Surfactant protein A is a required mediator of keratinocyte growth factor after experimental marrow transplantation

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Haddad, Imad Y., Carlos Milla, Shuxia Yang, Angela Panoskaltsis-Mortari, Samuel Hawgood, David L. Lacey, and Bruce R. Blazar. Surfactant protein A is a required mediator of keratinocyte growth factor after experimental marrow transplantation. Am J Physiol Lung Cell Mol Physiol 285: L602–L610, 2003. First published May 9, 2003; 10.1152/ajplung.00088.2003.—We reported an association between the ability of recombinant human surfactant protein A (SP-A) and to downregulate pulmonary inflammation; occurs after allogeneic bone marrow transplantation (BMT). To establish a causal relationship, rHuKGF (5 mg/kg) was administered subcutaneously for three consecutive days before irradiation to SP-A-sufficient mice. Early post-BMT weight loss was attenuated by rHuKGF in both SP-A(-/-) and SP-A (+/+ ) recipients. In the absence of supportive respiratory care, however, SP-A deficiency eventually abolished the ability of rHuKGF to prevent weight loss and to improve survival monitored for 1 mo after allogeneic BMT. In further experiments, the addition of cyclophosphamide (which is known to cause severe injury to the alveolar epithelium in donor T cell-recipient mice) to the conditioning regimen prevented rHuKGF-induced upregulation of SP-A and suppression of lung inflammation in both SP-A(+/-) and SP-A(-/-) mice. We conclude that endogenous baseline SP-A levels and optimal upregulation of SP-A are required for the anti-inflammatory protective effects of KGF after allogeneic transplantation.

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Importantly, rhuKGF ameliorates injury by a mechanism that is independent of conditioning-related toxicities. Instead, the mechanism of action of rhuKGF is via inhibition of allogeneic T cell-dependent immune responses in the absence of direct effects on T cells (26). Collectively, these results point to the generation of a KGF-induced epithelial cell-derived mediator with immunoregulatory functions.

Surfactant protein A (SP-A), a collectin that is associated with surfactant lipids, exhibits pulmonary immune modulatory effects (5, 6). Indeed, basal endogenous SP-A and enhanced alveolar SP-A levels (by instillation of purified human SP-A) attenuate the manifestations of IPS injury and decrease early mortality after allogeneic transplantation in rodents (35, 36). In addition, we have demonstrated a strong association between the abilities of rhuKGF to upregulate endogenous SP-A expression and suppress allogeneic T cell-dependent inflammation. For example, rhuKGF treatment increases SP-A expression in bronchoalveolar lavage fluid (BALF) collected on day 7 after allogeneic BMT, which is during the time that rhuKGF has been shown to suppress the generation of TNF-α and IFN-γ (15). However, the addition of cyclophosphamide [Cy, which is known to deplete antioxidant defenses and enhance oxidant-mediated injury to the alveolar epithelium (2, 43)] to the conditioning regimen prevents rhuKGF-mediated upregulation of SP-A and abolishes the ability of rhuKGF to attenuate the production of inflammatory mediators (37). A cause-and-effect relationship between rhuKGF-mediated SP-A induction and inhibition of early inflammatory responses after allogeneic BMT, however, was not established.

In this study, we utilized SP-A deletional mutant mice treated with rhuKGF to establish the critical role of SP-A in mediating the pulmonary protective effects of rhuKGF. We hypothesized that basal and/or optimal upregulation of SP-A are required for the protective anti-inflammatory functions of rhuKGF in the lung after allogeneic transplantation. Wild-type (SP-A-sufficient, SP-A+) and SP-A-deficient (SP-A−/−) mice were treated with rhuKGF before receiving lethal irradiation with or without Cy and were then given donor bone marrow plus GVHD/IPS-inducing allogeneic T cells. Results establish the important role of SP-A in facilitating rhuKGF-induced protective pulmonary responses.

