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Role of Integrin αvβ6 in Acute Lung Injury Induced by *Pseudomonas aeruginosa*▽

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Deletion of integrin αvβ6 has been associated with significant protection in experiments where lung injury was induced by bleomycin, lipophilic polysaccharides, and high tidal volume ventilation. This has led to the suggestion that antibody blockade of this integrin is a novel therapy for acute lung injury. We questioned whether β6 gene deletion would also protect against *Pseudomonas aeruginosa*-induced acute lung injury. Wild-type and littermate β6-null mice, as well as recombinant soluble TGF-β receptor type II-Fc (rsTGF-βRII-Fc) and anti-αvβ6 treated wild-type mice, were instilled with live *P. aeruginosa*. Four or 8 h after bacterial instillation, the mice were euthanized, and either bronchoalveolar lavage fluid or lung homogenates were obtained. Deletion of the β6 gene resulted in an overall increase in inflammatory cells in the lungs. Bacterial numbers from the lung homogenates of infected β6-null mice were significantly decreased compared to the numbers in the wild-type mice (1.6×10^6 CFU versus 4.2×10^6 CFU [$P < 0.01$]). There were no significant differences in *P. aeruginosa*-mediated increases in lung endothelial permeability between wild-type and β6-null mice. Similarly, pretreatment with the αvβ6 antibody or with rsTGF-βRII-Fc did not significantly affect the *P. aeruginosa*-induced lung injury or rate of survival compared to pretreatment with control immunoglobulin G. We conclude that deletion or inhibition of the integrin αvβ6 did *not* protect animals from *P. aeruginosa*-induced lung injury. However, these therapies also did not increase the lung injury, suggesting that host defense was not compromised by this promising new therapy.

αvβ6 is one member of the αv integrin subfamily (αvβ1, αvβ3, αvβ5, αvβ6, and αvβ8) that is expressed in the lungs, kidneys, and skin (1, 7, 8, 10). αvβ6 is one of the integrins known to be distributed in epithelial cells and is upregulated after injury or inflammation (1). The function of αvβ6 is through the activation of transforming growth factor β (TGF-β) by binding the latency-associated peptide (18). Transgenic mice deficient in integrin αvβ6 provide a useful tool to study lung phenotypes in animal experiments. Although β6 deletion induces hyperinflammation in the lungs in the basal state, the deletion of β6 protects against bleomycin-induced lung edema (19) and fibrosis (18), acute lung injury induced by lipopolysaccharide (LPS) instillation (19), and acute lung injury induced by high-tidal-volume ventilation (11).

Finally, deletion of β6 causes the development of emphysema that is associated with induced secretion of MMP-12 from macrophages and the development of larger macrophages in the lungs of knockout mice (17). The enlargement of macrophages suggests that the macrophages are activated and that innate immunity may be perturbed in lungs. αvβ6 integrin appears to play an important role in the modulation of the inflammatory response associated with several experimental models of lung injury, and we hypothesized that blockade of this integrin might also improve lung injury induced by *Pseudomonas aeruginosa*. Furthermore, if blocking αvβ6 integrin is not protective against *P. aeruginosa* pneumonia, it would be critical to demonstrate that this new treatment does not increase the vulnerability of the patients to nosocomial bacteria, such as *P. aeruginosa*, since this bacterium invariably colonizes the distal airways of patients with acute lung injury and is now the most common gram-negative bacterium responsible for the development of nosocomial pneumonia in mechanically ventilated patients (5).

β6 integrin deletion protects by suppressing TGF-β activation in the lung (2, 24, 25). TGF-β has been shown to increase endothelial (9) and epithelial permeability (19), decrease ENaC expression in the apical membrane of alveolar type II cells, and increase alveolar flooding (3). In LPS-induced lung injury experiments in mice, blockade of TGF-β with a soluble chimeric TGF-β type II receptor (rsTGF-βRII-Fc) led to protective results similar to those seen in mice missing the β6 gene (19). Overexpression of TGF-β increases apoptosis of airway epithelial cells and increases lung fibrosis induced by bleomycin (14), while blocking TGF-β reduced lung injury induced by LPS intratracheal instillation (19). These studies suggest that TGF-β plays an important role in inducing lung injury, and blockade of TGF-β has been considered as a therapy for patients with acute lung injury. Anti-inflammatory therapies, including corticosteroids and anti-tumor necrosis factor alpha (anti-TNF-α), have increased host vulnerability to infection

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(15, 16). Therefore, these studies were done to determine the effect of a blockade of TGF-β on bacterium-induced lung injury.

MATERIALS AND METHODS

Mice and genotyping. Mice were housed in an air-filtered, temperature-controlled (24°C), pathogen-free barrier with free access to food and water. Room humidity was controlled between 35 and 40%. Littermate wild-type mice (FVB/NJ, originally from Jackson Laboratories, body weight of ca. 22 to 26 g) and αvβ6 knockout mice (FVB/NJ background, 6 to 8 weeks old, body weight of ca. 21 to 25 g) were used in the present study. As done previously (19), all of the mice were confirmed with genotyping at day 5. The primers used for genotyping were 5′-CAGTAAATCGTTGTCAACAG-3′ (P1), 5′-AACCCTTGCAGGTAAGTG AG-3′ (P2), and 5′-TCTTCTGTCACGTCCTCTGA-3′ (P3). P1 is paired with P3 for targeted alleles, and P2 is paired with P3 for wild-type alleles. This study was approved by Institute of Animal and Use Committee at University of California, San Francisco.

Material and regents. αvβ6 antibody and control immunoglobulin G (IgG; 1 and 3 mg/kg, given intraperitoneally [i.p.] 16 h before bacterial challenge) were obtained from Biogen Idec (26). rsTGF-βRII-Fc (2 mg/kg, given i.p. 24 h before bacterial challenge) was also obtained from Biogen Idec (19). Enzyme-linked immunosorbent assay (ELISA) kits for mouse (KC, interleukin-10 [IL-10], TNF-α, and macrophage inflammatory protein 2 [MIP-2]) were purchased from R&D (Minneapolis, MN).

