Distinct Loci Influence Radiation-Induced Alveolitis from Fibrosing Alveolitis in the Mouse

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Abstract

Thoracic radiotherapy may produce the morbidity-associated lung responses of alveolitis or fibrosing alveolitis in treated cancer patients. The genetic factors that influence a patient's likelihood of developing alveolitis and the relationship of this inflammatory response to the development of fibrosis are largely unknown. Herein we use genetic mapping to identify radiation-induced lung response susceptibility loci in reciprocal backcross mice bred from C3H/HeJ (alveolitis response) and C57BL/6J (fibrosing alveolitis/fibrosis response) strains. Mice were treated with 18-Gy whole thorax irradiation and their survival, lung histopathology, and bronchoalveolar lavage cell types were recorded. A genome-wide scan was completed using 139 markers. The C3H/HeJ alveolitis response included mast cell infiltration and increased neutrophil numbers in the lavage compared with the level in the C57BL/6J strain, which developed fibrosis. In backcross mice, posttreatment survival was dictated by the development of an alveolitis response with increased mast cell, bronchoalveolar lavage total cell, and neutrophil numbers. Fibrosis was measured only in a subset of mice developing alveolitis and, in these mice, was associated with neutrophil count. Genotyping revealed coinheritance of C3H alleles (chromosomes 2, 4, 19, and X) and C57BL/6J alleles (chromosomes 1, 7, 9, and 17) to result in higher fibrosis scores in backcross mice. Mice that inherited C57BL/6J alleles at the putative alveolitis susceptibility loci were spared this response and lived to the end of the experiment. In this animal model, independent loci control the development of alveolitis from fibrosis, whereas fibrosing alveolitis occurs with the coinheritance of these factors. [Cancer Res 2007;67(22):10796–803]

Introduction

Radiation treatment of the thorax, which is frequently used as a therapy in breast, lung, and esophageal cancer (1–4) and in Hodgkin's disease (5, 6), produces alveolitis and fibrosis in the lungs of up to 30% of treated patients (7). These pathologies of excessive inflammation or deposition of extracellular matrix in the lung interstitium can lead to impaired lung function and, ultimately, respiratory failure. The ability to determine a priori which patients are at risk for the development of these treatment-induced effects would allow for the formulation of patient-specific regimens aimed at maximizing therapy while minimizing the incidence of potentially devastating side effects. Indeed, reports of Anscher and Vujaskovic (8) and Kong et al. (7) both indicate that non–small-cell lung cancer could be more aggressively and effectively treated with radiation if higher doses could be delivered to the lung without increasing the patient’s risk for developing the complications of alveolitis and fibrosis. This risk is dictated by radiation dose, tissue volume treated, and yet unknown inherent factors or genetic predisposition to adverse effects.

As the identification of causal genetic variations influencing lung disease susceptibility using clinical data alone can be confounded by the effects of multiple genes and their interactions on the phenotype and by the cancer and other treatment modalities, inbred mouse strains that vary in their propensity to develop alveolitis and fibrosis (9, 10) after radiation exposure can be evaluated to genetically dissect the lung response. Specifically, the three clinical outcomes of thoracic radiotherapy, with respect to adverse effects (fibrosing alveolitis, alveolitis, or a subsymptomatic response) and the times at which they occur (3 months posttherapy for alveolitis, 6 months posttherapy for fibrosis; refs. 2–4), are each represented in the radiation response of an inbred mouse strain. We (11) and others (9, 10) have reported that following high-dose whole thorax irradiation, C3H/HeJ (C3H) mice develop a diffuse lethal alveolitis, the C57BL/6J (B6) response is fibrosing alveolitis, whereas male mice of the congenic major histocompatibility strain B6.AKR-H2k present a minimal lung response at 6 months posttreatment (12). The phenotypic difference between B6 and C3H/KAM mice has been used to map three loci of susceptibility to radiation-induced pulmonary fibrosis, named Radp1, Radp2, and Radp3 (12). Susceptibility to the alveolitis response has not been mapped.

Defining the genetic basis for the radiation-induced inflammatory response of alveolitis has direct clinical implications as this response may be related to the severity of fibrosing alveolitis, a condition for which treatments are minimally effective (13). The relevance of the inflammatory response to the subsequent deposition of fibrosis is, however, not well understood. Hallahan et al. (14) showed intercellular adhesion molecule-1 knockout mice to have both fewer inflammatory cells and less fibrosis after thoracic radiation than did their wild-type littermates, and Adawi et al. (15) reported the administration of an anti-CD40 ligand antibody treatment to reduce both pulmonary inflammation and fibrosis in radiation-treated mice. Other studies have, however, shown that the lung injury of fibrosis can be separated from the alveolitis response (16, 17) and may be initiated by epithelial cell damage (18).

