Bleomycin-induced pulmonary injury in mice deficient in SPARC

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1Division of Neonatology, Department of Pediatrics and 2Division of Pulmonary Allergy and Critical Care, Department of Medicine, University of Pennsylvania, and 3The Wistar Institute, Philadelphia, Pennsylvania 19104

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Savani, Rashmin C., Zhao Zhou, Evgenia Arquiuri, Sunny Wang, Dinh Vu, Chin C. Howe, and Horace M. DeLisser. Bleomycin-induced pulmonary injury in mice deficient in SPARC. Am J Physiol Lung Cell Mol Physiol 279: L743–L750, 2000.—SPARC (secreted protein, acidic and rich in cysteine) is a component of the matrix that appears to regulate tissue remodeling. There is evidence that it accumulates in the lung in the setting of pulmonary injury and fibrosis, but direct evidence of its involvement is only now emerging. We therefore investigated the development of pulmonary fibrosis induced by bleomycin administered either intratracheally or intraperitoneally in mice deficient in SPARC. Bleomycin (0.15 U/mouse) given intratracheally induced significantly more pulmonary fibrosis in mice deficient in SPARC compared with that in wild-type control mice, with the mutant mice demonstrating greater neutrophil accumulation in the lung. However, in wild-type and SPARC-deficient mice given intraperitoneal bleomycin (0.8 U/injection \( \times \) 5 injections over 14 days), the pattern and severity of pulmonary fibrosis, as well as the levels of leucocyte recruitment, were similar in both strains of mice. These findings suggest that the involvement of SPARC in pulmonary injury is likely to be complex, dependent on several factors including the type, duration, and intensity of the insult. Furthermore, increased neutrophil accumulation in the peritoneal cavity was also observed in SPARC-null mice after acute chemical peritonitis. Together, these data suggest a possible role for SPARC in the recruitment of neutrophils to sites of acute inflammation.

secreted protein, acidic and rich in cysteine; inflammation; neutrophils; lung fibrosis

A LARGE NUMBER OF INHALED agents, systemic medications, autoimmuno processes and infections (local or distant), as well as unknown causes, may result in a pattern of lung injury that culminates in the development of pulmonary fibrosis (6, 12, 17). The host response to these insults involves stimulated or enhanced secretory, proliferative, and migratory activities on the parts of a number of cell types (2, 9, 25). This includes activation of peripheral blood leukocytes and their subsequent recruitment to sites of injury as well as increased fibroblast proliferation and collagen accumulation. These various cellular responses are dependent on interactions with constituents of the extracellular matrix, including fibronectin and hyaluronan (10, 21).

Another important element that appears to be involved in regulating cellular responses in the matrix of wounded and/or remodeling tissues is SPARC (secreted protein, acidic and rich in cysteine), also known as osteonectin or BM-40 (8, 18). SPARC and two other structurally dissimilar proteins, tenascin and thrombospondin, constitute a group of counteradhesive macromolecular molecules that mediate cell-matrix interactions but do not have a structural role in adult tissues. Platelets, macrophages, fibroblasts, and endothelial cells are sources of SPARC, and transforming growth factor-\( \beta \) and platelet-derived growth factor regulate its synthesis and secretion (5). Reported effects of SPARC on in vitro cell activity and function include the loss of focal adhesions, inhibition of cell spreading, chemotaxis, and proliferation and stimulation of matrix metalloproteases and plasminogen activator-1 (5, 8, 19). These observations have led to the proposal that SPARC regulates cell proliferation and migration in vivo. There is evidence that SPARC accumulates in the lung in the setting of pulmonary injury and fibrosis (7, 23), and it has recently been reported that mice deficient in SPARC demonstrate less collagen content in a murine model of pulmonary fibrosis induced by the intratracheal instillation of low-dose (0.075 U/mouse) bleomycin (23).