Bacterial culture and preparation. *P. aeruginosa* strains PA103 and PAK were used in the present study. PA103 is a standard lab strain, and PAK was obtained as a gift from Stephen Lory. PA103 is a nonmucoid strain that secretes the potent type III cytotoxin, ExoU, while PAK secretes the less-virulent ExoS cytotoxin. Both strains were cultured in duplicate in MINS medium overnight, washed twice, and resuspended in sterile Ringer lactate solution to a concentration of 10^9 CFU/ml. The *P. aeruginosa* suspension was diluted further with sterile phosphate-buffered saline (PBS) to obtain a final concentration of 2×10^7 CFU/ml or 1×10^8 CFU/ml in PBS.

Bacterial administration and lung injury measurement in mice. Mice were anesthetized with Avertin (250 mg/kg, i.p.). The skin around the neck area was sterilized with betadine and cut open to expose the jugular vein. A 0.1-ml portion of PBS (containing 0.1 μCi of ^{125}I-labeled albumin) was injected into the jugular vein, and then the skin was closed with 5-0 sutures. The mouse was laid on a board with its head elevated at 45°. Then, 50 μl of PBS (containing 1×10^6 CFU of PA103 or 5×10^6 CFU of PAK) was instilled into the left lung through the trachea via the mouth by using a 27G gavage needle (23). The mouse was allowed to recover for 15 min prior to replacement into the cage. Mice were active and appeared normal after 30 min. At 4 or 8 h after the bacterial instillation, a rectal temperature was recorded prior to euthanization with a larger dose of Avertin (500 mg/kg, i.p.). Blood samples were collected in a sterile fashion by using right ventricle punctures after thoracotomies had been done. The mouse lungs were removed, weighed, and homogenized for lung injury measurements. Excess lung water, endothelial permeability, and extravascular plasma equivalents were calculated as previously described (5). Radioactivity per gram of blood and lung was measured by using a gamma counter (Packard Instrument Company, Meriden, CT). For survival studies, 5×10^6 CFU of PAK was instilled into each mouse. Body weights and core temperatures were recorded at 1, 2, 3, 4, and 8 h. The time of death of each mouse was recorded.

BAL. Bronchoalveolar lavage fluid (BAL) was collected by infusing 1.5 ml of sterile PBS (containing 5 mM EDTA) into the lungs of the mice after tracheal cannulation. Gentle suction was applied, and ca. 85% of the fluid was withdrawn from the lungs. The collected fluid was centrifuged at 1,000 rpm for 10 min. The supernatant was stored immediately at −80°C for protein concentration and for cytokine measurements. The pellet was resuspended in 100 μl of PBS for cytocentrifuge preparation after hemolysis of the red blood cells; hemolysis was achieved by adding hypotonic PBS (200 mosmol for 20 s). The total BAL cell number was obtained by using a Beckman Coulter (Coulter Corp., Miami, FL), and the cells were analyzed after hematoxylin and eosin staining of the cytospun material. Blood neutrophils were counted by using a Hemavet (Drew Scientific, Inc., Oxford, CT).

Bacterial cultures from the lungs, spleen, and blood. Mouse blood, the spleen, and lungs were collected in a sterile fashion. The lungs and spleen were homogenized in sterile containers, and the homogenates were serially diluted and plated in triplicate on sheep blood agar plates. Blood was collected in sterile tubes containing 10% sodium citrate prior to serial dilution and plating in triplicate on sheep blood agar plates for bacterial colony counts.

In vitro macrophage isolation, culture, and quantification of bacterial phagocytosis. Alveolar macrophages were isolated by using a published protocol with some modifications (4). Briefly, the mouse lungs were lavaged with 1.5 ml of PBS (containing 5 mM EDTA) and centrifuged at 1,000 rpm for 10 min. The supernatants were discarded, and the pellets were resuspended in Dulbecco modified Eagle culture medium. A total of 2×10^5 cells were plated in 96-well plates, followed by incubation for 1 h. Trypan blue staining demonstrated 97% cell viability, and morphological analysis documented that more than 95% of the cells attached to the bottom of the wells were macrophages. After incubation for 1 h, the culture medium was replaced, and the same strain of *P. aeruginosa* utilized in the animal experiments, PA103, was added to each well (bacteria/cell ratio of 50:1), followed by incubation at 37°C for 1 h. The supernatant was then discarded, and macrophages were washed five times with sterile PBS. Macrophages were examined under microscopy (×60 oil). Phagocytosis by the macrophages was measured by counting the number of bacteria inside the macrophages. Approximately 100 macrophages from each group of mice were examined to quantify phagocytosis.

Pretreatment with β6 integrin blocking antibody. To determine the acute effects of β6 blockade, wild-type mice were pretreated with anti-αvβ6 blocking antibody, 3G9 (1 and 3 mg/kg, same dose for control antibody) 16 h prior to bacterial challenge (26). The dosages used were the same as the doses that had produced significant protection in experiments utilizing bleomycin and radiation (6, 20). Mice were then anesthetized and treated as they had been in the lung injury experiments (see above).

Pretreatment with murine rsTGF-βRII-Fc. Wild-type mice were pretreated with rsTGF-βRII-Fc (2 mg/kg, i.p.) or with control IgG (2 mg/kg, i.p.) 24 h before bacterial challenge, and lung injury was measured according to the protocol used for the β6 antibody experiments.

BAL and plasma cytokine measurement. BAL and plasma were collected as described above. BAL was diluted five times, and plasma was diluted two times for concentration measurements. ELISAs were carried out with MIP-2, KC (i.e., neutrophil chemotactic protein), TNF-α, and IL-10 kits (R&D) according to the manufacturer's protocol.

Statistical analysis. A Student *t* test was used for statistical analysis, and a *P* value of <0.05 was considered significant.

