Evidence for early fibrosis and increased airway resistance in bone marrow transplant recipient mice deficient in MMP12

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Evidence for early fibrosis and increased airway resistance in bone marrow transplant recipient mice deficient in MMP12. Am J Physiol Lung Cell Mol Physiol 301: L519–L526, 2011. First published July 22, 2011; doi:10.1152/ajplung.00383.2009.—Idiopathic pneumonia syndrome (IPS) is a significant cause of morbidity and mortality post-bone marrow transplantation (BMT) in humans. In our established murine IPS model in which lethally conditioned recipients are given allogeneic bone marrow and splenocytes, recruitment of host monocytes occurs early post-BMT, followed by donor T cells concomitant with development of severe lung dysfunction. Because matrix metalloproteinase 12 (MMP12) is important for macrophage infiltration and injury in other mouse models of lung disease such as emphysema, lethally conditioned MMP12−/− mice were used as allogeneic recipients to determine whether MMP12 plays a similar role in potentiating lung injury in IPS. Surprisingly, MMP12−/− mice developed IPS and exhibited an accelerated allogeneic T cell-dependent decrease in compliance compared with wild-type (WT) recipients. MMP12−/−, but not WT, mice also had allogeneic T cell-dependent elevated lung resistance post-BMT. Recruitment of monocytes and T cells into the lungs was not altered on day 7 post-BMT, but the lungs of MMP12−/− recipients had increased collagen deposition, a feature normally not seen in our IPS model. MMP12−/− mice had a compensatory increase in MMP2 in the lungs post-BMT, as well as increased β6-integrin compared with WT recipients, and only in the presence of allogeneic T cells. Levels of total transforming growth factor (TGF)-β1 protein in the lungs were elevated compared with WT recipients, consistent with the profibrotic function of β6-integrin as an activator of TGF-β. These data indicate that host-derived MMP12 may be important in limiting development of IPS by allowing proper remodeling of extracellular matrix and effective repair of BMT-related injury.

idiopathic pneumonia syndrome; matrix metalloproteinase 12

IDIOPATHIC PNEUMONIA SYNDROME (IPS) remains a major complication after bone marrow transplantation (BMT) and a significant cause of mortality (5). Increased risk of developing IPS is related to the intensity of the conditioning regimen used, the degree of alloreactivity of the donor graft, and the severity of graft-vs.-host disease (GVHD) (9, 44). Our murine IPS model is characterized by the influx of host monocytes and donor T cells into the lungs early postallogeneic BMT of lethally irradiated mice (28). The severity of IPS and the heightening of host macrophage activation in the lung in the early post-BMT period is dependent on allogeneic donor T cells and is enhanced by preconditioning with cyclophosphamide (Cy). The manifestations of lung dysfunction in our murine model include reduced specific compliance, decreased lung capacity, increased wet and dry lung weights, an influx of host monocytes and allogeneic T cells, increases in inflammatory mediators, and markers of oxidative stress (11, 27, 28).

The matrix metalloproteinases (MMPs) consist of 26 known human zinc proteases with essential roles in degrading components of the extracellular matrix (ECM) (25, 33, 38, 39, 42). MMPs are a family of secreted metalloendopeptidases that require zinc for catalytic activity and are inhibited endogenously by tissue inhibitors of metalloproteinases (4, 24). Macrophage metalloelastase (MME or MMP12) is characterized by its ability to hydrolyse numerous substrates and its macrophage-specific expression (34, 35). It was previously reported that lack of MMP12 in mice abrogates the development of smoke-induced emphysema by a mechanism involving failed recruitment of macrophages and lack of macrophage proteolytic activity (12). In recent murine studies, MMP12 inhibitors have been shown to reduce both the inflammatory process and airspace enlargement in lung tissue in chronic obstructive pulmonary disease (16, 18).