To investigate the role of SPARC in acute and chronic lung injury, we also studied the development of pulmonary fibrosis induced by bleomycin administered intratracheally or intraperitoneally to mice deficient in SPARC (14). We observed that high-dose bleomycin (0.15 U/mouse) given intratracheally induced more pulmonary fibrosis in SPARC-deficient mice than it did in wild-type control mice, with the mutant mice manifesting evidence of greater neutrophil accumulation in the lung. However, in wild-type and SPARC-deficient...
mice given intraperitoneal bleomycin (0.8 U/injection × 5 injections over 14 days), the pattern and severity of pulmonary fibrosis as well as the levels of leukocyte recruitment were similar in both strains of mice. These findings, along with those reported previously (23), suggest that the involvement of SPARC in pulmonary injury is likely to be complex and context specific, depending on a number of factors that include the nature, duration, and intensity of the insult. Additionally, because SPARC-deficient mice were found to have more neutrophils after acute chemical peritonitis than wild-type mice, our data implicate SPARC in the recruitment of neutrophils to sites of acute inflammation in the lung and abdomen.

**METHODS**

**Bleomycin-induced lung injury.** All animal experiments were approved by the Institutional Animal Care and Utilization Committee of the University of Pennsylvania. For these studies, pathogen-free 8- to 12-wk-old (weight, 18–22 g) matched male and female homozygous SPARC-null (SPARC(−/−)) mice (14) on a 129SvEv background (129SvEv × C57BL/6J backcrossed four times to 129SvEv) and 129SvEv wild-type SPARC(+/+) mice (Taconic, Germantown, NY) were used. The exception to this were the studies in which the mice were killed on day 10 after intratracheal bleomycin. These experiments were done with SPARC(+/+) and SPARC(−/−) mice maintained on a mixed genetic background (F2) of 129SvEv × C57BL/6J. The background of these latter mice was similar to those used by Strandjord et al. (23). These mice were treated with bleomycin sulfate (Bristol-Myers Squibb, Princeton, NJ). Some were injected intratracheally (4) (0.15 U/mouse in 50 μl of saline) after exposure of the trachea under ketamine anesthesia, and the mice were killed on days 7, 10, and 14 after treatment. Other mice received bleomycin injected intraperitoneally (4) (0.8 U/mouse in 500 μl of saline) on days 0, 3, 7, 10, and 14 and were killed on day 28. Control wild-type and null mice received an equivalent volume of saline alone administered in the same manner for each group. Preliminary experiments in mice of a comparable genetic background demonstrated that the doses of bleomycin used in our studies consistently produced pulmonary fibrosis with a low mortality rate (<10% for intraperitoneal and <20% for intratracheal bleomycin) that was very comparable to what other published reports (15, 16, 24) that used C57BL/6 mice have noted. After death, the main pulmonary artery of each animal was flushed with PBS to clear the lungs of blood before their removal from the chest.

**Histology.** For each animal, the lungs were inflated with 0.8–1.0 ml of Formalin, and the trachea was ligated and then fixed for 24 h in Formalin under vacuum. Five-micrometer sections were stained with hematoxylin and eosin for assessment of lung morphology or with trichrome to identify collagen.

**Hydroxyproline assay.** Lung hydroxyproline (HP) content was measured as an index of collagen content in whole mouse lungs according to methods previously described (28), with modifications. After perfusion of the lungs with PBS, they were removed, weighed and minced, and then hydrolyzed in 2 ml of 6 N HCl at 110°C overnight. The resulting hydrolysate was neutralized with 2 ml of 6 N NaOH filtered through a 0.45-mm nylon membrane. One hundred microliters of this solution were then added to 1 ml of 1.4% chloramine T (Sigma, St. Louis, MO), 10% n-propanol, and 0.5 M sodium acetate, pH 6.0. After a 20-min incubation at room temperature, 1 ml of Ehrlich’s reagent (1 M p-dimethylaminobenzaldehyde in 70% n-propanol and 20% perchloric acid) was added, and the resulting solution was incubated at 65°C for 15 min. Absorbance was then measured at 550 nm, and the amount of HP was determined against a standard curve produced with the use of known concentrations of HP.

**Myeloperoxidase assay.** Myeloperoxidase (MPO) activity was assessed with previously described methods (3). After perfusion to remove blood, whole lungs were removed, blotted dried, weighed, and then homogenized for 30 s in 1 ml of 0.1 mM phosphate (K2HPO4) buffer, pH 7.0. The homogenate was then centrifuged for 30 min at 40,000 g and 4°C, and the resulting pellet was resuspended in K2HPO4 buffer and stored at −70°C until the MPO assay was performed. Frozen samples were subsequently thawed, sonicated for 90 s, incubated for 1 h in a 60°C water bath, and then centrifuged at 12,000 g for 30 min at 4°C. One hundred microliters of the resulting supernatant were then mixed with 250 μl of 0.1 mM K2HPO4 buffer, pH 7.0, and 300 μl of Hanks’ balanced salt solution (HBSS) containing 0.25% BSA, followed by the addition of 50 μl of 1.25 mg/ml of o-dianisidine (Sigma) and 50 μl of 0.05% H2O2. The resulting solution was incubated at room temperature, and the reaction was terminated after 15 min by the addition of 50 μl of 1% sodium azide. The change in absorbance at 460 nm over this time period was taken as the index of MPO activity.