In the present study, a genome-wide scan of B6×C3H backcross mice was undertaken to identify loci influencing susceptibility to three radiation response outcomes: alveolitis, fibrosing alveolitis, and a sublethal response. Further, the correlation of inflammatory markers (mast cell influx and bronchoalveolar cell counts) to the development of each phenotype was investigated and the potential link between alveolitis and fibrosis severity was assessed.
Materials and Methods

Mice. Mice of the C57BL/6j and C3H/HeJ strains were purchased from The Jackson Laboratory. The radiation response phenotype of eight mice of each of the strains was measured as presented below. For the mapping experiment, 226 backcross mice were bred from B6 and C3H strain progenitors by mating B6 or C3H mice with B6C3F1 mice.

The experimental mice received radiation treatment at the McIntyre Facility of McGill University and were subsequently housed in the animal facility of the Meakins-Christie Laboratories. Animal use was approved by the McGill University Animal Care Committee and was in accordance with the guidelines of the Canadian Council on Animal Care.

Radiation treatment. Mice were treated at 8 weeks of age. Lung damage was elicited by whole thorax radiation exposure (18 Gy) using a gamma cell cesium-137 unit. For irradiation, the mice were anesthetized with i.p. injections of xylazine (5 mg/kg) and sodium pentobarbital (30 mg/kg) and placed in a 3-mm-thick Perspex box, which was aligned in the radiation unit. The dose rate to the lung was 0.7 Gy/min, whereas the rest of the body was shielded with 3 cm of lead to reduce the beam strength to 3% in this area.

Mice were irradiated, weighed weekly, and sacrificed when moribund or at 26 weeks posttreatment. Specifically, mice losing >20% of their body weight and exhibiting distress through ruffled fur, visibly accelerated breathing, and hunched posture were sacrificed. The control mice (n = 8 B6 mice, 8 C3H mice) were not exposed to radiation and were sacrificed at the 15- to 26-week time points. Twelve backcross mice were found dead after treatment and were not included in the analyses.

Histology and phenotype scoring. At autopsy, bronchoalveolar lavage was done by cannulating the trachea and retrieving cells from three 1-ml injections of PBS. Fluid was collected from B6 and C3H mice and from 180 of 214 backcross mice. The lungs were then removed and the single left lobe of each mouse was perfused with 10% neutral buffer formalin and, following standard histologic processing, stained with HE and Masson's trichrome. The area of the fibrosing phenotype for each mouse was quantified by image analysis of trichrome-stained histologic sections as described previously (19). Specifically, a user-drawn region surrounding the fibrosis was compared with the area of the entire lobe to yield the percentage of pulmonary fibrosis for individual mice. Images were collected at ×12.5 magnification using Image Pro software and a Quantex 251 microscope. To assess alveolitis, HE-stained left lung sections were evaluated by semiquantitative histology using a method adapted from Davidson et al. (20) and described in ref. 21. Alveolitis was scored subjectively on a scale of 0 to 6, with 0 being no alveolitis and 6 being extreme alveolitis (characterized by excessive thickening of the alveolar walls with cellular infiltrate and exudates present in the alveolar space of the entire lung section). Mast cell numbers were determined by counting the mast cells present in 10 fields of a lung histologic section stained with toluidine blue, at ×200 magnification, and calculating total mast cell count per square millimeter. All scoring was completed by a user blinded to mouse genotype and treatment. Differences in lung phenotype between groups were assessed with Student's t test. Correlations between the lung response phenotypes were calculated using Microsoft Excel software. Linkage was assessed using the standards proposed by Lander and Kruglyak (24) and the probabilities calculated from the χ2 test.

Results

B6 and C3H lung response phenotype. To characterize the B6/C3H strain difference in radiation-induced lung disease, we treated male and female mice of each strain with 18-Gy whole thorax irradiation and measured the lung response. C3H mice developed a lethal alveolitis at ~14 weeks after treatment (see Table 1) whereas the B6 strain response to radiation was fibrosing alveolitis, which occurred, on average, at 23.2 ± 2.0 weeks postirradiation in male mice and at 20.2 ± 1.5 weeks in female mice. No irradiated mice of the B6 or C3H strains survived to the end of the experiment. Bronchoalveolar lavage fluid was collected from each mouse at sacrifice and histologic evaluation of mast cell counts was completed to determine if either of these inflammatory cell counts differed in the inbred strain radiation response. As shown in Table 1, the lavage of C3H mice had a significantly greater neutrophil count (P = 3.6 × 10−4) and fewer macrophages (P = 0.003) than did B6 mice, and mast cell counts were also higher (P = 0.002) in the lung tissue of C3H mice. The average mast cell count in untreated mice was 0.6/mm2 of tissue and did not differ between the strains. The bronchoalveolar lavage counts of control mice also did not differ by strain and were, on average, 95% macrophages, 4.7% lymphocytes, and 0.3% neutrophils.