The role of MMP12 in the development and progression of IPS after allogeneic BMT is not known. We found that many of the inflammatory cells in the lungs of IPS mice expressed MMP12 after allogeneic BMT. Because host monocytes are the first inflammatory cell type to enter the lungs post-BMT, we reasoned that MMP12 might be necessary for the monocyte recruitment into the lungs and a critical mediator of IPS injury. With the use of MMP12−/− mice as recipients, our data indicate that, contrary to our hypothesis, deficiency of MMP12 does not hinder the development of IPS. Furthermore, unlike wild-type (WT) mice, MMP12−/− mice with IPS also had evidence of fibrosis and increased resistance associated with increased expression of integrin-β6, a potent activator of transforming growth factor (TGF)-β.

MATERIALS AND METHODS

Mice. C57BL/6 (termed B6; H2b) mice were purchased from the National Institutes of Health (Bethesda, MD). B10.BR (H2b) mice were purchased from Jackson Laboratories (Bar Harbor, ME). MMP12−/− mice backcrossed onto the B6 background (>10 generations) were bred from breeders provided by Dr. S. D. Shapiro (37). Mice were housed in microisolator cages in a specific pathogen-free facility at the University of Minnesota and cared for according to the Research Animal Resources guidelines of our institution. Experiments were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. Sentinel mice were found to be negative for infectious microorganisms known to cause pulmonary effects.
pathology such as pneumonia virus, K virus, and Sendai. For BMT, female donors were 8–12 wk of age and male recipients were used at 8–10 wk of age.

**BMT protocol.** Our BMT protocol has been described previously (28). B6 WT or MMP12–/– mice received Cy (Cytosan; Bristol Myers Squibb, Seattle, WA), 120 mg/kg per day intraperitoneally, as a conditioning regimen pre-BMT on days −3 and −2. All mice were lethally irradiated on the day before BMT (7.5 Gy total body irradiation) by X-ray at a dose rate of 0.41 Gy/min. Donor B10.BR bone marrow was T cell depleted (TCD) with anti-Thy.1 2 monoclonal antibody (MAb; clone 30-H-12, rat IgG2b; kindly provided by Dr. David Sachs, Charlestown, MA) plus complement (Nienfenege, Woodland, CA). B6 WT or MMP12–/– recipient mice were transplanted via caudal vein with 15 × 10^6 TCD B10.BR marrow with or without 15 × 10^6 spleen cells as a source of IPS-causing T cells.

**Lung hydroxyproline levels.** Mice were euthanized with pentobarbital sodium, and the thoracic cavity was partially dissected. Lungs were exsanguinated by perfusion with 1.0 ml of saline via the right common carotid artery. The lungs were inflated with 1.5 ml of saline via the right atrial vent using a ventilator. The maximum pressure was set at 30 cmH2O for P-V curves of air and liquid-filled lungs were determined as previously described using the Flexivent system (Scireq, Montreal, QC, Canada) (29). After intubation, the lungs were ventilated at 20 breaths/min, tidal volume of 0.15 ml, and end expiratory pressure remained constant around 2.5 cmH2O. Five 15-second BAL were performed on each mouse: snapshot-150, prime-8, prime-3, P-V, and TLC.

**Bronchoalveolar lavage.** The trachea was cannulated with a 19-gauge needle and infused with 0.5 ml of PBS, and the fluid was withdrawn. This was repeated twice, and a total of 1.5 ml of bronchoalveolar lavage fluid (BALF) was collected per mouse, centrifuged (1,000 g) at 4°C for 10 min to pellet the cells, and stored at −80°C.

**Lung protein extracts.** At the time of euthanasia, after exsanguination and BAL, the left lung was homogenized in 1 ml of PBS containing protease inhibitor cocktail (Roche, Indianapolis, IN) and centrifuged at 3,000 revolution/min for 10 min. The supernatant was filtered through a 1.2-μm syringe filter and stored at −80°C until assayed.

**Cheomokin/cytokine level determination.** BAL and serum levels of T cell/macrophage cytokines IL-2, IL-4, IL-5, IL-12p70 and VEGF; monocyte/macrophage attractants granulocyte/macrophage colony-stimulating factor and CCL2 (monocyte chemoattractant protein-1/ JE); predominant T cell attractants CCL3 [macrophage inflammatory protein (MIP)-1α], CCL4 (MIP-1β), and CXCL10 (IP-10); neutrophil attractants CXCL1 (KC) and CXCL2 (MIP-2); proinflammatory Th1-type cytokines IFN-γ, TNF-α, IL-1β, and IL-6; and anti-inflammatory Th2-type cytokines IL-13 and IL-10 were determined by Luminex method using mouse-specific kits (R&D Systems, Minneapolis, MN; sensitivity 1.5–3.0 pg/ml), and results were interpolated from standard curves of the relevant recombinant proteins (R&D Systems).