RESULTS

β6 deletion was not protective in acute lung injury induced by *P. aeruginosa* in mice. β6-null mice appeared grossly similar to wild-type mice, with a slightly lighter body weight at baseline (a difference of ca. 0.5 to 1 g). At 4 h after bacterial instillation, both groups of mice appeared similar; both showed reduced activity, and their body temperatures dropped from 37.5 to 38°C to approximately 28°C without significant body weight changes. Figure 1A and B show the hematoxylin and eosin staining of the cell cytocentrifuge preparations from the wild-type mice with or without bacterial challenge. Figure 1C and D show the BAL cell staining in β6-null mice with or without bacterial challenge. The macrophages from the β6-null mice were much larger than the macrophages from the wild-type mice, as expected (17). Figure 1E shows the total BAL cell number from the wild-type and β6-null mice at baseline and after bacterial challenge. Clearly, there were more cells from the lungs of β6-null mice at any time point, and the cell numbers (Fig. 1G) document that, at basal conditions, the majority of the cells were macrophages (53%), with 12% neutrophils and 35% lymphocytes. The cells from the BAL taken from the wild-type mice were 98% macrophages, and 1% were lymphocytes and neutrophils, respectively. After *P. aeruginosa* instillation, large numbers of neutrophils were recruited into the lungs of both wild-type and β6-null mice, but significantly more neutrophils were present in the lungs of the β6-null mice. Figure 1F shows that there were also more neutrophils in the blood of the β6-null mice at baseline, and the number of blood neutrophils in β6-null mice decreased to the same level as that

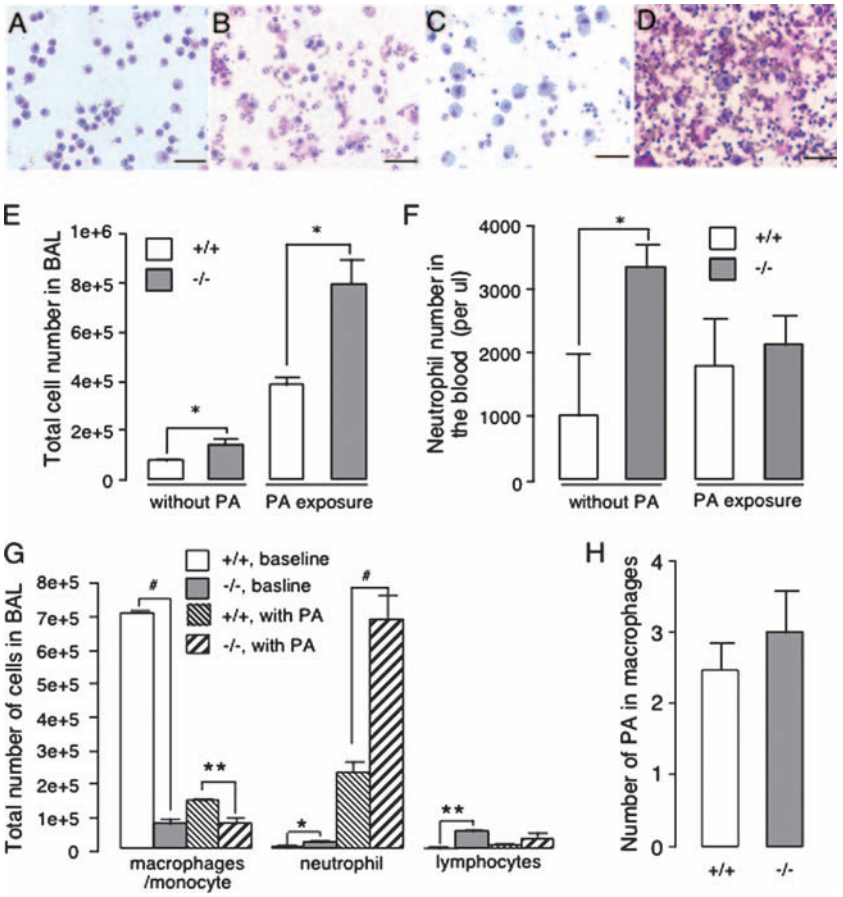

FIG. 1. Increased size and number but similar phagocytosis ability of alveolar macrophages before and after *P. aeruginosa* challenge in β6-null mice. (A and B) Cell cytospin image from wild-type mice before and after *P. aeruginosa* challenge; (C and D) cell cytospin image from β6-null mice before and after *P. aeruginosa* challenge; (E) total cell numbers from wild-type and β6-null mice after *P. aeruginosa* challenge; (F) neutrophil number in the blood in wild-type and β6-null mice before and after *P. aeruginosa* challenge; (G) total cell number from cytocentrifuge preparation of BAL fluid; (H) number of *P. aeruginosa* inside the macrophages. The open bar indicates the wild-type mice (+/+), and the shaded bar indicates the β6-null mice (−/−) in all figures except for panel G. Three to five mice were in each group. The data shown are means ± the standard error (SE). ⁎, $P < 0.05$; #, $P < 0.01$; ⁎⁎, $P < 0.02$. Scale bar: 100 μm. PA, *P. aeruginosa*.

in wild-type mice after *P. aeruginosa* challenge, suggesting that more neutrophils migrated into the lungs of the β6-null mice.

BAL protein has been used as an indicator for epithelial permeability (27). Figure 2A shows that the BAL protein concentrations were comparable between the wild-type and the β6-null mice before and after *P. aeruginosa* challenge. These results indicate that the deletion of β6 did not affect the lung epithelial permeability. Deletion of β6 also did not affect the lung endothelial permeability (Fig. 2B). Excess lung water was slightly higher in the β6-null mice at baseline and was significantly increased after the *P. aeruginosa* challenge (76.6 and 95.9 μl in wild-type and β6-null mice, respectively [*, $P < 0.05$]; Fig. 2C). Interestingly, although the percentage of positive blood cultures was the same in the two groups of mice (20% positive cultures, with similar numbers of bacterial CFU), the number of bacteria remaining in the lungs after 4 h was significantly reduced in the β6-null mice (1.6×10^6 CFU and 4.2×10^6 CFU in β6-null and wild-type mice, respectively [#, $P < 0.01$]; Fig. 2D).

Four cytokines—KC, IL-10, TNF-α, and MIP-2—were measured in the BAL and in the plasma samples from mice with or without bacterial instillation. We chose these cytokines because KC and MIP-2 are potent neutrophil chemoattractant chemokines, IL-10 is an anti-inflammation cytokine, and TNF-α is a known early proinflammatory cytokine that documents the severity of lung inflammation. Figure 3A demonstrates a higher KC concentration in the BAL of β6-null mice than that in the wild-type mice at basal conditions (*, $P < 0.05$). Although the BAL KC levels were higher in the β6-null mice after *P. aeruginosa* instillation, there were no significant differences compared to the results from the wild-type mice. BAL IL-10 concentrations were lower in the β6-null mice compared to the concentrations in the wild-type mice with or without *P. aeruginosa* instillations. There were no significant differences in plasma IL-10 concentrations at baseline and after the *P. aeruginosa* instillations between wild-type and β6-null mice (Fig. 3B). Figure 3C and D show similar levels of TNF-α and MIP-2 in the BAL and in the plasma samples obtained from wild-type and β6-null mice with or without *P. aeruginosa* instillations.