**METHODS**

**Mice.** Inbred B10.BR (H2b) and C57BL/6 mice, termed B6 (H2b), were purchased from Jackson Laboratories (Bar Harbor, ME). SP-A−/− mice were generated from embryonic stem cells derived from the 129/J mouse strain in which the mouse SP-A gene was disrupted by homologous recombination as previously described (22). SP-A−/− mice were backcrossed >10 generations to B6 mice. Mice were housed in micro-isolator cages in the specific pathogen-free facility of the University of Minnesota and were cared for according to the Research Animal Resources guidelines of our institution. For BMT, donors were 6–8 wk of age, and recipients were used at 8–10 wk of age. Sentinel mice were found to be negative for 15 known murine viruses including cytomegalovirus, K-virus, parvovirus, and pneumonia virus of mice.

**BMT procedure.** Either rhuKGF (Amgen, Thousand Oaks, CA) or PBS was administered to SP-A+ (+/+ ) and SP-A−/− B6 recipients (5 mg·kg−1·day−1 sc) on days 6, 5, and 4 before transplantation. Recipients were given heavy-dose total-body irradiation (TBI, 7.5 Gy) by X-ray at a dose rate of 0.41 Gy/min. Donor B10.BR bone marrow was T cell depleted with anti-Thy 1.2 monoclonal antibody (clone 30-H-12, rat IgG2a, kindly provided by Dr. David Sachs, Massachusetts General Hospital, Cambridge, MA) plus complement (Neomycin, Woodland, CA). For each experiment, a total of 5–10 recipient mice per treatment group were transplanted via caudal vein with 20 × 10^6 T cell-depleted bone marrow cells plus 15 × 10^6 GVHD/IPS-causing allogeneic spleen T cells (BMS) as previously described (27, 38). A cohort of mice was monitored for 1-mo survival and weight loss. In additional BMT experiments, recipients also received 120 mg·kg−1·day−1 ip of Cy (Cytoxan, Bristol Myers Squibb, Seattle, WA) on days –3 and –2 before allogeneic BMT (BMS + Cy).

**Bronchoalveolar lavage.** Mice were killed on day 7 after BMT. The thoracic cavity was partially dissected, and the trachea was cannulated with a 22-gauge angiocatheter. The catheter was infused with 1 ml of ice-cold sterile PBS that was then withdrawn. This was repeated once, and the return fluid was combined. BALF (10 μl) was used to count the number of inflammatory cells with a hemacytometer, and the remaining fluid was immediately centrifuged at 500 g for 10 min at 4°C to pellet cells. BALF cells were combined, resuspended in PBS, and centrifuged onto glass slides. Inflammatory cell types were identified by Wright-Giemsa staining and were counted based on four sample sets per group from two different experiments.

**BALF analysis.** Cell-free BALF TNF-α and IFN-γ levels were determined by sandwich ELISA using murine-specific commercial kits (sensitivity 1.5–3 pg/ml; R&D Systems, Minneapolis, MN). Nitrite in BALF was measured according to the Greiss method after the conversion of nitrate to nitrite with the reduced NADH-dependent enzyme nitrate reductase (Calbiochem, La Jolla, CA). BALF total protein was determined by the bicinchoninic acid (Sigma, St. Louis, MO) method using BSA as the standard.