Backcross mouse postradiation survival time and lung response phenotype. To identify mice developing radiation-induced alveolitis, fibrosing alveolitis, or spared a lethal lung response, B6 × C3H backcross mice, derived from breeding to either the B6 or the C3H parental strain, were exposed to 18-Gy thoracic irradiation and sacrificed when moribund or at 26 weeks posttreatment. In addition, to determine whether the identified strain differences in mast cell infiltration and neutrophils (PMN) in lavage following pulmonary irradiation were associated with the development of the alveolitis or fibrosis phenotypes assessed histologically, these measures were also completed in backcross mice.

The backcross mice of the 75% C3H alleles cohort (F1 and C3H parents) had, on average, a greater survival time postradiation than did C3H mice (see Table 1), with 36% of this cohort surviving to the end of the experiment. Consistent with the C3H lung response...
phenotype, the majority of the mice of this group, which were sacrificed due to the severity of their symptoms, developed an alveolitis with mast cell infiltration and limited fibrosis. Indeed, of the 72 mice that developed severe lung disease, only 7 had fibrosis scores of >3% of the lung. The phenotype of mice euthanized at the end of the experiment, in contrast, consisted of lower alveolitis scores and fewer mast and bronchoalveolar lavage cells than in sick mice, as shown in Table 1. For these mice that survived to the end of the experiment, there were no phenotypic differences between males and females (all P > 0.35; data not shown); for mice in respiratory distress, the males were euthanized, on average, later than females (20.5 ± 4.8 versus 18.3 ± 5.5 weeks; P = 0.07) and no other differences in lung phenotype by sex were identified (data not shown).

In the second cohort of mice, backcross mice with a B6 parent, a sex difference in survival was apparent as 45% (27 of 60) of male mice lived to the end of the experiment as did only 20% (8 of 41) of female mice. Of the mice sacrificed due to the severity of their symptoms, the lung phenotype was alveolitis or fibrosing alveolitis, as indicated in Table 1. Fibrosis of >3% of the lung was identified in 18 mice. As in the C3H cross, the moribund mice of the 75% B6 cohort also had, on average, greater numbers of mast cells and cells in the lavage than did mice sacrificed at the end of the experiment, and the only sex difference in phenotype was in posttreatment survival as distressed males were euthanized, on average, later than females (23.2 ± 2.6 versus 20.3 ± 3.8 weeks; P = 8.5 × 10^-3). Of the mice that survived to the end of the experiment, there were no phenotypic differences between males and females (all P > 0.16; data not shown).

To determine how each of the inflammatory measures (bronchoalveolar lavage cell differential and mast cell counts) is related to the development of each of alveolitis and fibrosis, and how these phenotypes are related to the postradiation treatment survival of the mice, correlation coefficients were calculated. As shown in Fig. 1, the postradiation survival of the backcross mice was negatively correlated with alveolitis score, and not the development of fibrosing alveolitis, in both cohorts. Alveolitis, in turn, was correlated with increased numbers of mast cells and lavage cells, particularly neutrophils. Finally, as shown in Fig. 1C, the amount of lung fibrosis increased with alveolitis severity, mast cell number, and lavage neutrophils in mice of the 75% B6 alleles cohort. To control for the presence of mast cell and neutrophil infiltration due to alveolitis in these mice, we grouped the moribund mice of the 75% B6 cross by fibrosis phenotype and assessed the inflammatory measures. The mice with a fibrosing alveolitis of >1% of the lung had more neutrophils in their lavage than did mice with minimal fibrosis (9.0 ± 1.8% versus 3.2 ± 0.8; P = 0.009) whereas mast cell numbers were similar in both groups (P = 0.41).

