Effects of Intercellular Adhesion Molecule 1 (ICAM-1) Null Mutation on Radiation-Induced Pulmonary Fibrosis and Respiratory Insufficiency in Mice

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Abstract: Therapy-induced inflammation and progressive fibrosis limit the efficacy of thoracic radiotherapy for lung neoplasms. However, mice bearing a null mutation of intercellular adhesion molecule 1 (ICAM-1) have previously been found to display no inflammatory cell infiltration into the lung following thoracic irradiation. We investigated the role of ICAM-1-mediated inflammation in the pathogenesis of radiation-induced pulmonary fibrosis in mice with a homozygous null mutation in the ICAM-1 gene (ICAM-1−/−) and in wild-type mice (ICAM-1+/+). Methods: Groups of 10 mice were each irradiated with total doses of 12.5, 14, 16, 17, or 18 Gy to the thorax or were mock irradiated. Inflammatory cell infiltration was measured by immunohistochemical staining of lung sections for leukocyte-common antigen (LCA). Dynamic pulmonary compliance was determined by plethysmography. Pulmonary fibrosis was evaluated by measuring alveolar septal wall thickness and the hydroxyproline content of lungs by immunohistochemical staining of lung sections for collagen III and by Masson's trichrome staining of lung sections. All statistical tests were two-sided. Results: Lungs of irradiated ICAM-1−/− mice had statistically significantly fewer LCA-positive cells than the lungs of irradiated ICAM-1+/+ mice at all radiation doses (P<.001). ICAM-1−/− mice had a higher mean dynamic pulmonary compliance than ICAM-1+/+ mice following irradiation. The incidence of respiratory insufficiency 18 months after thoracic irradiation was statistically significantly lower in ICAM-1−/− mice than in ICAM-1+/+ mice (odds ratio = 0.19 [95% confidence interval = 0.06 to 0.58], overall P = .0036). ICAM-1−/− mice had less pulmonary fibrosis and reduced thickening of the alveolar seaptum following thoracic irradiation than did ICAM-1+/+ mice. The lungs of irradiated ICAM-1−/− mice had less hydroxyproline than did the lungs of irradiated ICAM-1+/+ mice (P = .04). Conclusions: ICAM-1 and inflammation contribute to pulmonary fibrosis and impaired pulmonary compliance following thoracic irradiation. Agents that block ICAM-1 function or expression should be studied for their effects on the prevention of radiation-induced pulmonary fibrosis. [J Natl Cancer Inst 2002;94:733–41]
This experiment was repeated once.

Mouse PRC software (Lakeshore Technologies, Chicago, IL).

old ICAM-1+/+ or ICAM-1−/− mice were randomly assigned to thoracic irradiation as described above. Animal care was in accord with institutional guidelines.

Determination of the Lethal Dose of Thoracic Irradiation

Six-week-old ICAM-1+/+ or ICAM-1−/− mice were subjected to thoracic irradiation as described above. Ten mice of each genotype were randomly assigned to receive a total dose of 12.5, 14, 16, 17, or 18 Gy or mock irradiation (0 Gy). The mice were subsequently housed in a barrier facility for 18 months during which time they were monitored twice weekly for signs of respiratory distress, as defined by hypomotility, hunched backs, and tachypnea. Mice with respiratory distress were euthanized by an intraperitoneal injection of phenobarbital. The lungs of mice were dissected and fixed in 10% formalin (Histoprep; Fisher Scientific, Suwanee, GA) for 24 hours. Lungs were dehydrated and embedded in paraffin. Lungs were then sectioned into 5-μm-thick sections, mounted on glass slides, and stained.

