F, untreated experimental BALB/c animals with Scl GVHD (transplanted with B10.D2 bone marrow and spleen cells). C and G, experimental animals with Scl GVHD that were treated with anti-TGF-β Abs. D and H, control animals that were treated with anti-TGF-β Abs. (Scale bars: skin = 167 μm; lung = 83.5 μm).

**FIGURE 7.** Inhibition of skin thickening by anti-TGF-β Abs. Bars represent the mean and SD of the percentage decrease in alveolar space of (n) animals with Scl GVHD (untreated or anti-TGF-β treated) as compared with control animals (three animals) from one representative experiment (of three). p = 0.001 when using a paired t test to compare Scl GVHD animal (E) back skin thickness to control animal (C) back skin thickness; p = 0.0006 when experimental Scl GVHD animal back skin thickness and the back skin thickness of anti-TGF-β-treated experimental animals are compared. Skin thickness was determined by image analysis of hematoxylin and eosin-stained tissue sections using Optimas software as described in Materials and Methods.

**FIGURE 8.** Inhibition of lung fibrosis by anti-TGF-β Abs. Bars represent the mean and SD of the percentage of decrease in alveolar space of (n) animals with Scl GVHD (untreated or anti-TGF-β treated) as compared with control animals (three animals) from one representative experiment (of three). p < 0.0003 when using a paired t test to compare Scl GVHD animal (E) alveolar space with control (C) animal alveolar space. Paired t test analysis revealed that there was no significant difference between the Scl GVHD animals treated with anti-TGF-β Ab and the syngeneic control BMT animals indicating that the Ab treatment prevented lung fibrosis. The percentage of alveolar space was determined by image analysis of hematoxylin and eosin-stained tissue sections using Optimas software as described in Materials and Methods.
up-regulation of cutaneous collagen and TGF-β1 mRNA. Furthermore, the similarities between scleroderma and Scl GVHD, as well as the recent implication of microchimerism as a trigger for scleroderma, make the murine Scl GVHD model particularly appropriate for study. This model provides a system that can be manipulated to determine important variables in disease progression, and will allow us to test newly emerging therapeutic modalities at all stages of the disease, including the early stages when they may be more effective.

Central role of TGF-β1 in Scl GVHD: proof of the hypothesis

mRNA for the cytokine TGF-β1 is clearly up-regulated at early time points in animals with Scl GVHD. TGF-β1, a potent stimulus for increased collagen synthesis, is thought to be critical in the cutaneous and pulmonary fibrosis of scleroderma (32, 33), as well as in other models of fibrosis (34–38). TGF-β is made by multiple cell types, including monocytes, fibroblasts, and endothelial cells (39, 40), and can be converted to the active form by monocyte/macrophages (41). The three isoforms of human TGF-β (TGF-β1, TGF-β2, and TGF-β3) can be readily distinguished (42). These isoforms have pleuripotential effects on not only extracellular matrix homeostasis (32), but also on immune regulation (40, 43) and on epithelial growth (44). In human scleroderma, TGF-β1 is thought to be the critical isoform implicated in cutaneous and pulmonary fibrosis (45–47).

We have shown that anti-TGF-β Abs prevent the progression of skin and lung fibrosis in Scl GVHD at day 21, presumably by blocking TGF-β; however, we have not tested the effects of the anti-TGF-β Ab on other cytokines and the effect may be an indirect one. Because our Ab is polyclonal and inhibits all three isoforms of TGF-β, we cannot make any conclusions about the role of the individual isoforms in murine Scl GVHD. The striking results of the Ab inhibition studies suggest the need for further study of these TGF-β isoforms. Furthermore, anti-TGF-β therapy with a humanized mAb may be useful in preventing disease progression in human Scl GVHD and scleroderma. In addition to anti-TGF-β, experiments with Abs to other cytokines or to macrophage and T cell surface markers will be useful to further characterize the disease process in Scl GVHD and to identify other candidate molecules for immunotherapy.

Influence of genetic background on type, clinical manifestations, and effector cells of GVHD

Asai et al. (48) describe successful prevention of cytoxic GVHD due to transplantation across major histocompatibility loci using blocking Abs to TGF-β. The paradoxical results (TGF-β produced by transplanted donor NK cells protected against GVHD in this model) point out the importance of the genetic backgrounds of donor and recipient individuals in such transplantations, and the diversity of GVHD-like reactions. Several quite different models of murine GVHD exist (reviewed in Refs. 49 and 50). They include transplantation from parental to nonirradiated F1 hybrid offspring (P > F1), in which autoimmune features like those in lupus erythematosus are commonly seen; transplantation across major histocompatibility loci, which is often rapidly lethal; and transplantation across minor histocompatibility loci, which most closely approximates in severity and course allogeneic sibling bone marrow transplantation in humans. In this last type of GVHD, somewhat different forms of disease can be produced experimentally in mice in carefully controlled depletion experiments by selecting for different cell types including the following: cytotokcy CD8 T cells, CD4 T cells, and NK cells (49, 51–54). Therefore, the identity of effector cells may vary with the type of GVHD. Murine Scl GVHD is a subset of the last type of GVHD, exhibited by only a few transplantation pairs. It is not surprising, therefore, that different effector cells (presumably monocytes synthesizing TGF-β1, rather than cytotoxic T cells attacking epithelium) operate in this distinct fibrosing form of GVHD. The activation of host or donor monocytes by donor T cells is incompletely understood in this model, and will be an important parameter for examination in future experiments. Recently, Schlomchik et al. (55) determined that host APCs were required for the initiation of GVHD in a murine MHC-mismatch BMT model. Our model is also ideal for testing the involvement of host and donor APCs and T cells and the effects of chimerism in the development of Scl GVHD in an MHC-compatible BMT. Analysis of the diversity of murine GVHD-like reactions may be helpful in understanding the variability in type, development, and progression of human GVHD, and in understanding autoimmune disease itself.

In summary, we have shown that murine Scl GVHD faithfully reproduces the skin and lung fibrosis and up-regulation of cutaneous collagen and TGF-β1 mRNA that occurs in human scleroderma. Cutaneous CD11b+ mononuclear cell infiltrates and increased TGF-β1 and collagen mRNA precede dermal fibrosis and thickening, and the progression of early skin and lung disease can be inhibited with Abs to TGF-β. Analysis of the diversity of murine GVHD-like reactions may be helpful in understanding the variability in type, development, and progression of human GVHD, and in understanding a complex autoimmune disease such as scleroderma. Most importantly, murine Scl GVHD is a useful model for testing potential interventions for scleroderma and GVHD.

Note. After submission of our manuscript it was reported that Abs to TGF-β (the same R&D Ab (R&D Systems, Minneapolis, MN) used in our experiment) could reduce cutaneous sclerosis in a mouse model of bleomycin-induced scleroderma (56).

Acknowledgments

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