**Western blots and ELISA of pulmonary collectins.** Equal volumes (20 μl) of BALF were solubilized in 0.1 M Tris·HCl buffer (that contained 50 μM DTT, 0.01% bromophenol blue, 1% SDS, and 10% glycerol) and boiled for 5 min. The proteins were resolved by 10% SDS polyacrylamide gels, transferred to nitrocellulose paper, and probed with polyclonal anti-rabbit SP-A or SP-D antibodies (1:10,000 dilution) followed by alkaline phosphatase-conjugated goat anti-rabbit IgG (1:7,500 dilution) as the secondary antibody. Bound antibody was detected using nitro blue tetrazolium and a 5-bromo-4-chloro-3-indolyl-1-phosphate kit (Sigma). Densitometry was used to quantify the intensity of specific SP-A and SP-D bands (NIH Image, Scion, Frederick, MD). SP-A levels in cell-free BALF were determined using ELISA. Equal aliquots (1 μl) of BALF were serially diluted using 50 mM Na2CO3-NaHCO3 buffer at pH 9.6, coated to ELISA plates, and allowed to bind for at least 18 h at 4°C. Nonspecific binding sites were blocked with 1% BSA for 1 h at room temperature. The wells were then incubated with the anti-rabbit SP-A antibody (1:10,000 dilution) at 37°C for 1 h. Unbound antibody was removed by a series of washes with PBS-Tween 20 buffer. Horseradish peroxidase-conjugated goat anti-rabbit IgG (1:2,500 dilution; Sigma) was added as the secondary antibody. After serial washes, color was developed by adding...
o-phenylendiaminedihydrochloride (Sigma) and hydrogen peroxide to each well, and absorbance was read at 490 nm. Purified SP-A (0.125–3 ng) isolated from patients with alveolar proteinosis (as previously described) was used as a standard. Concentration of SP-A was calculated from the slope of each sample (absorbance/μl of BALF) and the slope of the standard curve (absorbance/ng of SP-A). Because human SP-A was used as a standard, results are considered relative and are not absolute concentrations.

Pulmonary function analysis. Pulmonary mechanics in pentobarbital sodium-anesthetized ventilated mice were measured using the method described by Diamond and O’Donnell (10) with slight modifications. In brief, after careful dissection of the neck, a short metal cannula was inserted into the trachea and secured with 3-0 silk. A polyethylene catheter was inserted orally into the lower third of the esophagus to estimate pleural pressure. The animal was then placed into a plethysmograph (Buxco Electronics, Sharon, CT) and connected to a mouse ventilator (Harvard Apparatus, March-Hugstetten, Germany) set at a respiratory rate of 150 breaths/min and a tidal volume of 200 μl. The respiratory flow response was measured through a flow transducer (SenSym SCXL004, Buxco Electronics) connected to the plethysmograph. Lung volume was obtained by electric integration of the flow signal. Intraseophageal and airway pressures were measured with a pressure transducer (Validyne DP45, Buxco Electronics) directly connected to their respective ports. These data were fed into a computer through a preamplifier (MaxII, Buxco Electronics), and the data were analyzed with BioSystem XA software (Buxco Electronics). When the signal was stable, delivered tidal volume was varied from 350 to 100 μl in 50-μl decrements, and for each delivered volume, the effective tidal volume, transpulmonary pressure, and dynamic compliance (lung compliance) values were measured. Body temperature was maintained at 37°C throughout the experiment. Volume vs. pressure plots were constructed for each treatment group.

Histology and immunohistochemistry. In one or two mice per group per experiment, a 3:1 mixture of 1 ml of optimal cutting temperature medium (Miles Laboratories, Elkhart, IN) and PBS was infused in the trachea. The lungs were snap-frozen in liquid nitrogen and stored at −80°C. Representative sections from each tissue block were stained with hematoxylin and eosin for assessment of airspace sizes using light microscopy. Frozen sections were cut 4 μm thick, mounted onto glass slides, and fixed for 5 min in acetone. After a blocking step in 10% normal horse serum (Sigma), sections were incubated for 30 min at 23°C with the anti-Mac-1 (clone M1/70, rat IgG2b, C with the anti-rabbit SP-D. Shown is a representative Western blot that was subjected to protein blotting on nitrocellulose transferred to the PVDF membrane, proteins were then fixed with 4% paraformaldehyde (PFA), blocked with 5% normal horse serum in TBST (0.1 M Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween 20), and incubated with the primary antibodies. SP-A was used as a standard, results are considered relative to the standard curve (absorbance/ng of SP-A). Because human SP-A was used as a standard, results are considered relative and are not absolute concentrations.