We used Masson’s trichrome stain to detect fibrosis as previously described (20). Briefly, tissue sections were rinsed in absolute alcohol followed by 95% alcohol and distilled water. Bouin’s fixative was heated to 60°C and dropped onto slides containing lung tissue sections, and the slides were then incubated for 3 minutes at 60°C. Sections were then cooled to room temperature, rinsed in running tap water for 5 minutes, and then rinsed once with distilled water. The sections were incubated in Weigert’s iron hematoxylin solution for 7 minutes at room temperature and then rinsed with distilled water. The sections were then incubated with Biebrich scarlet-acid fuchsin solution for 30 seconds followed by a distilled water rinse. Sections were then placed in a phosphotungstic acid solution for 105 seconds and then rinsed in distilled water. Sections were then placed in Aniline blue solution (Sigma Chemical Co., St. Louis, MO) for 3 minutes followed by a distilled water rinse. The sections were then placed in 1% acetic acid for 1 minute, followed by 95% alcohol, two changes of absolute alcohol, and two changes of xylene. The sections were mounted under coverslips with a xylene-soluble media (Sigma Chemical Co.). Randomly selected microscopic fields (at ×400 magnification) were photographed, and staining was quantified using ImagePro software (Media Cybernetics, Des Moines, IA).

Quantitation of Inflammatory Cell Infiltration

Inflammatory cell infiltration into the lungs of irradiated and mock-irradiated mice (10 mice per group) was quantified by indirect immunofluorescence microscopy using anti-leukocyte-common antigen (anti-LCA) antibody (Pharmining, San Diego, CA) to detect the presence of LCA in lung sections, as previously described (16). We counted the number of LCA-stained cells within 10 randomly selected fields of slides viewed at ×400. The fluorescence intensity of stained tissue was then quantified with NIH Image software.

Assessment of Type III Collagen Deposition in Lung Sections

Histologic sections of lungs from ICAM-1−/− and ICAM-1+/+ mice that had survived 18 months after thoracic irradiation were examined for evidence of fibrosis by staining with a goat anti-mouse collagen III immunoglobulin G (IgG) (Chemicon International, Temecula, CA). The lung sections were first rehydrated and incubated in 3% H2O2 for 1 hour at room temperature to quench endogenous peroxidases. The sections were then incubated in 5% goat serum in phosphate-buffered saline (PBS) for 1 hour at 37°C to block nonspecific binding. Goat anti-mouse collagen III IgG (20 μg/mL) was added to the blocking buffer, and the sections were incubated for 60 minutes at 37°C. The sections were then washed with blocking buffer at 37°C, and biotin-conjugated donkey anti-goat IgG (Accurate Chemicals, Westbury, NY) at 20 μg/mL in blocking buffer was added to the sections and incubated for 40 minutes at 37°C. The sections
were then washed, and ABC Reagent (Vector Laboratories, Inc., Burlingame, CA) was added in PBS, followed by reagents A and B for 30 minutes at room temperature, according to the manufacturer’s instructions (Vector Laboratories, Inc.). Sections were washed, peroxidase substrate was added, and the sections were incubated at room temperature for 10 minutes. The sections were then dehydrated in a graded alcohol series followed by xylene and mounted under coverslips.

**Determination of Hydroxyproline Content of Lung**

Groups of 10 ICAM-1+/+ or ICAM-1−/− mice were randomly assigned to receive mock irradiation (0 Gy) or thoracic irradiation with 14, 16, or 18 Gy. The mice were euthanized at 10 weeks, 9 months, and 18 months after mock or actual irradiation, and their lungs were removed, washed in PBS, and weighed. The total collagen content of each right lung was determined using a colorimetric assay to measure lung hydroxyproline content as previously described (21,22). Briefly, a minced aliquot of lung tissue was hydrolyzed overnight at 110 °C in 800 μL of 6 N HCl. To 200 μL of that hydrolysate were added 100 μL of 0.02% methyl red and 20 μL of 0.04% bromthymol blue. The sample volume was adjusted to 2 mL with a 0.5x assay buffer (0.024 M citric acid, 0.02 M acetic acid, 0.088 M sodium acetate, 0.085 M NaOH), and the pH was adjusted to 6.5–7.0. One milliliter of chloramine T solution (Sigma Chemical Co.) was added to each sample; the mixture was then incubated at room temperature for 20 minutes, after which 1 mL of dimethyl benzaldehyde solution (Sigma Chemical Co.) was added followed by incubation at 60 °C for 15 minutes. We measured the absorbance of each sample at 550 nm. Whole-lung hydroxyproline values, expressed as micrograms of hydroxyproline per lung, were determined by normalizing the hydroxyproline values obtained from this colorimetric assay of the minced lung aliquots to the wet weight of the whole right lung. Values were corrected for total lung wet weight.