**Linkage.** To isolate quantitative trait loci linked to the development of alveolitis or fibrosing alveolitis, we completed a genome scan, using 139 markers, of mice of reciprocal B6/C3H backcrosses. We initially identified the genomic regions of susceptibility to a lethal response to whole thoracic irradiation in 75% C3H/HeJ × 25% C57BL/6J backcross mice by comparing the set of genotypes of mice surviving to the end of the experiment to those of mice succumbing to lung disease. As shown in Table 2, by y^2 analysis, four genomic regions influenced the postradiation survival of the mice. Alleles from both the B6 and C3H backgrounds were shown to reduce the survival phenotype. The inflammatory lung response of alveolitis was also linked to these genomic regions (summarized in Table 3), which is consistent with the finding that the cause of distress in the mice of this cross is the development of alveolitis. The other inflammatory phenotypes, mast and total lavage cell counts, also generally differed by genotype at these markers of postradiation treatment survival, with the animals that develop more alveolitis having higher inflammatory cell counts. No other regions were identified as linked to the inflammatory phenotypes in the mice of this cross (data not shown).

An assessment of mice of the second cohort, backcross mice with one B6 parent, revealed the postradiation survival phenotype to be linked to three regions distinct from those identified in 75%

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**Table 1. Lung phenotypes of radiation-treated inbred strain and B6 × C3H backcross mice**

<table>
<thead>
<tr>
<th></th>
<th>C3H/HeJ</th>
<th>C57BL/6J</th>
<th>Backcross (75% C3H background)</th>
<th>Backcross (75% B6 background)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moribund mice (n = 8)</td>
<td>Moribund mice (n = 72)</td>
<td>End of experiment mice (n = 41)</td>
<td>Moribund mice (n = 66)</td>
</tr>
<tr>
<td><strong>Survival time (wk posttreatment)</strong></td>
<td>14.2 ± 3.6</td>
<td>21.7 ± 1.8</td>
<td>19.3 ± 5.3</td>
<td>26.1 ± 0</td>
</tr>
<tr>
<td><strong>Alveolitis score</strong></td>
<td>3.9 ± 1.0</td>
<td>3.3 ± 0.8</td>
<td>4.0 ± 0.6</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td><strong>%Fibrosis of lung</strong></td>
<td>0.4 ± 0.8</td>
<td>4.1 ± 3.0</td>
<td>0.6 ± 1.4</td>
<td>0.1 ± 0.4</td>
</tr>
<tr>
<td><strong>Mast cells/mm^2</strong></td>
<td>17.6 ± 3.6</td>
<td>4.3 ± 3.7</td>
<td>4.7 ± 4.2</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td><strong>Total cell no. (lavage × 10^6)</strong></td>
<td>16.3 ± 12.5</td>
<td>15.3 ± 11.3</td>
<td>14.2 ± 13.6</td>
<td>11.1 ± 0.8</td>
</tr>
<tr>
<td><strong>Macrophages (% of BAL)</strong></td>
<td>59.3 ± 15.7</td>
<td>83.5 ± 16.5</td>
<td>74.3 ± 10.9</td>
<td>75.9 ± 10.9</td>
</tr>
<tr>
<td><strong>Lymphocyte (% of BAL)</strong></td>
<td>19.8 ± 13.4</td>
<td>13.8 ± 15.3</td>
<td>19.7 ± 8.9</td>
<td>23.3 ± 11.0</td>
</tr>
<tr>
<td><strong>PMN (% of BAL)</strong></td>
<td>21.0 ± 9.2</td>
<td>2.8 ± 5.5</td>
<td>6.0 ± 9.7</td>
<td>0.8 ± 1.2</td>
</tr>
</tbody>
</table>

**NOTE:** Mice were treated with 18-Gy whole thorax irradiation and euthanized due to presentation of distress symptoms or at the end of the experiment (36 wk posttreatment). Phenotypes are presented as mean ± SD.

*Significant difference (P < 0.05) between C3H/HeJ and C57BL/6J mice.

†Significant difference (P < 0.05) between mice sacrificed due to severe symptoms and mice sacrificed at the end of the experiment.
C3H/HeJ × 25% C57BL/6J mice, and to a common locus on chromosome 2, as shown in Table 2. In this cross, the presence of a C3H allele at each of these markers was associated with a reduction in posttreatment survival and, as is shown in Table 3, an increase in alveolitis score. As was the case for mice of the first cohort, those with one C3H parent, the phenotypes of survival time, mast cell number, total lavage cell count, and percent PMN in lavage also, in general, differed between groups of mice in this second cohort, as defined by survival marker genotype. The markers for which a significant difference in survival time posttreatment was identified (Table 3) also influenced this trait in the subset of distressed mice only. This was determined by removing the mice surviving to the end of the experiment from the analysis and repeating the t test (data not shown). In the male mice of this cross (75% C57BL/6J/C2 25% C3H/HeJ) inheritance of a C3H allele in the proximal region of the X chromosome was significantly linked to the radiation response of neutrophils in the lavage as shown in Fig. 2.