RESULTS

Upregulation of SP-A by rHuKGF. Our previous results indicate that irradiated rHuKGF-treated mice infused with allogeneic spleen T cells (BMS) at the time of BMT exhibited upregulation of SP-A mRNA and protein assessed on day 7 after BMT (37). Western blotting on day 7 after BMT BALF confirmed enhanced (−2×) SP-A levels in BALF from rHuKGF-treated SP-A(+/+) BMS mice and the absence of SP-A from all SP-A(−/−) mice (Fig. 1A). Notably, upregulation of SP-A expression was observed 10 days after rHuKGF treatment only in recipients of allogeneic grafts but not in control (nonirradiated and nontransplanted) mice (37). To determine whether an early transient rHuKGF-induced upregulation of SP-A occurred in control mice, BALF was collected 3 days after rHuKGF treatment. BALF SP-A levels were 51 ± 8% higher in rHuKGF- compared with PBS-injected control mice (n = 4; P < 0.05). These results are consistent with our published data that the presence of alloreactive T cells

![Fig. 1. Western blots of equal bronchoalveolar lavage fluid (BALF) volume (20 μl) obtained on day 7 after bone marrow transplant (BMT) from surfactant protein A-sufficient [SP-A(+/+)] and -deficient [SP-A(−/−)] C57BL/6 mice injected with either PBS or recombinant human keratinocyte growth factor (KGF, 5 mg/kg sc) on days −6, −5, and −4 pre-BMT. All mice were total-body irradiated (7.5 Gy on day −1) and given T cell-depleted B10.BR mouse bone marrow cells (20 × 10^6 cells) plus inflammation-inducing donor T cells (15 × 10^6 cells). A: BALF SP-A was detected using anti-rabbit SP-A antibody. KGF treatment upregulated BALF SP-A protein. SP-A was undetectable in SP-A(−/−) mice. B: BALF SP-D was detected using anti-rabbit SP-D. Shown is a representative Western blot that was repeated twice.](http://ajplung.physiology.org/)

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can prolong the ability of exogenous rHuKGF to up-regulate the expression of SP-A in the lung (37). SP-D levels in BALF of experimental mice were determined using densitometry. Only minor modifications of SP-D levels were observed in SP-A(+/+) and SP-A(−/−) donor T cell-recipient irradiated mice in the presence or absence of rHuKGF (Fig. 1B).

SP-A is required for anti-inflammatory effects of rHuKGF in lung. BMT experiments were performed to determine the contribution of SP-A to the beneficial effects of rHuKGF after allogeneic BMT. rHuKGF was subcutaneously injected before irradiation into SP-A(+/+) and SP-A(−/−)-inbred mice given inflammation-inducing allogeneic spleen T cells (BMS) at the time of BMT. BALF was collected on day 7 after BMT, because our previous data indicate rHuKGF-mediated downregulation of donor T cell-dependent inflammation occurs at this time point (15, 24). Treatment of irradiated donor T cell-recipient SP-A(+/+) mice with rHuKGF suppressed day 7 after BMT BALF levels of TNF-α, IFN-γ, and nitric oxide. In contrast, the ability of rHuKGF to decrease the production of these inflammatory mediators in irradiated donor T cell-recipient SP-A(−/−) mice was abolished (Fig. 2). As previously reported (15, 24), rHuKGF did not significantly modify the number of inflammatory cells in BALF as determined by Wright stain (data not shown) or number of Mac-1-positive cells infiltrating the lungs of SP-A(+/+) and SP-A(−/−) mice. Mac-1-positive cells were 25 ± 4 and 23 ± 3% of nucleated cells in lung sections from PBS- and rHuKGF-injected SP-A(+/+) mice, and 33 ± 4 and 30 ± 6% of nucleated cells in lung sections of PBS- and rHuKGF-injected SP-A(−/−) mice, respectively (P > 0.05 comparing effects of rHuKGF treatment; n = 4 sections/group from 2 experiments). Taken together, these results indicate that SP-A is a required factor that mediates rHuKGF-induced downregulation of allogeneic T cell-dependent inflammatory responses without modification of cellular infiltration.