**Glucosaminidase assay.** To quantify the accumulation of macrophages, the activity of the macrophage lysosomal enzyme N-acetyl-β-glucosaminidase was determined. The activity of this enzyme has previously been used to measure macrophage-derived cell content in the brain (13). A spectrophotometric microtiter plate assay was used to quantify enzymatic release of p-nitrophenol as described previously for astroglial cells (13). Briefly, 20 μl of standards (purified N-acetyl-β-glucosaminidase at 0–200 μU total activity; Sigma) or lung samples prepared as described for the MPO assay were plated and mixed with 10 μl of Triton X-100. Twenty microliters of p-nitrophenol-conjugated N-acetyl-β-glucosaminide (a 24 mM solution prepared by combining 100 mg of the glucosaminide with 0.5 ml of n-propanol and 12.5 ml of McIlvain’s buffer, pH 4.6) (11, 20) were used as the substrate, and the plate was incubated at 37°C for 30 min. The reaction was stopped by the addition of 200 μl of 0.2 M sodium carbonate, and absorbance was measured at 405 nm. The standard curve generated was used to calculate the glucosaminidase activity of the samples.

**Thioglycolate-induced peritonitis.** For these studies, pathogen-free 8- to 12-wk-old (weight, 18–22 g) matched male and female SPARC(+/+) and SPARC(−/−) mice maintained on a mixed genetic background (F2) of 129SvEv × C57BL/6J were used. The procedures used to induce peritonitis were based on those described by Baron and Proctor (1). Three milliliters of calcium- and magnesium-free HBSS, pH 7.4, or thioglycolate broth (obtained from the Media Kitchen at the Wistar Institute, Philadelphia, PA) were injected intraperitoneally into each mouse. Four hours after intraperitoneal injection, the mice were killed by exposure to CO2, and the abdominal cavities were lavaged with 5 ml of ice-cold HBSS to recover the peritoneal cells. The volume recovered was noted, total cell concentration was determined with a Coulter counter (Hialeah, FL), and differential counts were performed on Wright-Giemsa-stained cytospin preparations.

**Statistical analysis.** Statistical comparisons between the various groups were carried out with ANOVA with Fisher’s exact test. P < 0.05 was considered significant.

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Fig. 1. Histopathology and lung collagen trichrome staining of lungs from wild-type [SPARC(+/+)] and SPARC-deficient [SPARC(−/−)] mice after intratracheal bleomycin or saline treatment. SPARC, secreted protein, acidic and rich in cysteine. Data are for mice killed on day 14 after treatment. A, C, E, and G: low magnification (originally ×100). B, D, F, and H: high magnification (originally ×200). Lung architecture was not altered after intratracheal saline treatment, and appreciable collagen staining was associated only with large vessels or airways (A–D, solid arrows). Bleomycin in SPARC(+/+) mice resulted in fibrosis characterized by subpleural and perivascular fibrotic lesions (E and F, open arrows), whereas in SPARC(−/−) animals, intratracheal bleomycin caused destruction of the normal alveolar organization with extensive collagen deposition and fibrosis (G and H).
RESULTS

Pulmonary fibrosis in SPARC(−/−) mice after intratracheal bleomycin administration. To investigate the potential involvement of SPARC in the host response to acute lung injury, we initially studied the development of pulmonary fibrosis in SPARC(+/+) and SPARC(−/−) mice 14 days after the intratracheal administration of bleomycin (0.15 U/mouse). Lung fibrosis in these mice was initially assessed histologically by the staining of paraffin-embedded tissue sections with hematoxylin and eosin (data not shown) and collagen-specific Masson’s trichrome stain. In mice (wild-type and mutant) given saline, the lung architecture was preserved, with appreciable collagen staining detected only in bands immediately adjacent to or associated with large vessels and airways (Fig. 1, A–D). This pattern did not differ from that in nontreated control mice (data not shown). For the SPARC(+/+) mice, intratracheal instillation of bleomycin resulted in loss of some of the normal alveolar organization, modest thickening of the alveolar walls, and focal fibrotic lesions, primarily in subpleural and perivascular areas (Fig. 1, E and F). In contrast to the wild-type mice, the SPARC(−/−) mice demonstrated a visibly greater fibrotic response that was characterized by marked distortion and destruction of the alveolar architecture, extensive collagen deposition and fibrosis, and multiple areas of collapsed lung (Fig. 1, G and H).