A potential interaction among loci influencing the lung response phenotype was uncovered, using marker covariate analysis in R/QTL, as controlling for the contribution of marker D4Mit9 to the alveolitis phenotype revealed suggestive linkage of this trait to marker D7Mit294 (LOD, 2.3). In detail, the alveolitis phenotype did not differ between the group of mice with genotype B6/B6 and those with B6/C3H at marker D4Mit9 (P = 0.74) when these mice also had the genotype B6/B6 at marker D7Mit294. A difference in alveolitis score was apparent, however, by D4Mit9 genotype.

Table 2. Genotype frequency in radiation-treated C3H/HeJ × C57BL/6J backcross mice

<table>
<thead>
<tr>
<th>Peak marker</th>
<th>Moribund mice</th>
<th>End of experiment mice</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>75% C3H/HeJ-25% C57BL/6J background</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D14Mit157</td>
<td>B6/C3H 29 1</td>
<td>C3H/C3H 43 10 4</td>
<td>1.2 × 10^-4</td>
</tr>
<tr>
<td></td>
<td>B6/C3H 23 4</td>
<td>C3H/C3H 10 13</td>
<td>3.5 × 10^-4</td>
</tr>
<tr>
<td>D2Mit66</td>
<td>B6/C3H 23 6</td>
<td>C3H/C3H 9 13</td>
<td>5.5 × 10^-4</td>
</tr>
<tr>
<td>D13Mit256</td>
<td>B6/C3H 13 8</td>
<td>C3H/C3H 20 5</td>
<td>6.9 × 10^-4</td>
</tr>
<tr>
<td>25% C3H/HeJ-75% C57BL/6J background</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2Mit327</td>
<td>B6/C3H 40 11</td>
<td>B6/B6 18 18</td>
<td>5.7 × 10^-4</td>
</tr>
<tr>
<td></td>
<td>B6/C3H 38 9</td>
<td>B6/B6 25 25</td>
<td>7.7 × 10^-6</td>
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<tr>
<td>D4Mit9</td>
<td>B6/C3H 39 12</td>
<td>B6/B6 21 20</td>
<td>1.3 × 10^-3</td>
</tr>
<tr>
<td>D19Mit90</td>
<td>C3H 15 3</td>
<td>B6 13 23</td>
<td>2.0 × 10^-11</td>
</tr>
</tbody>
</table>

*By χ² analysis, comparing the set of genotypes of mice developing a lethal lung disease to those surviving to the end of the experiment, 26 wk posttreatment.
1Number of mice with this genotype euthanized due to the severity of symptoms following 18-Gy whole thorax irradiation.
2Female mice only.
3Male mice only.
4Genotypes of C3H or B6 for male mice.

Figure 1. Correlations among lung response phenotypes in C3H × B6 backcross mice. Mice derived from a cross of F1 and C3H/HeJ mice (75% C3H) or from F1 and C57BL/6J mice (75% B6) were exposed to 18-Gy whole thorax irradiation and euthanized when moribund or at 26 wk posttreatment. Alveolitis, fibrosing alveolitis, and mast cell number were evaluated histologically and cellular influx was assessed by bronchoalveolar lavage cell typing (Mphage, macrophage; PMN, neutrophil; lymph, lymphocyte). Correlations of each phenotype to survival time postradiation treatment (A), alveolitis score (B), and fibrosis score (C) are given. The vertical dashed line indicates a significant correlation, with P < 0.05.
models of radiation-induced lung response, we showed the postthoracic cavity radiotherapy survival to be determined principally by the development of alveolitis. The complication of fibrosing alveolitis was found to occur only in mice that developed alveolitis and inherited fibrosis-promoting alleles. Separate loci were found to affect susceptibility to each of alveolitis and fibrosis, such that the mice inheriting promoting alleles at these loci developed fibrosing alveolitis and the mice inheriting the protective alleles at the alveolitis regions were spared lung disease and lived to the end of the experiment.

Consistent with previous reports (9–12, 27), the B6 mice of this study developed fibrosing alveolitis in response to radiation exposure whereas C3H strain mice developed the extensive inflammatory response of alveolitis only. The bronchoalveolar lavage data also reflect what has been reported for the radiation response of C3H (28) and B6 (29, 30) mice, and by comparing these profiles, we showed the C3H phenotype to include more neutrophils in the lavage than the B6 strain response. The inflammatory response of alveolitis only. The bronchoalveolar total and differential cell counts.

