Murine Susceptibility to Radiation-induced Pulmonary Fibrosis Is Influenced by a Genetic Factor Implicated in Susceptibility to Bleomycin-induced Pulmonary Fibrosis

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ABSTRACT

From evidence of interpatient variability in normal tissue sensitivity to radiotherapy and from radiation studies using inbred mouse strains, it is hypothesized that individual variation in susceptibility to radiation-induced pulmonary fibrosis is genetically controlled. A genetic model has been developed from the fibrosis-prone C57BL/6J and the fibrosis-resistant C3Hf/Kam mouse strains. Inheritance of the fibrotic phenotype was characterized in F1 and F2 (F1 intercross) generations derived from the parental strains. Genetic mapping was used to determine whether the quantitative trait loci (QTL), which influence susceptibility to bleomycin-induced lung fibrosis in these progenitor strains, could be implicated in susceptibility to radiation-induced lung fibrosis. Mice were treated with 14 or 16 Gy (60Co) to the whole thorax. The doses were selected to investigate the response at the LD50 and LD10 of C3Hf/Kam mice. The animals were sacrificed 33 weeks after treatment or when moribund. The percentage of lung with fibrosis for each mouse was quantified with image analysis of a histological section of the lung. For both the 14- and 16-Gy data sets, heritability was estimated at 38 ± 11%, and the number of genetic factors influencing susceptibility to pulmonary fibrosis was estimated to be one or two. Two hundred fifty-five F2 intercross mice were genotyped with markers at the bleomycin loci on chromosomes 11 and 17 (chromosome 17 marker is at the major histocompatibility complex). Genetic linkage was established for the marker on chromosome 17 (P = 3.0 × 10^-6), which accounts for 6.6% of the F2 phenotypic variance but not for the markers surrounding the QTL on chromosome 11 (P = 0.37). The inheritance data suggested that susceptibility to radiation-induced pulmonary fibrosis is a heritable trait controlled by two genetic loci, and through genomic mapping, a QTL on chromosome 17 was identified as one of the loci.

INTRODUCTION

Radiotherapy is dose limited by the development of late effects in the normal tissue. There are differences, however, within the patient population in susceptibility to the radiation-induced late effect of skin telangiectasia (1). Differences in patient response to radiation, measured as the late effect of pulmonary fibrosis, have been reported (2), but the genetic basis of interpatient variation in lung response has not been studied. To overcome clinical limitations, a mouse genetic model has been developed to investigate the hypothesis that individual variation in susceptibility to radiation-induced pulmonary fibrosis is, in part, genetically controlled.

Differences in radiation response between mouse strains have been measured in terms of pulmonary fibrosis (3–5), survival (6), and apoptosis of thymocytes (7, 8). The difference in lung response to radiation by mouse strain identified by Down and Steel in 1983 (9) was confirmed in a study of nine mouse strains by Sharplin and Franko in 1989 (3). In that work, the C3H/HeJ strain was classified as fibrosis resistant and the C57BL/6J strain as fibrosis prone following whole-thorax irradiation. In a follow-up inheritance study using backcross mice, Franko et al. (5) provided evidence that susceptibility to radiation-induced lung fibrosis is controlled by two autosomal genes that function additively.

Recently, a mouse genetic model of susceptibility to bleomycin-induced pulmonary fibrosis (10), which used C3H and B6 progenitor strains, was used to identify QTL on chromosomes 11 and 17 that influence that phenotype (11). Bleomycin treatment and radiotherapy to the thoracic cavity are similar in that both may lead to pulmonary fibrosis, which appears as a histologically similar lesion (11). It has also been shown that B6 mice develop lung fibrosis in response to ozone (12), hyperoxia (13), and silica (14) exposure, whereas C3H mice do not. Due to this consistent difference in lung response between C3H and B6 mice, it was further hypothesized that the loci that influence susceptibility to bleomycin-induced pulmonary fibrosis also influence susceptibility to radiation-induced pulmonary fibrosis.

MATERIALS AND METHODS

Mice. B6 mice were purchased from The Jackson Laboratory, and C3H mice were bred in the specific pathogen free animal colony of the Department of Experimental Radiation Oncology (University of Texas M. D. Anderson Cancer Center). The F1 (B6 female × C3H male = B6C3F1; C3H female × B6 male = C3B6F1) and F2 (F1 intercross) generations were bred at the M. D. Anderson Cancer Center.