Post-BMT weight loss and mortality are not modified by rHuKGF during SP-A deficiency. We next determined whether SP-A also alters the effects of rHuKGF treatment on the course of GVHD and/or BMT-related mortality. In our allogeneic BMT model, weight loss correlates with severity of GVHD (27). On the day of BMT, the mean weights of SP-A(+/+) and SP-A(−/−) mice were similar (23.4 ± 1.1 and 24.9 ± 1.3 g, respectively; P > 0.05). On day 7 after allogeneic BMT, rHuKGF attenuated weight loss in both SP-A(+/+) and SP-A(−/−) mice (Fig. 3A). However, when a cohort of mice was monitored for 1 mo, a major effect of SP-A deficiency on rHuKGF-induced modifications of post-BMT weight loss emerged. Although treatment of irradiated donor T cell-recipient wild-type mice with rHuKGF restored weight gain compared with PBS-injected mice (P = 0.028), rHuKGF failed to prevent the rapid weight loss in SP-A(−/−) mice after allogeneic transplantation (Fig. 3B). Notably, the difference in survival between rHuKGF-treated SP-A(+/+) and SP-A(−/−) recipients of allogeneic grafts was highly significant (P = 0.0005; Fig. 3C). In contrast, survival of PBS-injected SP-A(+/+) and SP-A(−/−) recipients was not different (P = 0.28). These results are consistent with the notion that the early systemic effects of rHuKGF are preserved in SP-A(−/−) BMS mice. However, possibly because of persistent lung inflammation, the ability of rHuKGF to limit weight loss beyond the first week and, more significantly, the ability of rHuKGF to decrease mortality after allogeneic BMT, are lost during SP-A deficiency.

Fig. 2. Impaired ability of recombinant human KGF to suppress the generation of TNF-α (A), IFN-γ (B), and nitric oxide (C) during SP-A deficiency after allogeneic BMT. SP-A(+/+) and SP-A(−/−) C57BL/6 mice were injected with either PBS (open bars) or recombinant human (rHu)KGF (5 mg/kg sc; solid bars) on days −6, −5, and −4 pre-BMT. Mice were total-body irradiated (7.5 Gy on day −1) and given T cell-depleted B10.BR bone marrow cells plus inflammation-inducing donor spleen T cells (BMS). BALF was collected on day 7 after BMT. TNF-α and IFN-γ values were measured using sandwich ELISA. Nitrate was reduced with nitrate reductase before nitrite measurement via the Greiss reaction. C, control (nonirradiated and nontransplanted). Shown are mean values ± SE for 10–15 mice/group from 2 separate experiments; *P < 0.05 comparing effects of KGF treatment in each treatment group; †P < 0.05 comparing effects of SP-A deficiency after allogeneic BMT.
Increased lung compliance in rHuKGF-treated SP-A(−/−) mice. Because the presence of SP-A is mainly limited to lung, we investigated whether severe lung injury may explain the reason for the impaired ability of rHuKGF to enhance survival of SP-A(−/−) mice after allogeneic BMT. Lung dysfunction was assessed on day 7 after allogeneic BMT by measurement of pulmonary function analysis in mice that were anesthetized and ventilated. Lung compliance values at a tidal volume of 200 μl in unmanipulated (nonirradiated and nontransplanted) SP-A(+/+) and SP-A(−/−) mice were similar and were not altered by rHuKGF treatment. After allogeneic BMT, compliance was significantly decreased in irradiated mice given donor T cells, which is consistent with development of IPS injury. Lung injury was exacerbated by SP-A deficiency, because compliance was lower in SP-A(−/−) compared with SP-A(+/+) mice given IPS-inducing donor T cells. Treatment with rHuKGF did not modify lung compliance in donor spleen T cell-recipient (BMS) wild-type mice; this was most likely because of persistent cellular lung infiltration. In contrast, rHuKGF-treated SP-A(−/−) BMS mice exhibited an unexpected increase in compliance to values similar to control (nonirradiated and nontransplanted) mice (Fig. 4A). Moreover, a leftward shift of the volume vs. pressure relationship was observed in rHuKGF-treated SP-A(−/−) donor T cell-recipient (BMS) mice compared with PBS-treated SP-A(−/−) BMS mice (Fig. 4B), and PBS- or rHuKGF-treated SP-A(+/+) BMS mice (data not shown). Because of the persistent presence of severe markers of inflammation and injury, these changes in the mechanical properties of the lungs of SP-A(−/−) BMS mice that were given rHuKGF are unlikely the result of improved pulmonary function. Hematoxylin and eosin-stained lung sections obtained on day 7 after allogeneic BMT were assessed for airspace sizes under low magnification (×10). Despite major differences in lung compliance, airspace sizes between rHuKGF-treated SP-A(−/−) mice and the remaining groups of control and experimental mice were similar (data not shown).