### Table 3. Effect of genotype on radiation-induced lung disease phenotype in C3H/HeJ × C57BL/6J backcross mice

<table>
<thead>
<tr>
<th>Peak marker</th>
<th>Genotype</th>
<th>Survival time (wk)</th>
<th>Alveolitis score</th>
<th>Fibrosis (% of lung)</th>
<th>Mast cells/mm²</th>
<th>PMN (% cells in lavage)</th>
<th>Total cell no. (lavage × 10⁵)</th>
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<tbody>
<tr>
<td>D14Mit157</td>
<td>C3H/C3H</td>
<td>21.0 ± 0.7</td>
<td>3.5 ± 0.1</td>
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<td>C3H/B6</td>
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<tr>
<td></td>
<td></td>
<td>0.13</td>
<td>5.0 × 10⁻³</td>
<td>0.16</td>
<td>0.01</td>
<td>0.52</td>
<td>0.22</td>
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<tr>
<td>D4Mit235</td>
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<td>2.8 ± 0.2</td>
<td>1.0 ± 0.4</td>
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<td></td>
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<td>2.9 × 10⁻³</td>
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<td>C3H/C3H</td>
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<td></td>
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<td>0.98</td>
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<td>0.52</td>
<td>0.01</td>
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<td>4.0 ± 0.3</td>
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<td>5.1 ± 1.3</td>
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<td>1.4 ± 0.6</td>
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<td></td>
<td></td>
<td>0.07</td>
<td>9.6 × 10⁻⁴</td>
<td>0.99</td>
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<tr>
<td>D2Mit327</td>
<td>B6/B6</td>
<td>24.6 ± 0.4</td>
<td>3.0 ± 0.2</td>
<td>1.8 ± 0.7</td>
<td>2.2 ± 0.4</td>
<td>2.9 ± 0.8</td>
<td>7.6 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>C3H/C3H</td>
<td>22.3 ± 0.6</td>
<td>3.6 ± 0.2</td>
<td>2.2 ± 0.7</td>
<td>2.8 ± 0.4</td>
<td>4.8 ± 1.1</td>
<td>12.5 ± 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6 × 10⁻³</td>
<td>0.035</td>
<td>0.72</td>
<td>0.29</td>
<td>0.18</td>
<td>0.058</td>
</tr>
<tr>
<td>D4Mit9</td>
<td>B6/B6</td>
<td>24.2 ± 0.4</td>
<td>3.0 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>1.9 ± 0.3</td>
<td>2.4 ± 0.5</td>
<td>8.7 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>C3H/C3H</td>
<td>22.1 ± 0.6</td>
<td>3.6 ± 0.2</td>
<td>2.8 ± 0.9</td>
<td>3.1 ± 0.4</td>
<td>5.5 ± 1.3</td>
<td>12.1 ± 1.8</td>
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<tr>
<td></td>
<td></td>
<td>4.3 × 10⁻³</td>
<td>0.01</td>
<td>0.10</td>
<td>0.02</td>
<td>0.03</td>
<td>0.18</td>
</tr>
<tr>
<td>D19Mit90</td>
<td>B6/B6</td>
<td>23.9 ± 0.6</td>
<td>2.9 ± 0.2</td>
<td>1.5 ± 0.7</td>
<td>1.9 ± 0.4</td>
<td>2.9 ± 0.6</td>
<td>5.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>C3H/C3H</td>
<td>22.7 ± 0.5</td>
<td>3.7 ± 0.2</td>
<td>2.5 ± 0.7</td>
<td>3.2 ± 0.4</td>
<td>4.9 ± 1.1</td>
<td>14.1 ± 2.0</td>
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<td></td>
<td></td>
<td>0.12</td>
<td>2.1 × 10⁻³</td>
<td>0.32</td>
<td>0.02</td>
<td>0.11</td>
<td>7.7 × 10⁻¹</td>
</tr>
<tr>
<td>DXMit68</td>
<td>B6</td>
<td>25.4 ± 0.2</td>
<td>2.8 ± 0.2</td>
<td>1.4 ± 0.7</td>
<td>1.7 ± 0.4</td>
<td>1.5 ± 0.3</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>C3H</td>
<td>22.8 ± 0.7</td>
<td>3.9 ± 0.3</td>
<td>2.4 ± 0.9</td>
<td>3.6 ± 0.6</td>
<td>4.9 ± 1.1</td>
<td>20.5 ± 4.0</td>
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<tr>
<td></td>
<td></td>
<td>1.4 × 10⁻³</td>
<td>2.0 × 10⁻³</td>
<td>0.4</td>
<td>0.02</td>
<td>8.9 × 10⁻¹</td>
<td>6.8 × 10⁻¹</td>
</tr>
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NOTE: Mice were treated with 18-Gy whole thorax irradiation and euthanized due to presentation of distress symptoms or at the end of the experiment (26 wk). Lung disease phenotype, presented as the mean ± SE, was assessed by survival posttreatment; histologic evidence of alveolitis, fibrosis, and mast cells and bronchoalveolar total and differential cell counts.