**Statistical Analysis**

We used a general linear model to test for associations between the numbers of leukocytes present in the lungs, dynamic compliances (mL/cm H2O), ICAM-1 genotype (wild type versus homozygous null), and the doses of irradiation the mice received (0, 14, 16, and 18 Gy). The general linear model (logistic regression analysis) was also used to test the association between the respiratory insufficiency (yes/no), ICAM-1 genotype (wild type versus homozygous null), and the doses of irradiation the mice received (12.5, 14, 16, 17, and 18 Gy). We applied the Bonferroni method to adjust the overall statistical significance levels to 5% for the multiple comparisons in this study. All statistical tests were two-sided, and differences were considered statistically significant for P<.05. SAS software version 8.1 (SAS Institute Inc., Cary, NC) was used for all statistical analyses.

**RESULTS**

**Radiation-Induced Inflammatory Cell Infiltration Into the Lungs of Mice**

Pulmonary inflammation precedes radiation-induced fibrosis. To determine whether there is a causal relationship between radiation-induced pneumonitis and subsequent fibrosis, we studied these processes in ICAM-1−/− mice, which do not display radiation-induced pulmonary inflammation. Anti-LCA antibody binds to all leukocytes and provides the means of quantifying neutrophils, lymphocytes, and monocytes within the irradiated lung. As shown in Fig. 1, at 5 weeks after mock irradiation, the lungs of ICAM-1−/− mice had statistically significantly fewer LCA-positive cells than the lungs of ICAM-1+/+ mice. For example, the mean numbers of LCA-positive cells/field in lung sections from ICAM-1+/+ mice subjected to 0 Gy (four LCA-positive cells/field), 14 Gy (six LCA-positive cells/field), 16 Gy (eight LCA-positive cells/field), and 18 Gy (seven LCA-positive cells/field) of radiation, respectively (P<.001 for each; difference in mean number of LCA-positive cells/field between ICAM-1+/+ mice and ICAM-1−/− mice subjected to mock irradiation [0 Gy] = nine cells/field [95% CI = seven cells/field to 11 cells/field], between ICAM-1+/+ mice and ICAM-1−/− mice subjected to 14 Gy = 44 cells/field [95% CI = 31 cells/field to 57 cells/field], between ICAM-1+/+ mice and ICAM-1−/− mice subjected to 16 Gy = 46 cells/field [95% CI = 34 cells/field to 58 cells/field], and between ICAM-1+/+ mice and ICAM-1−/− mice subjected to 18 Gy = 54 cells/field [95% CI = 43 cells/field to 65 cells/field]). LCA-positive cells were present in both the perivascular and alveolar spaces of the lungs of all irradiated ICAM-1+/+ mice.

**Pulmonary Compliance in Mice Subjected to Thoracic Irradiation**

Diminished ventilation and perfusion are two aspects of impaired lung function that are a consequence of thoracic irradiation. The onset of impaired ventilation occurs within weeks of...
irradiation; thus early pathologic events, such as cell loss, edema, diminished surfactant, and inflammation, have been implicated in radiation-induced impairment of lung function. One physiologic measure of these events is dynamic pulmonary compliance, which is a function of lung volume, surface tension, and the elasticity of lung parenchyma. We therefore evaluated dynamic pulmonary compliance, as measured by changes in plethysmographic pressure, in ICAM-1+/+ and ICAM-1−/− mice 10 weeks after they had received 14, 16, and 18 Gy of thoracic irradiation to determine whether the absence of inflammation in irradiated lung had an impact on dynamic pulmonary compliance.