**Frozen tissue preparation.** A mixture of 0.5 ml of optimal cutting temperature compound (OCT; Miles, Elkhart, IN):PBS (3:1) was infused via the trachea into the lungs. Lung tissue was embedded in OCT, frozen in liquid nitrogen, and stored at −80°C.

**Histochernistry.** For general morphology, cryosections (6 μm) were fixed in acetone and stained with hematoxylin/eosin. For collagen staining, Masson’s trichrome staining kit (HT15) was purchased from Sigma-Aldrich (St. Louis, MO). Briefly, nuclei are stained with Weigert’s iron hematoxylin, and cytoplasm and muscle are then stained with Beirich scarlet-acid fuchsin. After treatment with phosphotungstic and phosphomolybdic acid, collagen is demonstrated by staining with aniline blue. Accustain Elastin staining kit (HT25) was purchased from Sigma-Aldrich. Tissue sections are overstained in hematoxylin-iodine-ferric chloride solution in a dye-lake reaction, which stains elastin black. Van Gieson solution is used as a counterstain to stain collagen fibers red and other components yellow.

**Immunohistochemistry.** Cryosections (6 μm) were fixed in acetone and immunoperoxidase-stained using biotinylated mAbs with avidin-biotin blocking reagents, avidin-biotin complex peroxidase conjugate, and diaminobenzidine chromogen purchased from Vector Laboratories (Burlingame, CA). The biotinylated mAbs were used for the following markers: CD4, CD8, Gr-1, and M170, all purchased from BD Pharmingen (San Diego, CA). Stained lung sections were counterstained with methyl green and examined under ×200 and ×400 magnification in coded fashion and photographed using an RT Spot camera mounted on an Olympus BX51 microscope (Olympus, Hamburg, Germany).

**Immunofluorescence.** Cryosections (6 μm) were fixed in acetone and incubated with rabbit-anti-mouse β6-integrin antibody (Santa Cruz Biotechnology, Santa Cruz, CA) with Cy3-labeled donkey-anti-rabbit secondary (Jackson ImmunoResearch, West Grove, PA). Sections were mounted in Vectashield containing DAPI (Vector Laboratories) and imaged by confocal microscopy (Olympus FluoView).

**In situ hybridization.** Cryosections (6 μm) were hybridized with digoxigenin-labeled antisense RNA probes corresponding to the murine MMP12 sequence 1–318 bp. Immunological detection of digoxigenin-labeled RNA duplexes was accomplished with antidigoxigenin antibody (alkaline-phosphatase conjugated; Roche) and detected with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate complex. Stained tissue was analyzed on an Olympus BX50 WI microscope with a Spot RT camera (Diagnostic Instruments, Sterling Heights, MI) for capturing images.

**MMP assay.** MMP2 and MMP9 levels in BALF on day 7 post-BMT were determined by Luminex method using the MMP2 bead sets of the human MMP MultiAnalyte Profiling Kit (R&D Systems) that cross-react with mouse MMP2.

**Quantitative real-time RT-PCR (qRT-PCR).** Total RNA was extracted from total lung tissue with Trizol (Invitrogen, Carlsbad, CA) and cDNA was generated using 1 μg DNase-treated RNA with oligo-dT primers and TermoScript polymerase (Invitrogen). PCR was performed by using premade primers/probes (GAPDH Mm9999991_g1, β1-integrin Mm01306375_m1, β6-integrin Mm00434375_m1, TGF-β1 Mm01178820_m1 all from Applied Biosystems, Foster City, CA) with TaqMan Real-Time PCR premix and run on an Applied Biosystems 7500 real-time PCR System. PCR conditions were standard for the Applied Biosystems premade probe sets: 95°C for 15 s; 60°C for 60 s; 72°C for 2 min; 40 cycles. Data were analyzed by ANOVA or Student’s t-test. P values ≤ 0.05 were considered statistically significant.
RESULTS

Deficiency of host MMP12 does not affect inflammatory response in mice with IPS. In our initial description of murine IPS post-BMT (28), lung injury was highly correlated with increased host monocyte and donor T inflammatory cell influx. To determine whether MMP12 might be necessary for the monocyte recruitment, and a critical mediator of IPS injury following BMT, lethally conditioned WT or MMP12−/− recipient mice were transplanted with allogeneic B10. BR marrow with splenocytes.