Pulmonary fibrosis was further evaluated by determination of the total lung HP content, an index of collagen deposition. After 14 days, intratracheal instillation of bleomycin resulted in a significant increase (P < 0.05) in lung collagen in both wild-type and mutant mice (Fig. 2). The increase in collagen induced by intratracheal bleomycin, however, was significantly greater in SPARC(−/−) mice than in SPARC(+/+) animals (326 ± 44 vs. 214 ± 27 µg HP/lung; P < 0.005). Thus SPARC(−/−) mice showed a 2.0 ± 0.3-fold increase in HP content after injury, whereas SPARC(+/+) mice had a 1.4 ± 0.1-fold increase compared with saline control mice of each type (P < 0.05; Table 1).

Inflammatory cell recruitment in SPARC(−/−) mice after intratracheal bleomycin administration. Because inflammatory responses to acute lung injury contribute significantly to the fibrotic process, we next investigated whether the differences in the fibrotic response between wild-type and mutant mice after intratracheal bleomycin injury were associated with differences in inflammatory cell recruitment. Total lung MPO and glucosaminidase activities were used as indexes of neutrophil and macrophage accumulation, respectively (Table 1). Data are expressed as the ratio of MPO or glucosaminidase activity for bleomycin treatment to that of saline treatment for each mouse type. When assessed at days 7 and 14 after treatment, intratracheal bleomycin treatment, compared with saline instillation, resulted in a 2- to 3-fold increase in the number of lung neutrophils and macrophages in both wild-type and mutant mice. However, significantly more neutrophils were present in the lungs of the SPARC(−/−) mice than in the lungs of the SPARC(+/+) animals, with comparable levels of macrophage accumulation in both types of mice at both time points (Table 1).

The finding of enhanced neutrophil accumulation by MPO activity was confirmed by determination of neutrophil counts in bronchoalveolar lavage fluid obtained 10 days after bleomycin administration. At this time point, it was noted that significantly more neutrophils were recovered from SPARC(−/−) mice compared with those from SPARC(+/+) mice: 21,800 vs. 10,500 neutrophils/ml, respectively (P < 0.05; n = 3–5 animals).
animals of both mouse types, neutrophil concentration was <800 neutrophils/ml.

Lung injury in SPARC(−/−) mice after intraperitoneal bleomycin administration. Pulmonary fibrosis may also develop after the intraperitoneal administration of bleomycin. Given this and our finding that mice deficient in SPARC have an exuberant fibrotic response after intratracheal bleomycin instillation, we also investigated whether the absence of SPARC would alter the development of fibrosis in the intraperitoneal model of bleomycin-induced lung fibrosis. In these studies, the mice were given intraperitoneal injections of bleomycin (0.8 U/mouse) on days 0, 3, 7, 10, and 14, with subsequent death of the animals on day 28 (24). With this protocol, we observed that, in contrast to what was noted after intratracheal bleomycin, intraperitoneal administration of bleomycin resulted in similar patterns and degree of injury in SPARC(+/+) and SPARC(−/−) mice. Specifically, involved areas were characterized by disruption of the normal alveolar architecture with a pattern of fibrosis consisting of thickening of the alveolar walls and/or subpleural or perivascular fibrotic lesions (Fig. 3). Collagen deposition as assessed by the HP content was comparable in

Table 1. Effect of bleomycin on lung collagen and leukocyte recruitment

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<td>SPARC(−/−)</td>
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Values are means ± SE expressed as the ratio of hydroxyproline (HP) content, myeloperoxidase (MPO) activity and glucosaminidase (GA) activity for bleomycin treatment to those for saline treatment (n = 3–8 mice). The increase in HP content and MPO activity in secreted protein, acidic and rich in cysteine-deficient [SPARC (−/−)] mice was significantly greater than that in wild-type [SPARC (+/+) ] mice after intratracheal bleomycin treatment (*P < 0.05).