At 10 weeks after mock irradiation, ICAM-1+/+ mice had a mean dynamic pulmonary compliance of 0.054 mL/cm H₂O and ICAM-1−/− mice had a mean dynamic pulmonary compliance of 0.051 mL/cm H₂O. Ten weeks after receiving 14 Gy of thoracic irradiation, ICAM-1+/+ mice had a mean dynamic pulmonary compliance of 0.043 mL/cm H₂O, which was statistically significantly lower than that of the mock-irradiated ICAM-1+/+ mice (P<.001; difference in mean dynamic pulmonary compliance between mock-irradiated and irradiated ICAM-1+/+ mice = 0.011 mL/cm H₂O [95% CI = 0.006 mL/cm H₂O to 0.016 mL/cm H₂O]) (Fig. 2). By contrast, at 10 weeks after receiving 14 Gy of thoracic irradiation, the ICAM-1−/− mice had a mean dynamic pulmonary compliance of 0.049 mL/cm H₂O, which was not statistically significantly different from that of the mock-irradiated ICAM-1−/− mice (P = .17; difference in mean dynamic pulmonary compliance between mock-irradiated and irradiated ICAM-1−/− mice = 0.002 mL/cm H₂O [95% CI = 0.001 mL/cm H₂O to 0.004 mL/cm H₂O]) (Fig. 2).

Incidences of Respiratory Distress in Mice Following Thoracic Irradiation

To determine whether the radiation-induced reduction in pulmonary compliance we observed was associated with respiratory distress, mice were monitored for signs of tachypnea, hunched backs, and hypomotility—three indicators of respiratory distress—twice weekly for 18 months after receiving thoracic irradiation with 12.5, 14, 16, 17, and 18 Gy. These mice began to display signs of respiratory distress 6 months after thoracic irradiation. We compared the incidence of respiratory distress in ICAM-1−/− mice with that in ICAM-1+/+ mice that had received the same dose of radiation. At 18 months after irradiation, 20% (95% CI = 0% to 44%) of the ICAM-1−/− mice that received a dose of 14 Gy, 50% (95% CI = 19% to 81%) of the ICAM-1+/+ mice that received a dose of 16 Gy, and 80% (95% CI = 56% to 100%) of the 10 ICAM-1−/− mice that received a dose of 18 Gy displayed respiratory distress (Fig. 3, A). By contrast, at this same time point, 0% (95% CI = 0% to 31%) of the ICAM-1−/− mice that received a dose of 14 Gy, 20% (95% CI = 0% to 44%) of the ICAM-1+/+ mice that received a dose of 16 Gy, and 50% (95% CI = 19% to 81%) of the ICAM-1−/− mice that received a dose of 18 Gy displayed respiratory distress (Fig. 3, A). The overall incidence of respiratory distress at 18 months after thoracic irradiation was statistically significantly lower in all groups of irradiated ICAM-1−/− mice than it was in all groups of irradiated ICAM-1+/+ mice (overall P = .0036, odds ratio [OR] = 0.19; 95% CI = 0.06 to 0.58).

The doses of radiation that produced respiratory insufficiency in 50% of the mice (LD₅₀) at 18 months were 16 Gy for ICAM-1+/+ mice and 18 Gy for ICAM-1−/− mice, respectively. Thus, the ICAM-1−/− mice required higher doses of thoracic irradiation than did the ICAM-1+/+ mice to display respiratory distress.