In vitro analysis shows that macrophage phagocytosis was not different between wild-type and β6-null mice. The isolated macrophages from the β6-null mice were larger than those

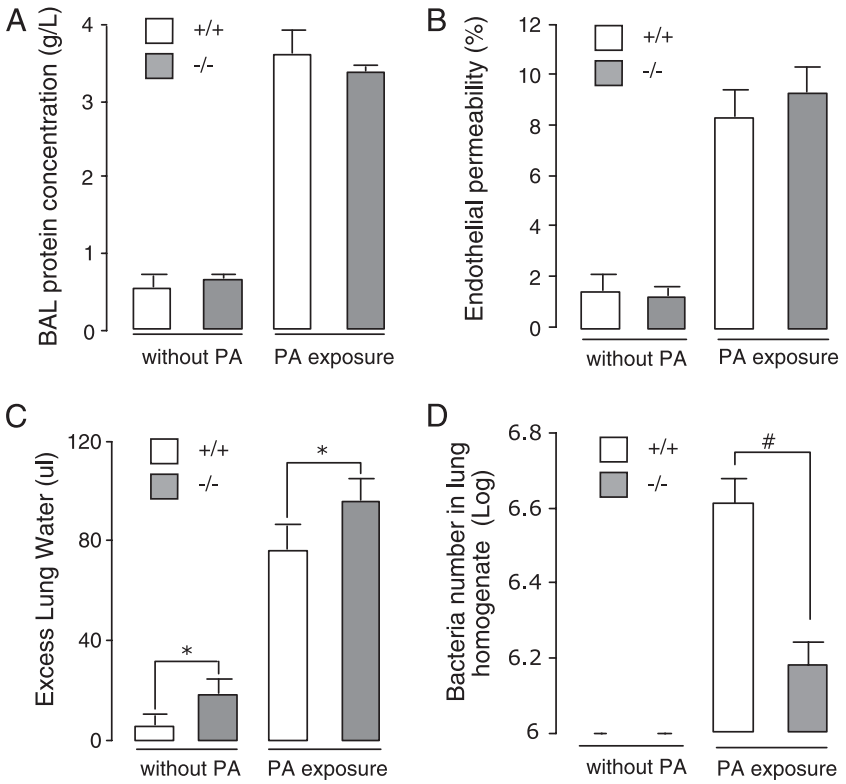

FIG. 2. β6 deletion was not protective in acute lung injury induced by *P. aeruginosa* instillation. (A) BAL protein concentration in wild-type and β6-null mice with or without *P. aeruginosa* challenge; (B) endothelial permeability in wild-type and in β6-null mice with or without *P. aeruginosa* challenge; (C) excess lung water in wild-type and in β6-null mice with or without *P. aeruginosa* challenge; (D) bacterial number in lung homogenates from wild-type and from β6-null mice with or without *P. aeruginosa* challenge. The open bar indicates wild-type mice (+/+), and the shaded bar indicates β6-null mice (−/−). Five mice were in each group. The data shown are means ± the SE. *, $P < 0.05$; #, $P < 0.01$. PA, *P. aeruginosa*.

from wild-type mice, as has been demonstrated previously (17). After 1 h of incubation, more than 95% of the macrophages were still attached to the bottom of the plates. Coculture of the macrophages with *P. aeruginosa* showed that ca. 20 to 30% of the macrophages detached from the plates. The macrophages remaining on the bottom of the plates were used to quantify the phagocytosis of the bacteria. Although the macrophages isolated from the β6-null mice were larger, their ability to ingest the PA103 was not significantly increased (Fig. 1H).

Pretreatment with αvβ6 integrin blocking antibody was not protective in acute lung injury induced by *P. aeruginosa*. There were no significant differences between the endothelial permeability, excess lung water, and bacterial colony counts in lung homogenates and in blood samples obtained from wild-type mice treated with β6 blocking or treated with control antibody that had been instilled with *P. aeruginosa* PA103 (Fig. 4). When the mice were pretreated with a high dose of β6 blocking antibody (3 mg/kg), there were still no significant differences in the severity of lung injury between the animals that had received the control antibody or that had received β6 blocking antibody pretreatment (data not shown). The percentages of positive blood cultures, as well as the average numbers of bacteria CFU, were comparable between the control mice and the αvβ6 antibody-treated mice. Acute αvβ6 integrin inhibition did not protect the animals from the acute lung injury induced by *P. aeruginosa* PA103.

To exclude the possibility that the PA103 strain so massively damaged the lungs of the animals that the possible protective effect from αvβ6 inhibition was obscured, we repeated the experiments with a less virulent strain, PAK, which secretes ExoS but not ExoU. We also studied the animals instilled with PAK for a longer duration (8 h). The results indicate that there were no differences in the *P. aeruginosa*-induced lung injury measured in mice that had received control antibody or in those that had received β6 blocking antibody (Fig. 5). Furthermore, survival rates were the same between β6 antibody- and control antibody-treated mice (Fig. 6).

Pretreatment with soluble chimeric TGF-β receptor was not protective in acute lung injury induced by *P. aeruginosa*. There were no significant differences between the endothelial permeability, excess lung water, and bacterial colony counts in the lung homogenates and blood samples obtained from the mice that were pretreated with rsTGF-βRII-Fc and the mice that had received the control antibody (Fig. 7). The percentages of positive blood cultures, as well as the average numbers of bacteria CFU, were comparable between the control mice and the soluble chimeric TGF-β receptor-treated mice. These results indicate that the inhibition of TGF-β did not protect against *P. aeruginosa*-induced lung injury and also that the injury was not worse in mice that received the anti-inflammatory therapeutic treatment.