Fig. 3. Histopathology and lung collagen trichrome staining of lungs from SPARC(+/+) and SPARC(−/−) mice after intraperitoneal bleomycin or saline treatment (original magnification, ×200). Data are for mice killed on day 28 after treatment. Lung architecture was not altered after saline treatment in both mouse types (A and C), and collagen staining was restricted to the large vessels or airways (solid arrows). For both mouse types, intraperitoneal bleomycin treatment resulted in fibrosis characterized by thickened alveolar walls and subpleural and perivascular fibrotic lesions (B and D, open arrows).
both mouse types (Table 1). Also, assessment of leukocyte infiltration on day 28 revealed no significant differences between the mutant and wild-type mice in neutrophil and macrophage accumulation after intraperitoneal instillation of bleomycin (Table 1). These data suggest that the absence of SPARC does not alter the development of pulmonary fibrosis or the associated inflammatory response that follows intraperitoneal bleomycin administration.

Neutrophil accumulation in SPARC(−/−) mice during thioglycolate-induced chemical peritonitis. The finding that intratracheal but not intraperitoneal bleomycin was associated with significantly more lung neutrophils suggested that the loss of SPARC in these mice may result in increased and/or more persistent neutrophil accumulation after acute inflammatory insults. To test this proposal, we studied leukocyte recruitment in wild-type and mutant mice after intraperitoneal injection with thioglycolate. In this model of chemical peritonitis, the inflammatory cell infiltrate within the peritoneal cavity 4–6 h after thioglycolate administration consisted overwhelmingly of neutrophils and thus represents a pure model of neutrophilic inflammation. We observed that after thioglycolate administration, significantly more intraperitoneal neutrophils were recovered from SPARC(−/−) than from SPARC(+/+) mice (Fig. 4), findings that are consistent with the hypothesis that loss of SPARC augments neutrophil accumulation after an acute insult.

DISCUSSION

To begin to understand the potential role of SPARC in lung injury, we studied the development of pulmonary fibrosis induced by bleomycin in mice deficient in SPARC. It was observed that bleomycin administered intratracheally induced a more exuberant inflammatory and fibrotic response in the lungs of SPARC-deficient than in wild-type control mice. In contrast, the pattern and severity of pulmonary fibrosis as well as the degree of leukocyte accumulation were similar in wild-type and mutant mice after intraperitoneal bleomycin treatment. Furthermore, increased neutrophil accumulation was observed in SPARC-deficient mice after acute chemical peritonitis. Together, these findings not only provide evidence of a role for SPARC in the host response to pulmonary insults that is likely to be complex and context specific, but they also imply SPARC in modifying the recruitment of neutrophils into sites of acute inflammation.

SPARC, an ~40-kDa protein secreted by a number of cell types including macrophages and fibroblasts, is found in the matrix of tissues responding to or recovering from injury (18). Its importance in these processes is suggested by a variety of in vitro studies that have shown that SPARC disrupts cell adhesion, modifies the extracellular matrix, inhibits the cell cycle, and antagonizes the effects of growth factors (5, 8). Although its specific role in the host response to lung injury is not known, the recent observation by Strandjord et al. (23) that intratracheal bleomycin-induced lung injury in mice resulted in an ~3-fold increase in SPARC mRNA suggests that SPARC may participate in these processes. However, understanding the physiological role(s) of SPARC has proved difficult because of a lack of readily available reagents capable of antagonizing the function of SPARC in vivo. Thus the generation of mice deficient in SPARC provides an important vehicle for studying the function of this protein in vivo.

Pulmonary injury induced by bleomycin delivered intratracheally or intraperitoneally provides two well-established models of lung fibrosis (4, 24). With these systems, we observed that the fibrosis that results from the intratracheal instillation of bleomycin (assessed histologically and by collagen content) was greater in mice deficient in SPARC compared with that in wild-type animals, whereas the absence of SPARC did not alter the pattern and severity of fibrosis that developed after the intraperitoneal administration of bleomycin. These findings suggest that the role of SPARC in lung injury is dependent on the type, intensity, and duration of insult. To evaluate this proposal further, pulmonary fibrosis in other models of lung injury, e.g., silica-induced injury (15), need to be the subject of future investigations.