To determine whether the difference in incidence of respiratory distress in response to irradiation between ICAM-1+/+ and ICAM-1−/− mice was time-dependent, we repeated this experiment and monitored the mice over a shorter time period for signs of respiratory distress (Fig. 3, B). At 9 months after irradiation, 10% (95% CI = 0% to 29%) of the ICAM-1−/− mice that received a dose of 14 Gy and 40% (95% CI = 10% to 70%) of the ICAM-1+/+ mice that received a dose of 18 Gy developed tachypnea and hypomotility (Fig. 3, B). By contrast, at this same time point, 0% (95% CI = 0% to 31%) of the ICAM-1−/− mice that received a dose of 14 Gy, 10% (95% CI = 0% to 29%) of the ICAM-1+/+ mice that received a dose of 16 Gy, and 20% (95% CI = 0% to 44%) of the ICAM-1−/− mice that received a dose of 18 Gy developed tachypnea and hypomotility (Fig. 3, B). Although the overall incidence of respiratory distress at 9 months after thoracic irradiation was lower in all groups of irradiated ICAM-1−/− mice than it was in all groups of irradiated ICAM-1+/+ mice, that difference was not statistically significant (overall P = .07; general linear model). These findings indicate that the ICAM-1 null mutation may attenuate radiation-induced respiratory distress, but that it does not entirely eliminate radiation-induced pulmonary injury. Moreover, the onset of respiratory insufficiency was time-dependent in both the ICAM-1+/+ and ICAM-1−/− mice.

Mouse Lung Histology Following Actual or Mock Thoracic Irradiation

To determine whether the wild-type ICAM-1 genotype was associated with radiation-induced alveolar thickening and pul-

Fig. 2. Dynamic pulmonary compliance in intercellular adhesion molecule 1 (ICAM-1)+/+ and ICAM-1−/− mice. Plethysmography was performed by mechanical ventilation through a metal cannula at 10 weeks after mice were mock-irradiated (control) or irradiated with the indicated doses. Pressures were measured with a Whiter reference chamber by means of a differential pressure transducer. Dynamic compliance was calculated from volume and pressure signals recorded digitally. Shown are the mean dynamic pulmonary compliances (95% confidence intervals are shown) for five mice in each group. Solid and open bars correspond to ICAM-1+/+ mice and ICAM-1−/− mice, respectively. * indicates statistically significant reduction in mean dynamic pulmonary compliance compared with mock-irradiated mice of the same genotype (P<.001).
Collagen Deposition in the Lungs of Irradiated Mice

We used an antibody to collagen III to stain lung sections prepared from ICAM-1+/+ and ICAM-1−/− mice at various times after thoracic irradiation to determine the patterns of collagen deposition in response to radiation-induced pulmonary injury. The lungs of irradiated ICAM-1+/+ mice had higher collagen deposition, primarily in the perivascular regions, 12 months after irradiation with 16 Gy than did the lungs of mock-irradiated mice and ICAM-1−/− mice at 12 months after irradiation (Fig. 5).

To confirm that the lungs of ICAM-1+/+ mice had less radiation-induced collagen deposition than the lungs of ICAM-1−/− mice, we determined the hydroxyproline content, which provides a more quantitative assessment of collagen content (21,22), of the lungs of mice at 9 and 18 months after thoracic irradiation. ICAM-1+/+ and ICAM-1−/− mice were mock irradiated (0 Gy) or irradiated with 14, 16, or 18 Gy to the whole thorax (Fig. 6). The four groups of mock-irradiated mice had similar mean levels of hydroxyproline in their lungs: ICAM-1+/+ and ICAM-1−/− mice had mean levels of 211 μg/lung and 201 μg/lung, respectively, at 9 months after mock irradiation and 219 μg/lung and 201 μg/lung, respectively, at 18 months after mock irradiation. Because the mean hydroxyproline values assayed at 9 months after irradiation were similar to those assayed at 18 months after irradiation for each ICAM-1 genotype, we discuss only the values assayed at 18 months after irradiation. The mean hydroxyproline content in the lungs of ICAM-1+/+ mice showed no increase in alveolar septal wall thickening and no increase in Masson’s trichrome staining as compared with mock-irradiated mice (Fig. 4, C).