Contrary to our expectations, deficiency of MMP12 in recipient mice did not affect perivascular or alveolar inflammation compared with WT on day 7 post-BMT [Fig. 1; allogeneic bone marrow (BM) and splenocytes (BMS) panels]. In situ hybridization analysis of cryosections of lungs taken on day 7 post-BMT confirmed the presence of MMP12+ cells in both the WT and MMP12−/− recipients (Fig. 1, bottom). Therefore, it appears that both host and donor-derived cells contribute to the production of MMP12 in the post-BMT lung, at least in an MMP12-deficient host. Lung sections were stained for various...
cell type markers on day 7 following post-BMT. Positive cells were counted and quantified as a percent of nucleated cells as we previously described (28). There was no significant difference in the numbers of CD4+, CD8-, or Gr-1-positive cells between the WT or MMP12+/− recipients receiving BMS on day 7 post-BMT (WT vs. MMP12+/−: CD4: 7.6 ± 2.0% vs. 10.2 ± 2.0%; CD8: 2.2 ± 0.3% vs. 2.6 ± 0.8%; Gr-1: 0.4 ± 0.4% vs. 0.5 ± 0.5%; n = 6/group). There was also no significant difference in Mac-1 (M170)-positive cells (16.1 ± 4.7% vs. 18.5 ± 9.1%), which is in agreement with lung fibrosis in other MMP12+/− model systems (22). We also found no significant difference in the frequencies of cells expressing donor or host major histocompatibility complex class II (Host I-Aβ: 11.4 ± 2.9% vs. 14.4 ± 0.6%; Donor I-Aβ: 2.3 ± 0.2% vs. 2.1 ± 0.6%).

Analysis of a large panel of inflammatory cytokines and chemokines (see MATERIALS AND METHODS) in lung protein extracts and BAL fluid on days 3 and 7 post-BMT revealed no statistical differences between MMP12+/− mice and WT recipients.

Post-BMT lung dysfunction is accelerated in MMP12+/− mice. We have previously demonstrated that decreased compliance and TLC are part of the IPS manifestation in our model (28). Lung function tests showed that the allogeneic T cell-dependent decrease in compliance and TLC was accelerated in MMP12+/− mice (Fig. 2). Kinetic analysis of the same mice over time showed that MMP12+/− mice had decreased lung compliance and increased elasticity at the time of transplant, i.e., as a result of conditioning. However, this returned to normal levels by day 3 post-BMT in MMP12+/− mice receiving only allogeneic BM (i.e., no splenocytes), indicating that the subsequent decline in lung function is dependent on allogeneic T cells. TLC was also statistically decreased compared with BM controls earlier in MMP-12+/− recipients. By day 7 post-BMT, both the MMP12+/− and WT recipients had decreased specific compliance and TLC to the same degree. Interestingly, MMP12+/− but not WT recipients of BMS, had a T cell-dependent increase in lung resistance as early as day 3 post-BMT. This is a feature normally not seen in our acute IPS model but is seen in our chronic model, where mice develop lung fibrosis and obliterative bronchiolitis (29).

MMP12+/− mice have increased deposition of collagen in the lungs post-BMT. To further elucidate the mechanism behind the reduction in lung compliance and because compliance is inversely correlated to collagen levels, collagen content was evaluated in the lungs post-BMT. Collagen levels as measured by hydroxyproline were significantly increased in MMP12+/− lungs on day 7 post-BMT compared with the B6 WT recipients (Fig. 3A). Therefore, host-derived MMP12 may be important in downregulating IPS by allowing proper remodeling of ECM and effective repair of BMT-related injury. Masson’s trichrome staining of cryosections of mouse lungs for collagen showed that, on day 7 post-BMT, lungs of MMP12+/− recipients had evidence of increased collagen deposition (Fig. 3C), a feature normally not seen in our IPS model with WT mice. Because collagen production can be induced by TGF-β signaling, levels of TGF-β in lung protein extracts were measured. Total (free + latent) TGF-β levels were elevated in MMP12+/− recipients (Fig. 3B) and correlated with OH-proline levels (Fig. 3A). Interestingly, the levels of free TGF-β were similar in all groups (not shown), indicating that any signaling by TGF-β may be via a mechanism that does not release free TGF-β.