Radiation Treatment. The mice were necessarily treated with either an X-ray unit or a Cobalt 60 unit because the animals were housed in separate colonies. For irradiations, unanesthetized mice were restrained in plastic jigs (15) to immobilize the thoracic region of the animal. Mice housed in the Department of Veterinary Medicine were irradiated to the whole thorax with a single dose of 60Co γ-rays (Eldorado Unit AECL, 1.25 MeV, 0.80 Gy/min) at a skin-to-surface distance of 80 cm. Irradiations were performed through a 25 x 22-mm portal, and the remainder of the body was shielded with 5.1 cm of lead to reduce the beam strength to 4.7% outside the field. Using the same portal size and a skin-to-surface distance of 35.5 cm, mice housed in the Department of Experimental Radiation Oncology were irradiated using a Phillips 250-kV X-ray unit (1.1 Gy/min, 250 kVp, 15 mA, 80% output) to a radiobiologically equivalent single dose. Five mm of lead shielding were used with the X-ray unit in order to reduce the beam strength to 3.1% outside the thorax. An experiment was performed previously to determine the RBE ratio of 60Co to 250 kV X-ray as 0.91. In this report, the radiation doses are given as 60Co. Ten male C3H mice and 10 male B6 mice were treated on each unit to confirm the RBE derived dose. Twenty B6, 20 C3H, and 10 F2 mice were sham irradiated to serve as controls.

Experimental Design. A dose of 14 Gy (LD50 of C3H mice) or 16 Gy (LD100 of C3H mice; Ref. 15) was delivered to C3H, B6, B6C3F1, C3B6F1,

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2 To whom requests for reprints should be addressed, at Department of Experimental Radiation Oncology, Box 66, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030.

3 The abbreviations used are: C3H, C3Hf/Kam; B6, C57BL/6J; F1, first filial generation; F2, second filial generation; QTL, quantitative trait locus (loci); RBE, radiobiological effectiveness.


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and F2 mice, representing all four possible F1 intercrosses. The F1 intercross was selected for the generation of F2 mice because this method is the most efficient for subsequent linkage studies due to the segregation of markers in both parents (16). The inheritance of the fibrosing phenotype was investigated at two dosages to determine whether the mode of inheritance is influenced by dose. The number of animals treated for each sex and dose is given in Table 1. The mice were checked daily for signs of distress (ruffled fur, hunched appearance, and lethargy) and sacrificed by cervical dislocation, when moribund or at 33 weeks after irradiation. The end of the experiment was chosen to encompass the later onset of lung response in the B6 strain (4).

**Histology and Fibrosis Scoring (Phenotype).** At autopsy, the lungs were removed and submitted for histology. The lungs were perfused with 10% neutral buffered formalin and fixed for at least 24 h in formalin. Tissue sections were stained with H&E to evaluate alveolitis and with Masson’s Trichrome to identify the site(s) of collagen deposition in the lung.

The fibrosis phenotype was defined as the fibrotic scar appearing in lungs of B6 mice following radiation treatment and is shown in Fig. 1A. This lesion consists of subpleural foci of collapsed apposed alveolar walls with collagen superimposed.

The fibrotic lesion was quantified with image analysis of histological sections as described elsewhere (10). Specifically, the area of fibrosis in each lung lobe was determined from a user-drawn region of interest surrounding the lung lobe, which was calculated as the area of the alveolar region above the fibrotic lesion, excluding the area of fibrosis in each section through the whole lung, calculated as the weighted (by the area of the lobe) percentage of fibrosis measured in each lung lobe. One section per mouse was analyzed as was shown to be sufficient in previous studies (4, 10) and by others (17).

To confirm that all mice sacrificed before the planned end of the experiment were in respiratory distress, each lung section was assessed for the extent of alveolitis. All mice sacrificed before the end of the experiment were included in the analyses only if the histological section indicated a severe alveolitis response.

**Genotyping with Microsatellite Markers.** DNA from F2 animals was prepared, using a DNA extraction kit, from liver samples collected at necropsy. DNA concentration was determined from spectrophotometric readings at 260 nm.

To genotype F2 intercross progeny, PCRs were performed with one radioactive labeled primer and one unlabeled primer (Research Genetics, Huntsville, AL), and the products were visualized using autoradiography of polyacrylamide gels. For the reactions, the forward primer was end labeled with [32P]dATP (10 mCi/ml) using a T4-polynucleotide kinase. A 15-µl reaction volume containing 45 ng of genomic DNA, 70 mM Tris-hydrogen chloride, pH 7.6, 50 mM potassium chloride, 1.5 mM magnesium chloride, 0.3 units of Taq polymerase, 2 pmol of each unlabeled primer, 0.06 pmol of labeled primer, and 200 μM of each dNTP were prepared in a 96-well plate. The reactions were overlaid with 30 ml of light mineral oil, and PCR was done in an Ericorn thermocycler with the following cycle conditions: 94°C for 5 min, 32 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min, with a final elongation step at 72°C for 10 min.