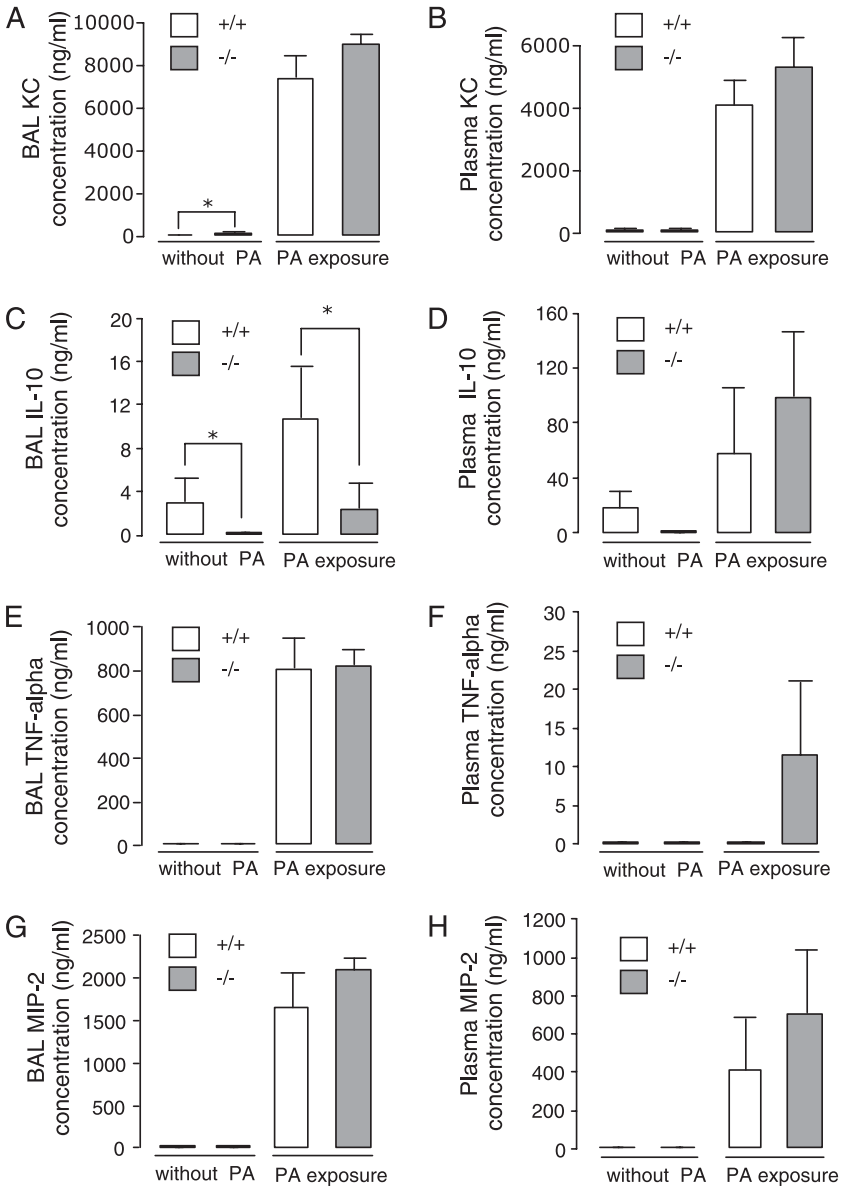

FIG. 3. Cytokine concentrations in BAL and blood from wild-type and β6-null mice before and after *P. aeruginosa* challenge. (A, C, E, and G) Cytokine concentrations in BAL samples; (B, D, F, and H) cytokine concentrations in plasma samples. Panels A and B show KC chemokine concentrations, panels C and D show TNF-α concentrations, panels E and F show IL-10 concentrations, and panels G and H show MIP-2 concentrations. The open bar indicates wild-type mice (+/+), and the shaded bar indicates β6-null mice (−/−). Three to five mice were in each group. The data shown are means ± the SE. *, $P < 0.05$. PA, *P. aeruginosa*.

DISCUSSION

The main finding of this study is that integrin β6 deletion or antibody blockade of TGF-β did not increase the susceptibility of the host to *P. aeruginosa* pneumonia, nor do these interventions protect mice from *P. aeruginosa*-induced lung injury. The role of αvβ6 integrin in lung injury and/or fibrosis has been investigated using β6-null mice and by acutely blocking αvβ6 with an antibody. Deletion of the β6 gene protected against lung injury induced by bleomycin, LPS, and high tidal volume ventilation (11, 18, 19). Why then was β6 gene deletion not protective against lung injury induced by live *P. aeruginosa*? First, live bacteria cause lung injury through several mechanisms that cannot be replicated by the instillation of endotoxin alone (20), and LPS does not lead to the same degree of lung injury as seen with live bacteria (13). Therefore, LPS-induced lung injury is not a good surrogate for infection-induced lung injury.

It appears that more bacteria were eliminated in the lungs of the β6-null mice, since significantly fewer bacteria were cultured in the lungs of the β6-null mice after 4 h. The increased killing might have occurred because of the increased number of inflammatory cells, including macrophages and neutrophils, in the lungs of these mice. We could not demonstrate that phagocytosis by the β6-null macrophages was superior or inferior to that of the wild-type macrophages. However, the

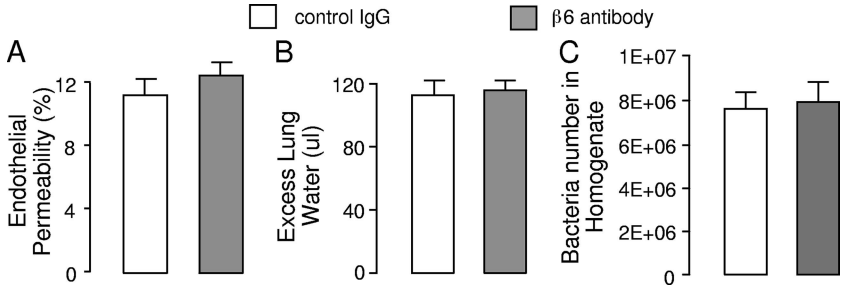

FIG. 4. αvβ6 inhibition was not protective in acute lung injury induced by *P. aeruginosa*. (A) Endothelial permeability in wild-type mice treated with or without αvβ6 antibody (1 mg/kg); (B) excess lung water in wild-type mice with or without αvβ6 antibody pretreatment (1 mg/kg); (C) *P. aeruginosa* number in lung homogenate 4 h after *P. aeruginosa* instillation. The open bar indicates the control IgG group, and the shaded bar indicates the αvβ6 antibody group. Eight to ten mice were in each group. The data shown are means ± the SE.