![Fig. 2. MMP12+/− recipients of BMS exhibit accelerated lung dysfunction compared with WT B6 recipients. MMP12+/− mice also have decreased lung compliance and increased elastance on day 0 in response to pre-BMT conditioning. BM, bone marrow; MME, macrophage metalloelastase (i.e., MMP12). Results shown are representative of 3 experiments (n = 8–24 per group). A: *P < 0.05 for MMP12+/− BMS vs. all other groups. B: #P < 0.05 for MMP12+/− BMS vs. MMP12+/− and B6 BM control and #P < 0.05 for B6 BMS vs. B6 BM control. C and D: +P < 0.05 for MMP12+/− groups vs. B6 groups, *P < 0.05 for MMP12+/− BMS vs. all other groups, #P < 0.05 for BMS groups vs. their respective BM controls. Standard deviations ranged from 4.9 to 15.2% of values.](http://ajplung.physiology.org/)
Elevated levels of MMP2 in MMP12−/− mice with IPS. To elucidate the reasons behind the accelerated lung dysfunction and increased deposition (or lack of degradation) of collagen in the lungs post-BMT, the levels of MMP2 and MMP9 were evaluated. BAL fluids from day 7 post-BMT MMP12−/− mice were compared with B6 WT recipients. No difference in MMP9 levels was found (not shown), but, as seen in Fig. 3D, MMP2 was significantly increased in the BAL fluid in MMP12−/− mice on day 7 post-BMT. These data therefore show that, despite a compensatory increase in MMP2 in the lungs of MMP12−/− recipient mice with IPS, it is not sufficient to contain the increased collagen deposition. It appears that either host-derived MMP12 is required to limit ECM deposition in post-BMT lungs, or the compensatory increase in MMP2 is associated with post-BMT lung fibrosis.

Increased expression of β6-integrin in MMP12−/− mice post-BMT. Because latent TGF-β can be activated by αβ6-integrin leading to upregulation of collagen (41), without releasing free TGF-β, β6-integrin expression was evaluated in the lungs on day 7 post-BMT. As shown in Fig. 4A, β6-integrin gene expression was dramatically increased in the lungs of MMP12−/− recipients of BMS. This was confirmed by Western blot analysis (Fig. 4B). As a control, no changes were seen in expression of β1-integrin, which is constitutively expressed (not shown). Immunofluorescence staining for β6-integrin (Fig. 4C) showed dramatic expression of this integrin in the bronchiolar epithelium and to a lesser extent in the distal alveolar regions. Thus activation of TGF-β by β6-integrin may play an important role in signaling collagen production and the development of fibrosis and increased resistance in the lungs of MMP12−/− mice post-BMT.

DISCUSSION

In this article, we have demonstrated that, after allogeneic BMT, IPS was not prevented by deficiency of MMP12 in recipients. MMP12−/− mice exhibited an accelerated decrease in lung compliance, higher lung resistance, increased collagen deposition in the lungs, and higher levels of β6-integrin gene expression.
expression along with increased total TGF-β1 and MMP2 protein levels when compared with WT recipients. Therefore, the increased levels of TGF-β1, but not free TGF-β, concomitant with an increase in β6-integrin and collagen is consistent with a β6-integrin-TGF-β pathway of fibrosis.