Pulmonary fibrosis is associated with increased alveolar septal wall thickness. We measured alveolar septal wall thickness in the lungs of ICAM-1+/+ and ICAM-1−/− mice 12 months after they had received thoracic irradiation. The average thickness measurements from 10 randomly selected microscopic fields are shown in Fig. 4, D. Alveolar septal wall thickness in the lungs of ICAM-1+/+ mice irradiated with 14, 16, and 17 Gy was an average of 3.1-fold, 3.2-fold, and 3.3-fold higher, respectively, than in the lungs of mock-irradiated ICAM-1+/+ mice. By contrast, alveolar septal wall thickness was an average of 1.5-fold, 1.9-fold, and 1.9-fold higher in the lungs of ICAM-1−/− mice irradiated with 14, 16, and 17 Gy, respectively, than in the lungs of mock-irradiated ICAM-1−/− mice. There was a statistically significant difference in the fold increase in alveolar septal wall thickness between ICAM-1+/+ mice and ICAM-1−/− mice after each dose of radiation (difference in the fold increase in alveolar septal wall thickness between ICAM-1+/+ mice and ICAM-1−/− mice after 14 Gy = 1.6-fold [95% CI = 1.2-fold to 2.0-fold, \( P<.001 \)]; between ICAM-1+/+ mice and ICAM-1−/− mice after 16 Gy = 1.3-fold [95% CI = 0.9-fold to 1.7-fold, \( P = .008 \)]; and between ICAM-1+/+ mice and ICAM-1−/− mice after 17 Gy = 1.4-fold [95% CI = 0.9-fold to 1.9-fold, \( P = .024 \)]).
was 290 mg/lung at 18 months after irradiation with 14 Gy. Moreover, higher doses of radiation resulted in even higher mean hydroxyproline levels in the lungs of the ICAM-1+/+ mice. For example, the lungs of ICAM-1+/+ mice irradiated with 16 Gy and 18 Gy had mean hydroxyproline levels of 382 mg/lung and 433 mg/lung, respectively. By contrast, ICAM-1−/− mice subjected to 16 Gy and 18 Gy of radiation had mean hydroxyproline levels of 259 mg/lung and 275 mg/lung, respectively, at 18 months after irradiation, which were statistically significantly lower than those of ICAM-1+/+ mice irradiated with the same dose of radiation (difference in mean values between ICAM-1+/+ mice and mock-irradiated control mice of the same genotype, * indicates a statistically significant difference in the fold increase in alveolar septal wall thickness in the lungs of ICAM-1+/+ mice compared with the lungs of ICAM-1−/− mice in the same radiation dose group (P = .008, general linear model).

**DISCUSSION**

We have investigated the relationship between radiation-induced inflammation and fibrosis by studying the lungs of irradiated mice that contain or lack the gene for ICAM-1. Here we confirm our previous findings, that inflammation occurs 4–5 weeks after irradiation in the lungs of ICAM-1+/+ mice but not in those of ICAM-1−/− mice. In the present study, we observed that all irradiated mice displayed reduced pulmonary compliance, which worsened even after inflammation was resolved. Because we did not observe radiation-induced pneumonitis in mice bearing a null mutation of ICAM-1, we used the ICAM-1−/− mice as a model system to study the physiologic significance of radiation-induced inflammatory injury in the lung. The hypothesis tested in this work was that mice bearing an ICAM-1 null mutation would display attenuated radiation-induced fibrosis and a subsequent reduction in pulmonary compliance. However, we found that irradiated ICAM-1−/− mice had lower levels of hydroxyproline in their lungs and improved pulmonary com
pliance than did irradiated ICAM-1+/+ mice. Moreover, a greater dose of thoracic irradiation was required to induce tachypnea and hypomotility in ICAM-1−/− mice than in ICAM-1+/+ mice. Although these findings support the hypothesis that inflammatory injury is the primary contributor to the reduction in pulmonary compliance following irradiation, they also show that inflammation is not essential for reduced dynamic pulmonary compliance.