Our findings of increased injury and fibrosis in SPARC-null mice after intratracheal bleomycin treatment differ from those of Strandjord et al. (23), who reported that intratracheal instillation of bleomycin in these mutant mice resulted in less fibrosis as assessed...
by HP content. The reasons for the differing results of the two studies are not clear, but there are at least three possibilities. First, the SPARC-null mice used in our lung injury experiments (with the exception of the day 10 experiments) were generated by 129SvEv × C57BL/6J backcrossed four times to 129SvEv (see METHODS). Because the C57BL/6 murine strain is known to be fibrosis prone (22), it is possible that the mutant mice may have retained some increased C57BL/6 sensitivity to bleomycin-induced injury relative to wild-type animals. We believe this is unlikely because 1) in our study, the mortality and the fibrotic response of the 129SvEv wild-type mice after intratracheal bleomycin treatment were very comparable to what other published reports of studies that used C57BL/6 mice have noted (15, 16, 24), suggesting that our wild-type mice were not less sensitive to the effects of bleomycin, and 2) no differences were seen after intraperitoneal bleomycin administration in the same strains of mice that were used for the intratracheal experiments.

Second, the genetic background of the mice used by Strandjord and associates (23) was 129SvEv × C57BL/6J, whereas the mice in the majority of the experiments in this report had a 129SvEv background. Thus differences in the species and/or strains of mice employed in the two studies may be a factor. We would note, however, that our studies, which investigated inflammation 10 days after intratracheal bleomycin, and the thioglycolate experiments were both performed with mice that were similar to those used by Strandjord et al. (23). As indicated above, these experiments yielded results that were consistent with the data initially obtained from mice with the 129SvEv background. Consequently, species and/or strain differences are unlikely to account entirely for the differing results between the two reports.

Finally, in our model, 0.15 U bleomycin/mouse was administered, whereas in the studies of Strandjord and associates (23), each mouse received only 0.075 U of bleomycin. The divergent results may therefore be related to differences in the amount of bleomycin given to the animals. If this is correct, the two reports are not necessarily conflicting. Specifically, if considered together, they provide further evidence for the proposal that the role and involvement of SPARC depends on the intensity of the injury. The varying and often opposite effects of different peptides derived from SPARC on processes relevant to wound repair, such as angiogenesis, cell proliferation, and migration (19), may provide clues as to the basis of the variable effects of this pleiotropic molecule within the same model of lung injury.

The reasons for the greater fibrotic response in the SPARC-deficient mice after intratracheal bleomycin are unclear. However, altered inflammatory cell recruitment may be one possibility; neutrophil accumulation was greater in mutant mice compared with the wild-type controls. This effect on neutrophil accumulation may not be limited to acute pulmonary inflammation because intraperitoneal neutrophils were present in increased numbers during the initial stages of thioglycolate peritonitis.

A critical event in the host response to pulmonary insults is the recruitment and activation of inflammatory and immune effector cells. Neutrophil recruitment can be caused by a number of events including chemokine and growth factor expression by damaged epithelial cells and activation of endothelial cells to promote adhesion and by the extracellular matrix (26). Some chemotactic agents also activate the polymorphonuclear neutrophils, causing them to secrete proteases and toxic oxygen- and nitrogen-based free radicals, which may contribute further to tissue damage and thus provide a mechanism for the maintenance of alveolitis (26, 27). Our data therefore suggest that, in the setting of injury induced by intratracheal bleomycin, SPARC may act to limit the accumulation of neutrophils. This could potentially be mediated by the inhibition of the release of chemotactic and other proinflammatory mediators that regulate leukocyte-endothelial and leukocyte-matrix interactions. Of course, a direct effect of SPARC on fibroblasts and collagen expression also remains a possibility (23). These effects await the further in vitro analysis of individual cell types from wild-type and null mice.

In summary, the enhanced fibrotic response in the lungs of SPARC-deficient mice after intratracheal but not intraperitoneal bleomycin suggests that the role of SPARC in the host response to pulmonary injury is very likely to be complex, dependent on a variety of factors including the type, duration, and severity of the insult. Understanding the participation of matrix proteins such as SPARC in the process of recovery from lung injury may lead to the development of new therapeutic interventions that promote healing after pulmonary insults.

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