On the basis of MMP12 elastin-cleavage studies (17, 19), our initial hypothesis was that MMP12−/− mice would do significantly better post-BMT, compared with WT, as a result of hindered influx of host monocytes because of the lack of degradation of matrix. However, mice lacking MMP12 exhibited no differences in the degree of inflammatory cell influx compared with their WT counterparts. This was unexpected, as these results are contradictory to the inhibition of monocyte influx in the emphysema mouse model (12). However, our findings are in agreement with the lack of effect on cellular infiltration seen in the lungs of MMP12−/− mice after Fas-Ligand-induced lung fibrosis (22), as well as with the finding that MMP12 deficiency does not affect bleomycin-induced inflammatory cell infiltration and pulmonary fibrosis (20). Furthermore, in the Manoury report (20), baseline levels of collagen mRNA were lower in the MMP12−/− compared with the WT but were the same by day 14 post-bleomycin, indicating that there was higher induction of collagen in the MMP12−/−.

Interestingly, MMP12+ cells were seen in the lungs of MMP12−/− mice postallogeneic BMT in our model. This indicates that donor-derived monocytes can enter the lungs early post-BMT if host monocytes are insufficient in this capacity. Host- and donor-derived antigen-presenting cells differ in their capacities to induce GVHD reactions in different target organs (1), but this has not been studied in relation to the lungs post-BMT. Although pulmonary dendritic cells are the most potent antigen-presenting cells in the lungs (21, 31), they can be regulated by host macrophages in the lung (13). It is not known whether expression of MMP12 affects this downregulatory capacity of lung macrophages in vivo or whether host vs. allogeneic macrophages alter the type of lung injury manifested post-BMT (i.e., nonfibrotic vs. fibrotic injury as we has seen using MMP12−/− recipients). In support of a role for donor-derived macrophages in fibrotic injury, we have previously reported in a model of obliterative bronchiolitis that occurs later post-BMT (as opposed to the early occurring acute IPS studied in this paper) that most of the macrophages in the lungs were donor derived (29).

As an adjunct to these studies, GVHD studies on mice conditioned with total body irradiation alone (i.e., without Cy) showed that MMP12−/− recipient mice exhibited an accelerated lethality post-BMT (mean survival 28 days for MMP12−/− vs. 55 days for WT, P = 0.002, n = 16/group, with 100% survival of BM controls for both recipient types). GVHD scores of typical target organs (liver and colon) did not differ statistically between groups (data not shown). However, evidence of pulmonary embolism was found in several MMP12−/− mice (example in Fig. 1, inset in BMS panel). This is also a feature not seen in WT mice with GVHD. This may have been a contributing factor to the increased rate of death in MMP12−/− recipients receiving BMS.

The relevance of the accelerated lung dysfunction and evidence of fibrosis seen in the MMP12−/− mice post-BMT is exemplified by the observation that acute IPS develops in patients over a range of time frames, as early as 3 wk to several

Fig. 4. Increased β6-integrin expression in the lungs of MMP12−/− mice receiving allogeneic splenocytes. Lungs from day 7 post-BMT mice were evaluated for the levels of β6-integrin by qRT-PCR (A), *P < 0.05, n = 3 per group. B: Western blot, showing 3 mice per transplant group as indicated with relative quantitation. C: immunofluorescence with anti-β6-integrin antibody as described in MATERIALS AND METHODS. MMP12−/− recipients of BMS have significant β6 expression on bronchiolar epithelium, as well as some alveolar epithelium consistent with alveolar type II cells, compared with the BM control, and the B6 recipients. A nonimmune rabbit primary antibody control staining of a serial section of lung of MMP12−/− recipient of BMS is also shown (magnification ×200). D: serial sections of lungs shown in C (from day 7 post-BMT mice) were stained by H&E (magnification ×100).
months post- hematopoietic stem cell transplantation. The reason for this is unknown, but there is a disease spectrum to IPS (26), and use of the MMP12−/− in our model may have provided a clue to some of the different disease manifestations and times of onset seen in IPS.

It has been proposed in previous studies that MMP12 is primarily responsible for strict cleavage of lung elastase causing numerous lung diseases (8, 18, 37). It has also been proposed that MMP12 could play a role in the regulation of inflammation and fibrosis by nonelastase means (7, 16, 40). For example, MMP12 can orchestrate cell trafficking of neutrophils and macrophages into the lungs attributable to cleavage of CCL and CXCL chemokines, creating more potent chemotactic fragments (7). However, in our BMT studies with MMP12−/− recipients, we did not find differences in the degree of cellular infiltration or in the amounts of a large panel of cytokines and chemokines in the BALF or lung protein extracts at different time points post-BMT (data not shown). In this article, we propose that there may be a novel role for MMP12 in post-BMT lung injury that is dependent on regulation of profibrotic gene expression.