presence of these inflammatory cells may have also led to increased lung injury in the infected β6-null mice. KC and MIP-2 are two potent chemoattractant chemokines that recruit neutrophils into the alveolar spaces in mice (21). Cytokine concentration measurements from the BAL documented elevated basal KC BAL concentrations in the β6-null mice compared to levels in the BAL from wild-type mice, which may help explain why more neutrophils were in the lungs at baseline in the β6-null mice. BAL IL-10 concentrations in the β6-null mice were lower than in the BAL from the wild-type mice before and after the *P. aeruginosa* instillations, which may also explain why there were more neutrophils in the lungs of the β6-null mice given the nature of IL-10 as an anti-inflammatory cytokine. We have found that recombinant IL-10 pretreatment improved oxygenation and hemodynamics, decreased bacteremia, and improved survival in rabbits instilled with the same *P. aeruginosa* strain, PA103, that was utilized in the present experiments (22). Therefore, the decreased IL-10 levels in the β6-null mice may also explain why these animals did not have an improved outcome with this infection. The TNF-α and MIP-2 plasma levels were comparable between the wild-type and β6-null mice before and after bacteria challenge. However, there were more inflammatory cells, elevated KC concentrations, decreased IL-10 concentrations, and increased numbers of neutrophils in the blood of the β6-null animals; all of these factors may have contributed to the lack of protection against lung injury after *P. aeruginosa* instillation in the β6-null mice.

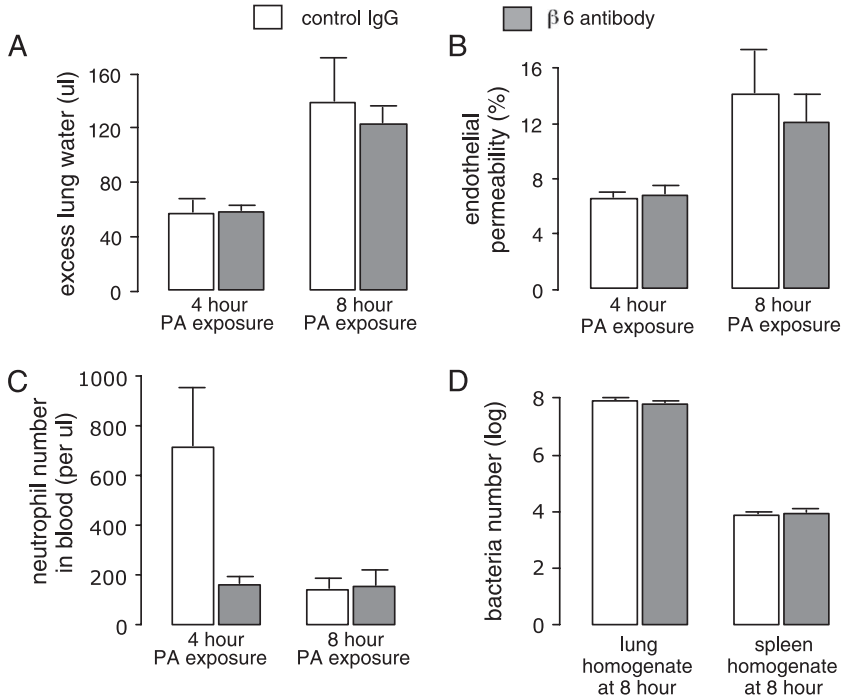

FIG. 5. αvβ6 inhibition was not protective in acute lung injury induced by *P. aeruginosa* (PAK strain). (A to C) Excess lung water, endothelial permeability, and neutrophil number in blood in control IgG- and β6 antibody-treated mice at 4 and 8 h after bacterial instillation; (D) bacterial number in lung and spleen homogenate in control and αvβ6 antibody-treated mice at 8 h after bacterial instillation. The open bar indicates the control IgG group, and the shaded bar indicates the αvβ6 antibody-treated group. Four to five mice were in each group. The data shown are means ± the SE. PA, *P. aeruginosa*.

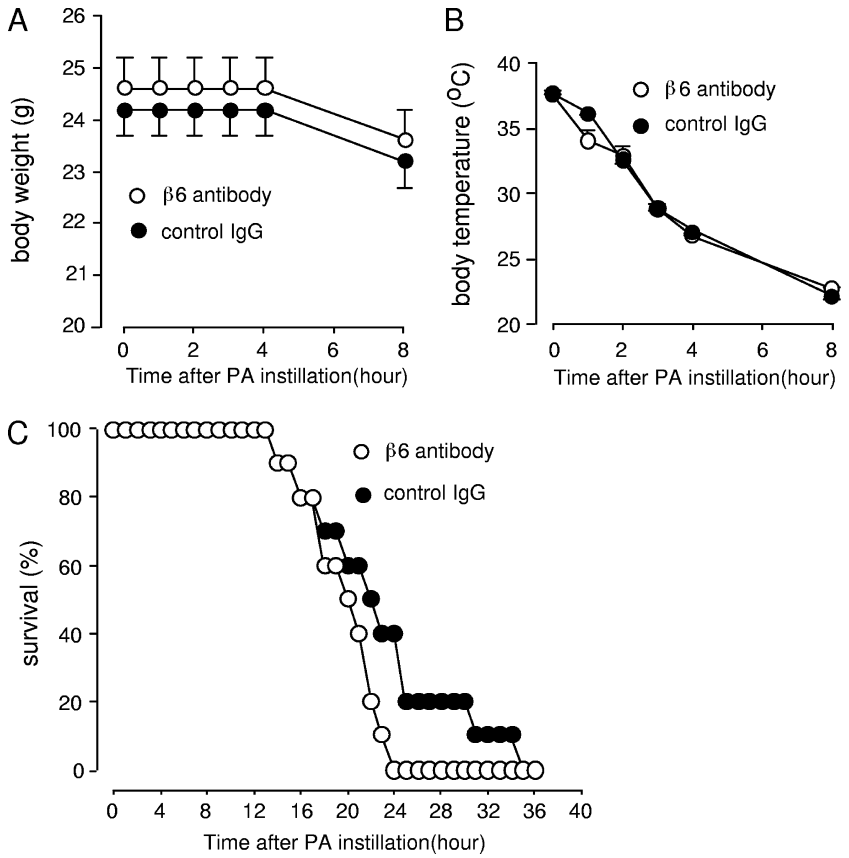

FIG. 6. Survival study in control IgG and β6 antibody-treated mice after *P. aeruginosa* (PAK strain) instillation. (A) Body weight changes in control IgG- and β6 antibody-treated mice; (B) body temperature changes in control IgG- and β6 antibody-treated mice; (C) survival curves in control IgG- and β6 antibody-treated mice. Symbols: ○, β6 antibody-treated mice; ●, control IgG-treated mice. Ten mice were in each group. The data shown are means ± the SE. PA, *P. aeruginosa*.