Effects of Cy/TBI conditioning on rHuKGF responses after allogeneic BMT. To obtain additional evidence for the hypothesis that rHuKGF-induced upregulation of SP-A is critical for the anti-inflammatory effects of rHuKGF in the lung after allogeneic BMT, rHuKGF responses in SP-A(+/+) and SP-A(−/−) irradiated mice given Cy on days −3 and −2 before allogeneic BMT were compared. We previously reported that injection of Cy into TBI mice given allogeneic T cells at the time of BMT depletes antioxidant defenses and enhances the generation of oxidative and/or nitritative stress (16, 43). Generation of oxidative and/or nitritative stress was associated with an impaired ability of rHuKGF to upregulate SP-A expression and suppress donor T cell-dependent inflammation (15). The rHuKGF treatment attenuated Cy/TBI- and donor T cell-mediated weight loss measured on day 7 after BMT [average percent weight loss, −29.1 ± 2.3 and −21.1 ± 2.0 in PBS- and rHuKGF-treated SP-A(+/+) mice].
mice, respectively; *P < 0.05; n = 4; and −34.2 ± 1.9 and −26.5 ± 2.8 in PBS- and rHuKGF-treated SP-A(−/−) mice, respectively; *P < 0.05; n = 4], which is consistent with the early systemic beneficial effects of rHuKGF in all treated mice. In lung, however, rHuKGF treatment failed to upregulate SP-A levels contained in the BALF collected on day 7 after allogeneic BMT in both SP-A(+/+) and SP-A(−/−) mice (Fig. 5A). Moreover, the ability of rHuKGF to prevent permeability edema (Fig. 5B) and decrease the production of the proinflammatory cytokines TNF-α (Fig. 5C) and IFN-γ (Fig. 5D) in both SP-A(+/+) and SP-A(−/−) BMS + Cy mice was impaired.

**DISCUSSION**

Utilizing SP-A(−/−) mice, we show herein that the absence of SP-A impairs the ability of rHuKGF to suppress the generation of inflammatory mediators contained in BALF collected on day 7 after allogeneic transplantation. Persistent lung inflammation in the absence of SP-A may eventually abolish the systemic beneficial effects of rHuKGF on mortality. Additional BMT experiments showed that failure to upregulate SP-A in the rHuKGF-treated Cy/TBI-donor T cell-recipient (BMS + Cy) wild-type and SP-A-deficient mice also resulted in failure of rHuKGF to suppress donor T cell-dependent pulmonary inflammation. Collectively, these studies establish the critical role of SP-A and optimal upregulation of SP-A in mediating the protective anti-inflammatory effects of KGF in the lung. Furthermore, the absence of major modifications of SP-D during SP-A deficiency after BMT supports a more specific role of SP-A in rHuKGF-mediated pulmonary effects.