MMP12−/− recipient mice exhibited an accelerated progression of IPS early post-BMT, which included decreases in specific compliance and TLC, as well as increased elastase and resistance in the lung. These changes in lung function were associated with increases in MMP2, collagen, TGF-β, and β6-integrin. We also evaluated the level of the profibrotic factor PDGF and found no difference between MMP-12−/− and WT mice either before or after BMT (data not shown). These data indicate the importance of MMP12 in normal lung function specifically related to basal membrane turnover. Recipient expression of MMP12 may be required for the correct processing of both collagen and elastin in the lungs post-BMT to inhibit fibrosis. Although donor-derived MMP12+ cells are found in the lungs of MMP12−/− recipients post-BMT, it appears to be either not sufficient or not present in the correct context to replace the host deficiency. Alternatively, MMP12−/− mice may have other phenotypic changes, not previously described in other models of injury, that are conducive to development of lung fibrosis in the BMT setting. It is difficult to know whether the increase in MMP2 levels seen in the lungs of MMP12−/− mice receiving BMS is attributable to compensatory increase by the host cells or increased MMP2 secretion by the donor cells in the MMP12−/− environment. Nevertheless, these data indicate that the increase in MMP2 protein secretion does not seem to be robust enough to overcome the insufficiency of MMP12, leaving collagen and elastin deposition to go unchecked.

TGF-β plays a critical role in the development of pulmonary fibrosis (2, 15). Latent TGF-β is activated by αVβ6-integrin, leading to upregulation of collagen deposition via the Smad pathway in fibroblasts and subsequent fibrosis (3, 6, 30, 41, 43). It has recently been reported that inhibition of αVβ6-integrin prevents bleomycin- and radiation-induced pulmonary fibrosis in mice (14, 32). αVβ6-Integrin is significantly upregulated on injured epithelial cells (36), and we have previously documented that pulmonary epithelial cells express TGF-β1 early post-BMT (28), thus setting the stage for local activation of fibroblasts once they have migrated to the site of epithelial cells that have been sufficiently injured to the point of expressing β6-integrin. Even though WT mice receiving BMS had a significant increase in lung TGF-β1 mRNA, this was not accompanied by an increase in β6-integrin or an increase in fibrosis. Although β8-integrin can also activate TGF-β through a metalloprotease-dependent pathway (23), it results in the release of free TGF-β. In our analyses of lungs for TGF-β, we found increases in total TGF-β, not the free form, in MMP12−/− recipients, thus indicating that the increased collagen deposition is via activation by β6-integrin, not β8-integrin (36). There is some discrepancy between the OH-proline data and the trichrome stains. Trichrome staining interpretation is more subjective than the quantitative OH-proline biochemical assay. The increased OH-proline levels of the MMP12−/− given BM alone do correspond to increased TGF-β levels, but the trichrome stain does not illustrate this. Because collagen is present in all the matrix of the lung (bronchiolar as well as distal alveolar areas), smaller increases in the more diffuse alveolar areas would be difficult to discern by trichrome. What is, however, significant is that only the MMP12−/− mice with IPS exhibited increased integrin-β6 (very prominent in the bronchiolar epithelium) and increased resistance (which is typically attributable to bronchiolar changes). TGF-β can be activated by many means to signal collagen production, but it seems that activation by β6-integrin results in an airway pattern of collagen deposition that leads to increased resistance.

The demonstration of dramatic upregulation of β6-integrin being associated with fibrosis early post-BMT in MMP12−/− recipients in our acute model of IPS, which normally does not include fibrosis, opens up a new avenue for targeted therapy of obliterative bronchiolitis, which is a serious problem of fibrotic lung injury associated with chronic GVHD post-BMT. Furthermore, it raises caution for therapies aimed at targeting MMPs for preventing lung injury because such treatments may interfere with proper remodeling during the repair phase.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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