*Female mice only.
†Male mice only.
‡Genotypes of C3H or B6 for male mice.

Discussion

In this study, we identified regions of the genome linked to the development of alveolitis and fibrosing alveolitis, the common treatment-related pathologies that limit thoracic cavity radiotherapy for cancer patients. In mice derived from established inbred models of radiation-induced lung response, we showed the postthoracic cavity radiotherapy survival to be determined principally by the development of alveolitis. The complication of fibrosing alveolitis was found to occur only in mice that developed alveolitis and inherited fibrosis-promoting alleles. Separate loci were found to affect susceptibility to each of alveolitis and fibrosis, such that the mice inheriting promoting alleles at these loci developed fibrosing alveolitis and the mice inheriting the protective alleles at the alveolitis regions were spared lung disease and lived to the end of the experiment.

Consistent with previous reports (9–12, 27), the B6 mice of this study developed fibrosing alveolitis in response to radiation exposure whereas C3H strain mice developed the extensive inflammatory response of alveolitis only. The bronchoalveolar lavage data also reflect what has been reported for the radiation response of C3H (28) and B6 (29, 30) mice, and by comparing these profiles, we showed the C3H phenotype to include more neutrophils in the lavage than the B6 strain response. The inflammatory response of the inbred strains also differed in mast cell influx, as we identified that the C3H radiation response of the inbred strains also differed in mast cell influx, as we identified that the C3H radiation response.
B6 response. The previously uncharacterized strain difference in mast cells may contribute to the C3H lung response of alveolitis because this cell type has been shown to secrete chemokines and cytokines, such as interleukin 6 (IL-6), which recruit inflammatory cells (31). Mast cells also release enzyme-rich granules and active amines, which, by permeating surrounding vessels, would promote inflammatory cell infiltration characteristic of alveolitis (32).

The responses of the mice studied here show similarities to that which has been reported for clinical radiation-induced lung disease. In particular, the alveolitis phenotype of mast cells has been identified to be a feature of clinical radiation-induced lung disease (33) and the time for the mice to develop alveolitis or fibrosing alveolitis is similar to that of patients (1–4). Whether alveolitis contributes to fibrosis development clinically is not known, but Giotopoulos et al. (34) have shown the risk of lung fibrosis to be increased in breast cancer patients who develop an early acute reaction (skin inflammation) to radiation. The second fibrosis risk factor identified by Giotopoulos et al. (34) was to have inherited the transforming growth factor-β1 (TGF-β1→509T) single-nucleotide polymorphism (and having undergone radiotherapy), a gene which maps to one of our putative fibrosis linkage intervals. Finally, studies of cancer patients undergoing radiation therapy have revealed bronchoalveolar lavage (35) and serum (8, 36, 37) levels of TGF-β and IL-6 to be associated with the development of a radiation response, which supports the concept that the mouse pathology correlates with the clinical side effects seen with therapeutic radiation in the cancer patients.