Previous studies (1–3) have failed to clarify the role of inflammation in the development of pulmonary fibrosis. For example, C57BL/6 mice, which are prone to radiation-induced fibrosis, develop less inflammation than C3H mice, which are prone to radiation-induced inflammation. Conversely, the inflammation-prone mice develop less fibrosis than the fibrosis-prone mice. Glucocorticoids reduce inflammation but not subsequent fibrosis in the lungs of irradiated rats (23). However, steroids have many nonspecific effects that influence lung physiology (24). The purpose of the present study was to specifically study the role of ICAM-1 in radiation-induced lung injury.

We found that hydroxyproline content and collagen staining—two markers of fibrosis—increased in the lungs of ICAM-1+/+ mice following thoracic irradiation. These findings indicate that fibrosis may account for the diminished pulmonary compliance observed in these mice following thoracic irradiation. The role of fibrosis in radiation-induced reduction in pulmonary compliance is also supported by results from previous studies (5,25,26).

Fibrosis may develop in response to inflammation (12). Leukocytes release cytokines, such as tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β), which stimulate collagen production. Fibrosis occurs in foci that sur-
round a nidus of inflammatory cell infiltration, thus implicating inflammatory cells in both the early (pneumonitic) and the late (fibrotic) phases of radiation-induced lung injury. However, inflammatory cells are not the only source of TGF-β and TNF-α, and the elimination of inflammation does not entirely prevent the reduced pulmonary compliance and respiratory compromise, as shown in this study. Although ICAM-1 knockout mice serve as a model to study the role of inflammation (27) in the pathogenesis of treatment-related pulmonary fibrosis, this model has limitations. For example, injection of an ICAM-1-blocking antibody into ICAM-1−/− mice is more efficient than a homozygous knockout of the ICAM-1 gene at preventing tissue injury, indicating that other cell adhesion molecules, such as P-selectin, may also contribute to inflammation (28). This finding suggests that redundant mechanisms of inflammatory cell trafficking may compensate for the effects of a null mutation in the ICAM-1 gene.

In the present study, the reduction in dynamic pulmonary compliance occurred 10 weeks after irradiation of wild-type mice, even though pulmonary inflammation occurred at 5 weeks. This late progression to impaired pulmonary compliance may indicate that radiation-induced injury is multifactorial and related to inflammation, edema, and diminished surfactant. This speculation is supported by the finding that irradiated ICAM-1−/− mice also have diminished pulmonary compliance, albeit to a lesser extent than wild-type mice. We propose that the ICAM-1 null mutation reduces the inflammatory component of lung injury but does have effect on the cytotoxic effects of irradiation. This hypothesis is supported by a recent study (29) that showed that endothelial cell cytotoxicity is the predominant event in the pathophysiology of radiation-induced organ injury.

The present study shows that the incidence of tachypnea and hypomotility increases between 9 and 18 months after thoracic irradiation in both ICAM-1−/− and ICAM-1−/+ mice. The radiation doses required to induce a 50% incidence of respiratory distress at 18 months were 18 Gy in ICAM-1−/+ mice but only 16 Gy in ICAM-1−/− mice. Taken together with the findings that mice bearing the ICAM-1 null mutation showed essentially no radiation-induced pulmonary fibrosis but had attenuated impairment of pulmonary compliance, these results suggest that ICAM-1 expression contributes to the pathogenesis of treatment-related lung injury. ICAM-1 is required for radiation-induced pneumonitis, which supports the link between inflammation and collagen deposition in the irradiated lung. These findings suggest that agents that block ICAM-1 function or expression should be studied for their effects on the prevention of radiation-induced pulmonary fibrosis. In addition, other means of protecting lungs from the cytotoxic effects of ionizing radiation should be considered.

REFERENCES

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NOTES

Supported by Public Health Service grants R01-CA58508, R01-CA70937, R01-CA89674, R21-CA89888, P30-CA68485, and P50-CA90949 (to D. E. Hallahan) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The authors acknowledge technical assistance from S. Virudachalam, P. Padrid, and Jason Dugger.

Manuscript received July 10, 2001; revised February 22, 2002; accepted March 14, 2002.