TGF-β is an important factor involved in extracellular matrix deposition and tissue repair after the acute phase of lung injury. TGF-β suppresses the activation of macrophages in the alveolar space under normal conditions and is also involved in inflammatory response upon various insults. Integrin αvβ6 has been shown to bind and activate TGF-β (18). TGF-β has three isoforms: TGF-β1, TGF-β2, and TGF-β3. TGF-β is synthesized as a latent form at a high concentration in extracellular matrix and can be activated through many pathways, including thrombospondin and the αvβ6 pathway (11, 18). The main functions of TGF-β include the inhibition of epithelial proliferation, the induction of gene expression of extracellular matrix genes, and the inhibition of metalloprotease genes (2, 18). The deletion of TGF-β is lethal and causes widespread tissue inflammation (12). Overexpression of TGF-β increases endothelial permeability, decreases apical expression of ENaC, and

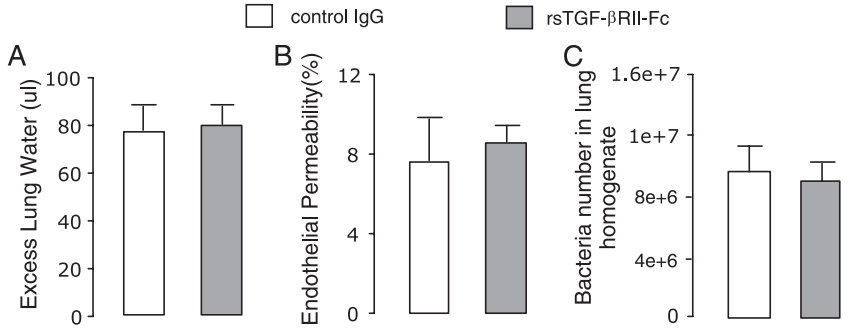

FIG. 7. TGF-β inhibition with rsTGF-βRII-Fc was not protective in acute lung injury induced by *P. aeruginosa*. (A) Excess lung water in wild-type mice with or without rsTGF-βRII-Fc pretreatment; (B) endothelial permeability in wild-type mice with or without rsTGF-βRII-Fc pretreatment; (C) bacterial number in lung homogenate with or without rsTGF-βRII-Fc pretreatment. The open box indicates the control IgG group, and the shaded box indicates the rsTGF-βRII-Fc group. Eight to ten mice were in each group. The data shown are means ± the SE.

increases apoptosis of alveolar epithelial cells, suggesting that the blockade of TGF-β may be beneficial in acute lung injury. Interestingly, our study has shown that the deletion of β6 or blocking either αvβ6 or TGF-β did not protect against lung injury induced by two different strains of *P. aeruginosa*. PA103 is a laboratory strain that secretes the potent virulence factor ExoU, whereas PAK secretes ExoS. There is a possibility that massive damage of the lungs induced by PA103 could have masked a protective effect of αvβ6 inhibition. However, we did not see any protection with the inhibition of αvβ6 when mice were challenged with either bacterial strain, suggesting that the lack of protection from lung injury does not depend on the type of *P. aeruginosa* strain.

In summary, inflammation is a necessary component of bacterial killing and, therefore, prevention of inflammation can cripple host defense. Although acute blockade of αvβ6 and TGF-β did not protect the mice from *P. aeruginosa*-induced lung injury, the lung injury was not significantly increased, nor was bacterial dissemination increased in these short-term or survival experiments. Anti-inflammatory agents should always be tested in experiments in which live bacteria are utilized to determine their effect on host defense.