The data from the backcross mice suggest that the alveolitis response occurred with an increase in mast cells in tissues and PMN in lavage. The mast cell numbers in the lungs of moribund backcross mice were similar to the counts of the B6 strain (i.e., lower than that of C3H mice), suggesting this level to be sufficient for an alveolitis response. From the fact that distinct loci of the alveolitis response were mapped in 75% C3H backcross mice from 75% B6 backcross mice, it is inferred that different genetic combinations can lead to alveolitis in mice. Indeed, several loci of lung inflammation in response to different irritants have been mapped (38), and our data overlap with previously identified linkages to chromosome 2 (39) and chromosome 17 (40). One specific combination is likely sex specific as males of the 75% B6 backcross mice, it is inferred that different genetic combinations can lead to alveolitis in mice. Indeed, several loci of lung inflammation in response to different irritants have been mapped (38), and our data overlap with previously identified linkages to chromosome 2 (39) and chromosome 17 (40). One specific combination is likely sex specific as males of the 75% B6 backcross only inheriting the C3H allele on the proximal X chromosome developed alveolitis whereas X linkage was not detected in female mice. In addition, a sex difference in response was apparent as the male backcross mice tended to develop respiratory distress symptoms later than female mice, as did mice of the B6 parental strain. Further, in the linkage data providing the map locations of genetic variants dictating the B6/C3H radiation response, the inflammatory phenotypes of the mice grouped by putative alveolitis marker can provide information on mechanisms. Specifically, the majority of the markers that are linked to the development of alveolitis also predict for mast cell levels potentially indicating that the genetic variant underlying alveolitis susceptibility at these loci is related to mast cell recruitment or activity. Supporting this possibility, mast cell proteases 1, 2, 4, and 8 and mast cell chymase 1 have been mapped to the putative alveolitis region of chromosome 14. In addition, genetic variation of chromosomes 4 and X may contribute to the development of an alveolitis response through neutrophil recruitment. Both mast cell number in tissue and percent PMN in lavage. The mast cell numbers in the lungs of moribund backcross only inheriting the C3H allele on the proximal X chromosome developed alveolitis whereas X linkage was not detected in female mice. In addition, a sex difference in response was apparent as the male backcross mice tended to develop respiratory distress symptoms later than female mice, as did mice of the B6 parental strain. Further, in the linkage data providing the map locations of genetic variants dictating the B6/C3H radiation response, the inflammatory phenotypes of the mice grouped by putative alveolitis marker can provide information on mechanisms. Specifically, the majority of the markers that are linked to the development of alveolitis also predict for mast cell levels potentially indicating that the genetic variant underlying alveolitis susceptibility at these loci is related to mast cell recruitment or activity. Supporting this possibility, mast cell proteases 1, 2, 4, and 8 and mast cell chymase 1 have been mapped to the putative alveolitis region of chromosome 14. In addition, genetic variation of chromosomes 4 and X may contribute to the development of an alveolitis response through neutrophil recruitment. Both mast cell number in tissue and percent PMN in lavage were shown to correlate with alveolitis in radiation-treated mice, but whether these inflammatory changes directly contribute to pathology remains unknown.

The fibrosing alveolitis response uniquely occurred in mice inheriting both alveolitis-enhancing alleles and B6 alleles within regions of chromosome 1, 7, 9, or 17. Thus, the combination of
sufficient alveolitis development and fibrosis-promoting alleles was necessary for the radiation-induced response of fibrosing alveolitis. The importance of the alveolitis response to the development of fibrosis is indicated by the fact that only 2 of the 35 mice from the B6 cross that survived to the end of experiment had fibrosis in >50% of the lung. In the remaining 33 mice, the alleles at fibrosis markers (given in Table 4) were found at Mendelian ratios, but fibrosis did not occur in all mice with the genotype B6/B6 at these loci. Significant linkage to the fibrosis phenotype, and indeed significant amounts of lung fibrosis, could only be detected in mice with two B6 alleles at the specific loci, and we were unable to map this phenotype in the 75% C3H backcross data set alone.

The backcross mice developing fibrosis were also found to have more neutrophils in their lavage than did mice developing alveolitis alone. Further, a subset of these 75% B6 backcross mice developed more fibrosing alveolitis than the fibrosis-prone strain B6, which indicates that C3H alleles could contribute to the development of this phenotype, possibly through neutrophil recruitment. PMN infiltration in tissue and percent PMN of lavage to potentially contribute to tissue remodeling (44) or may indirectly affect fibrosis development by cleaving cytokines or chemokines to more or less active forms (45).

In summary, we have mapped loci of susceptibility to radiation-induced alveolitis and fibrosing alveolitis in the mouse. The genetic factors influencing each of these phenotypes were identified to be distinct and to interact to produce a response of either alveolitis or fibrosing alveolitis in the lung, or to spare the mice the development of radiation-induced lung disease. Inflammatory response phenotyping revealed alveolitis severity to be correlated with mast cell infiltration in tissue and percent PMN of lavage to potentially augment the fibrosis response. Despite the inbred strain difference in radiation-induced percent PMN in lavage, lavage cell types alone were not predictive of an alveolitis versus fibrosis response, but rather differentiated those animals developing respiratory distress from those that do not. The genetic basis of the complex phenotype of radiation-induced lung disease can be dissected in mice, and studies elucidating specific genetic variants underlying these susceptibility regions may lead to the development of diagnostic screening tools designed to guide patient-specific therapies and minimize potentially life-threatening complications.

### Acknowledgments

Received 7/18/2007; revised 9/11/2007; accepted 9/18/2007.

Grant support: Canadian Institutes of Health Research grant MOP62846, Fonds de la Recherche en Sante Quebec, and McGill University Health Science Centre.

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### References


Loci of Radiation-Induced Lung Disease


Distinct Loci Influence Radiation-Induced Alveolitis from Fibrosing Alveolitis in the Mouse

Christina K. Haston, Michelle Begin, Genevieve Dorion, et al.


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