After the initial report by Panoskaltis-Mortari and co-workers that showed that rHuKGF prevents GVHD/IPS and improves survival of irradiated mice infused with inflammation-inducing donor T cells at the time of BMT (25), studies have focused on determining the protective mechanisms of rHuKGF. For example, rHuKGF can accelerate the proliferation of alveolar type II pneumocytes assessed on day 3 after BMT (24) and prevent depletion of glutathione-reducing potential (43). Other potential protective mechanisms include repair of microvascular endothelial and epithelial barrier functions (13, 31). Because of the multiple beneficial functions of KGF, we expected the absence of SP-A to partially prevent rHuKGF-induced pulmonary protective effects. Instead, rHuKGF-treated SP-A(−/−) mice exhibited severe lung inflammation comparable to that of PBS-injected SP-A(−/−) mice. A possible argument for the failure of rHuKGF action in SP-A(−/−) mice is increased baseline lung inflammation and injury during SP-A deficiency after allogeneic BMT, which might render any treatment including rHuKGF less effective. However, we have previously shown that intratracheal instillation of human SP-A on day 4 after BMT was enough to suppress donor T cell-dependent inflammation and attenuate IPS injury during severe lung dysfunction in irradiated mice injected with Cy and donor T cells (35). In support of a direct relationship between KGF and SP-A are the observations that KGF upregulates SP-A expression in vitro and in vivo (34, 40), and evidence that KGF and SP-A exhibit survival signaling in alveolar type II cells via activation of the phosphatidylinositol 3-kinase/Akt pathway (30, 33).

The major effect of SP-A on rHuKGF responses in our model of allogeneic T cell-dependent inflammation may represent the important role of SP-A in regulation of the adaptive immune system. Accumulating evidence indicates that the pulmonary collects modulate T cell immune responses (12, 17). For example, SP-A and SP-D suppress lymphocyte proliferation and
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cytokine production in children with asthma (29). In addition, increased expression of markers of T and B lymphocyte activation has been observed in the lung and spleen of influenza-infected SP-A(−/−) mice (21). Recent studies indicate that SP-A and SP-D can suppress T cell proliferation by at least two distinct mechanisms: a direct IL-2-independent mechanism that involves attenuation of cytosolic free calcium and a second IL-2-dependent mechanism that requires activation via the T cell-receptor complex in the presence of accessory cells (7). Whether a similar dominant function of SP-A in mediating the protective effects of rHuKGF will be observed in other models of lung injury remains to be determined.

An important finding of this study is that SP-A deficiency also eliminated the ability of rHuKGF to prevent weight loss and death after allogeneic BMT. Notably, however, early weight loss in the first week after allogeneic transplantation was attenuated in rHuKGF-treated SP-A(+/+) and SP-A(−/−) mice compared with PBS-injected mice. Clearly, rHuKGF administered subcutaneously exerts multiple systemic effects that can limit GVHD-related injury. For example, rHuKGF administration from day −3 to day 7 ameliorates the severity of GVHD in the gastrointestinal tract most likely by protecting the intestinal epithelium and limiting lipopolysaccharide leakage (20). In the thymus, pre-BMT injection of rHuKGF between days −3 and 3 of GVHD restores thymic architecture and normal thymopoiesis, which probably occurs via enhanced production of thymic epithelial cell-derived IL-7 (4). These extrapulmonary beneficial functions of rHuKGF are unlikely to be altered by SP-A deficiency.

In the context of persistent inflammation in the lungs of SP-A(−/−) mice, however, severe immune responses, in the absence of respiratory support, may eventually lead to rapidly progressive respiratory failure, multiple organ failure, and death. These results underscore the major influence of lung inflammation and injury on final outcome. It is also possible that, had respiratory supportive care been provided as is routinely performed in IPS patients, the systemic protective effects of rHuKGF treatment might have been preserved.

SP-A protein has also been observed in rabbit sinus and middle ear mucosa (11). Akiyama and colleagues (3) thoroughly investigated SP-A expression in 8-12-wk-old C57BL/6 mice. Although SP-A mRNA was detected in lung, uterus, ovary, and lacrimal gland, SP-A protein was detected only in lung. In the absence of evidence for SP-A protein in GVHD target organs in mice, it is unlikely that extrapulmonary SP-A had a role in the differences in weight loss and survival between rHuKGF-treated SP-A(+/+) and SP-A(−/−) mice after allogeneic transplantation (see Fig. 3).