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REFERENCES

1. **Breuss, J. M., J. Gallo, H. M. DeLisser, I. V. Klimanskaya, H. G. Folkesson, J. F. Pittet, S. L. Nishimura, K. Aldape, D. V. Landers, W. Carpenter, et al.** 1995. Expression of the beta 6 integrin subunit in development, neoplasia and tissue repair suggests a role in epithelial remodeling. J. Cell Sci. **108:** 2241–2251.
2. **Dhainaut, J. F., J. Charpentier, and J. D. Chiche.** 2003. Transforming growth factor-beta: a mediator of cell regulation in acute respiratory distress syndrome. Crit. Care Med. **31:**S258–S264.
3. **Frank, J., J. Roux, H. Kawakatsu, G. Su, A. Dagenais, Y. Berthiaume, M. Howard, C. M. Canessa, X. Fang, D. Sheppard, M. A. Matthay, and J. F. Pittet.** 2003. Transforming growth factor-β1 decreases expression of the epithelial sodium channel alpha ENaC and alveolar epithelial vectorial sodium and fluid transport via an ERK1/2-dependent mechanism. J. Biol. Chem. **278:**43939–43950.
4. **Giannoni, E., T. Sawa, L. Allen, J. Wiener-Kronish, and S. Hawgood.** 2006. Surfactant proteins A and D enhance pulmonary clearance of *Pseudomonas aeruginosa*. Am. J. Respir. Cell Mol. Biol. **34:**704–710.
5. **Hijazi, M. H., and N. R. MacIntyre.** 2000. Advances in infection control: ventilator-associated pneumonia. Semin. Respir. Crit. Care Med. **21:**245–262.
6. **Horan, G. S., S. Wood, V. Ona, D. J. Li, M. E. Lukashev, P. H. Weinreb, K. J. Simon, K. Hahm, N. E. Allaire, N. J. Rinaldi, J. Goyal, C. A. Feghali-Bostwick, E. L. Matteson, C. O'Hara, R. Lafyatis, G. S. Davis, X. Huang, D. Sheppard, and S. M. Violette.** 2008. Partial inhibition of integrin αvβ6 prevents pulmonary fibrosis without exacerbating inflammation. Am. J. Respir. Crit. Care Med. **177:**56–65.
7. **Huang, X. Z., J. F. Wu, D. Cass, D. J. Erle, D. Corry, S. G. Young, R. V. Farese, Jr., and D. Sheppard.** 1996. Inactivation of the integrin beta 6 subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin. J. Cell Biol. **133:**921–928.
8. **Huang, X., J. Wu, W. Zhu, R. Pytela, and D. Sheppard.** 1998. Expression of the human integrin β6 subunit in alveolar type II cells and bronchiolar epithelial cells reverses lung inflammation in beta6 knockout mice. Am. J. Respir. Cell Mol. Biol. **19:**636–642.
9. **Hurst, V. I. V., P. L. Goldberg, F. L. Minnear, R. L. Heimark, and P. A. Vincent.** 1999. Rearrangement of adherens junctions by transforming growth factor-β1: role of contraction. Am. J. Physiol. **276:**L582–L595.
10. **Hynes, R. O.** 1992. Integrins: versatility, modulation, and signaling in cell adhesion. Cell **69:**11–25.
11. **Jenkins, R. G., X. Su, G. Su, C. J. Scotton, E. Camerer, G. J. Laurent, G. E. Davis, R. C. Chambers, M. A. Matthay, and D. Sheppard.** 2006. Ligation of protease-activated receptor 1 enhances αvβ6 integrin-dependent TGF-β activation and promotes acute lung injury. J. Clin. Investig. **116:**1606–1614.
12. **Kulkarni, A. B., C. G. Huh, D. Becker, A. Geiser, M. Lyght, K. C. Flanders, A. B. Roberts, M. B. Sporn, J. M. Ward, and S. Karlsson.** 1993. Transforming growth factor β1-null mutation in mice causes excessive inflammatory response and early death. Proc. Natl. Acad. Sci. USA **90:**770–774.
13. **Kurahashi, K., O. Kajikawa, T. Sawa, T., M. Ohara, M. A. Gropper, D. W. Frank, T. R. Martin, and J. P. Wiener-Kronish.** 1999. Pathogenesis of septic shock in *Pseudomonas aeruginosa* pneumonia. J. Clin. Investig. **104:**743–750.
14. **Lee, C. G., S. J. Cho, M. J. Kang, S. P. Chapoval, P. J. Lee, P. W. Noble, T. Yehualaeshet, B. Lu, R. A. Flavell, J. Milbrandt, R. J. Homer, and J. A. Elias.** 2004. Early growth response gene 1-mediated apoptosis is essential for transforming growth factor β1-induced pulmonary fibrosis. J. Exp. Med. **200:**377–389.
15. **Lin, J., D. Ziring, S. Desai, S. Kim, M. Wong, Y. Korin, J. Braun, E. Reed, D. Gjertson, and R. R. J. Singh.** 2008. TNFα blockade in human diseases: an overview of efficacy and safety. Clin. Immunol. **126:**13–30.
16. **Lipiner-Friedman, D., C. L. Sprung, P. F. Laterre, Y. Weiss, S. V. Goodman, M. Vogeser, J. Briegel, D. Keh, M. Singer, R. Moreno, E. Bellissant, D. Annane, et al.** 2007. Adrenal function in sepsis: the retrospective Corticus cohort study. Crit. Care Med. 2007 **35:**1012–1018.
17. **Morris, D. G., X. Huang, N. Kaminski, Y. Wang, S. D. Shapiro, G. Dolganov, A. Glick, and D. Sheppard.** 2003. Loss of integrin αvβ6-mediated TGF-β activation causes MMP12-dependent emphysema. Nature **422:**169–173.
18. **Munger, J. S., X. Huang, H. Kawakatsu, M. J. Griffiths, S. L. Dalton, J. Wu, J. F. Pittet, N. Kaminiski, C. Garat, M. A. MAtthay, D. B. Rifkin, and D. Sheppard.** 1999. The integrin αvβ6 binds and activates latent TGF-β1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell **96:**319–328.
19. **Pittet, J. F., M. J. Griffiths, T. Geiser, N. Kaminiski, S. L. Dalton, X. Huang, L. A. Brown, P. J. Gotwals, V. E. Koteliansky, M. A. Matthay, and D. Sheppard.** 2001. TGF-β is a critical mediator of acute lung injury. J. Clin. Investig. **107:**1537–1544.
20. **Puthawala, K., N. Hadjiangelis, S. C. Jacoby, E. Bayongan, Z. Zhao, Z. Yang, M. L. Devitt, G. S. Horan, P. H. Weinreb, M. E. Lukashev, S. M. Violette, K. S. Grant, C. Colarossi, S. C. Formenti, and J. S. Munger.** 2008. Inhibition of integrin αvβ6, an activator of latent TGFβ, prevents radiation-induced lung fibrosis. Am. J. Respir. Crit. Care Med. **177:**82–90.
21. **Rovai, L. E., H. R. Herschman, and J. B. Smith.** 1998. The murine neutrophil-chemoattractant chemokines LIX, KC, and MIP-2 have distinct induction kinetics, tissue distributions, and tissue-specific sensitivities to glucocorticoid regulation in endotoxemia. J. Leukoc. Biol. 64:**4:** 494–502.
22. **Sawa, T., D. B. Corry, M. A. Gropper, M. Ohara, K. Kurahashi, and J. P. Wiener-Kronish.** 1997. IL-10 improves lung injury and survival in *Pseudomonas aeruginosa* pneumonia. J. Immunol. 15:**159:**2858–2866.
23. **Sawa, T., T. L. Yahr, M. Ohara, Kurahashi, K., M. A. Gropper, J. P. Wiener-Kronish, and D. W. Frank.** 1999. Active and passive immunization with the *Pseudomonas* V antigen protects against type III intoxication and lung injury. Nat. Med. **5:**392–398.
24. **Sheppard, D.** 2004. Roles of αv integrins in vascular biology and pulmonary pathology. Curr. Opin. Cell Biol. **16:**552–557.
25. **Sheppard, D.** 2006. Transforming growth factor β: A central modulator of pulmonary and airway inflammation and fibrosis. Proc. Am. Thorac. Soc. **3:**413–417.
26. **Weinreb, P. H., K. J. Simon, P. Rayhorn, W. J. Yang, D. R. Leone, B. M. Dolinski, B. R. Pearse, Y. Yokota, H. Kawakatsu, A. Atakilit, D. Sheppard, and S. M. Violette.** 2004. Function-blocking integrin αvβ6 monoclonal antibodies: distinct ligand-mimetic and nonligand-mimetic classes. J. Biol. Chem. **279:**17875–17887.
27. **Wesselkamper, S. C., L. C. Chen, and T. Gordon.** 2001. Development of pulmonary tolerance in mice exposed to zinc oxide fumes. Toxicol. Sci. **60:**144–151.

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