We expected to find severe lung dysfunction and therefore low lung compliance in rHuKGF-treated donor T cell-recipient SP-A(−/−) mice. Instead, we observed that SP-A(−/−) mice given rHuKGF and IPS-causing T cells to exhibit increased lung compliance and a leftward shift of the volume vs. pressure relationship compared with PBS-treated SP-A(−/−) mice. Interestingly, a spontaneous age-dependent increase in lung compliance has been described in SP-D(−/−) mice and SP-A/SP-D double-knockout mice [SP-A/D(−/−)] but not control or experimental SP-A(−/−) mice (18, 32). The mechanism of high compliance in SP-D(−/−) mice involves oxidant-induced activation of...
NF-κB and increased production of matrix metalloproteinases (42). Degradation of elastin and collagen leads to enlarged airspace and development of emphysema. We were unable to demonstrate increased airspace sizes in lung sections from rHuKGF-treated SP-A(−/−) mice, perhaps because of the early time point that was chosen to assess for signs of emphysema. Additional studies are required to determine the time course and mechanisms of increased compliance in rHuKGF-treated donor T cell-recipient SP-A gene mutant mice.

Addition of Cy to the conditioning regimen prevented rHuKGF-induced upregulation of SP-A in wild-type mice most likely because of severe oxidant injury to the alveolar epithelium (37). Failure to upregulate SP-A also abolished the anti-inflammatory protective effect of rHuKGF in lung. These data indicate that basal levels of endogenous SP-A may not be enough to modulate donor T cell immune responses. Optimal upregulation of SP-A is required to limit the severe inflammation after allogeneic BMT. When the alveolar epithelium is severely injured, SP-A replacement therapy to enhance airspace levels may be necessary to limit the destructive T cell-dependent inflammatory responses. It is important to mention that compared with their respective wild-type recipients, the absence of SP-A caused considerably more severe lung inflammation and IPS injury in BMS + Cy (given Cy) than BMS mice (without Cy). These findings may clarify the reason we observed accelerated mortality on day 8 after BMT in SP-A(−/−) BMS + Cy mice compared with SP-A(+/+) BMS + Cy mice as previously reported (35) but no significant difference in the survival of PBS-injected SP-A(+/+) and SP-A(−/−) BMS mice (see Fig. 3C).

In conclusion, our results provide evidence that rHuKGF may suppress T cell immune responses and may attenuate the manifestations of IPS by upregulating SP-A in lung. These data indicate that basal levels of endogenous SP-A may not be enough to modulate donor T cell immune responses. Optimal upregulation of SP-A is required to limit the severe inflammation after allogeneic BMT. When the alveolar epithelium is severely injured, SP-A replacement therapy to enhance airspace levels may be necessary to limit the destructive T cell-dependent inflammatory responses. It is important to mention that compared with their respective wild-type recipients, the absence of SP-A caused considerably more severe lung inflammation and IPS injury in BMS + Cy (given Cy) than BMS mice (without Cy). These findings may clarify the reason we observed accelerated mortality on day 8 after BMT in SP-A(−/−) BMS + Cy mice compared with SP-A(+/+) BMS + Cy mice as previously reported (35) but no significant difference in the survival of PBS-injected SP-A(+/+) and SP-A(−/−) BMS mice (see Fig. 3C).

In conclusion, our results provide evidence that rHuKGF may suppress T cell immune responses and may attenuate the manifestations of IPS by upregulation of SP-A. Clearly, SP-A plays an important role in lung mesenchymal-epithelial cellular interactions. Exposure to signals of inflammation may induce mesenchymal cells to secrete KGF that can enhance the expression of SP-A. One potential function of SP-A is downregulation of allogeneic T cell-dependent inflammatory responses associated with IPS injury and potentially other T cell-mediated pulmonary diseases.

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DISCLOSURES

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