**Data Analysis.** Tests for differences in fibrotic area (the phenotype) and sacrifice time between groups were done by Student’s t test; tests among groups were done by single-factor ANOVA. The analyses were completed separately for each of the 14- and 16-Gy data sets.

Analyses of the area of fibrosis data set were completed to evaluate the effect of the presence of an X-linked factor, genomic imprinting, or mitochondrial inheritance on the data. To investigate X-linkage, the level of fibrosis in the F1 generation males was compared, as was the level of fibrosis in F1 females. To determine mitochondrial inheritance, the phenotypic data in offspring of a B6C3FI female and offspring of a C3B6FI female were compared. ANOVA was also used to identify any difference in the phenotype among the four F2 types dependent on the F1 cross used in derivation.

An estimate of the number of genetic factors controlling susceptibility to radiation-induced pulmonary fibrosis was obtained using Wright’s formula (18) as amended by Lander and Botstein (16) for F2 by intercross.


\[
k = \frac{\mu_{EF} - \mu_{CE}}{\sigma_{CE}^2}
\]

\[
H = 1 - \left(\frac{\sigma_{CE}^2}{\sigma_{EF}^2}\right)
\]

\[
SE_H = (1 - H)(2/n_e + 1/n_o)^{1/2}
\]

where \( n_e \) and \( n_o \) are the number of individuals contributing to the estimated variances. For the estimates of \( H \) and \( k \), the contribution of each of the parental and F1 strains to the environmental variance was estimated as the mean of the inbred strains, and \( \sigma_{CE}^2 \) is the phenotypic variance of the F2 generation.

The SE of \( H \), \( SE_H \), was estimated from the following equation:

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SE_H = (1 - H)(2/n_e + 1/n_o)^{1/2}
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Linear regression analysis was completed to determine whether the area of fibrosis in F2 mice was correlated with the time of sacrifice.

**Linkage Analysis.** F2 mice were genotyped with markers D11Mit5 and D11Mit320 to surround the peak of the chromosome 11 QTL identified in the bleomycin study and marker D17Mit13, at the peak of chromosome 17 QTL. All of the F2 mice included in the phenotypic analyses were genotyped except mice sacrificed at the end of the experiment, which exhibited no lung damage, due to the ambiguous phenotype. Mice that lack a measurable lung response are not useful for subsequent genotyping because these mice can not be classified as having the C3H or B6 phenotype. To investigate possible linkage between bleomycin markers and the radiation-induced phenotype, the F2 mice were grouped by genotype, and differences in the mean phenotypic level between groups were assessed by Student’s t test. With the calculated \( P \), linkage was assessed using the standards proposed by Lander and Kruglyak.

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**Table 1 Fibrotic phenotype and sacrifice time by mouse strain and dose**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Mean area of fibrosis, % (variance)</th>
<th>Mean sacrifice time, weeks (variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>16 Gy</td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>Males</td>
<td>3.5 (6.9)</td>
<td>30.6 (4.9)</td>
</tr>
<tr>
<td>C3HF/Kam</td>
<td>Males</td>
<td>0.0 (0)</td>
<td>14.8 (1.5)</td>
</tr>
<tr>
<td>B6C3FI</td>
<td>Males</td>
<td>0.3 (0.9)</td>
<td>32.2 (7.1)</td>
</tr>
<tr>
<td>C3B6FI</td>
<td>Males</td>
<td>0.3 (0.5)</td>
<td>26.0 (28.6)</td>
</tr>
<tr>
<td>F2</td>
<td>Males</td>
<td>1.0 (4.6)</td>
<td>25.1 (40.0)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>7.6 (21.1)</td>
<td>23.5 (3.6)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.0 (0)</td>
<td>12.8 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.3 (0.3)</td>
<td>24.9 (17.7)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.5 (1.6)</td>
<td>25.2 (30.7)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>1.0 (4.2)</td>
<td>21.5 (37.5)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>7.5 (10.7)</td>
<td>26.0 (6.5)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.0 (0)</td>
<td>26.2 (49.6)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.5 (1.3)</td>
<td>26.7 (25.2)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.6 (1.3)</td>
<td>28.6 (19.3)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>1.0 (5.7)</td>
<td>24.1 (43.8)</td>
</tr>
</tbody>
</table>

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Table 1 summarizes the fibrotic phenotype and sacrifice time by mouse strain and dose.
GENETIC MODEL OF RADIATION-INDUCED LUNGFIBROSIS

Fig. 1. Histological sections of the left lung from male mice following 16-Gy whole-thorax irradiation. A, section from B6 mouse exhibiting the fibrotic phenotype; lung damage consists of subpleural foci of collapsed alveolar walls with superimposed collagen and inflammatory response of cells in airspace surrounds the fibrotic lesion. B, section from C3H mouse with no fibrosis. The lesion is alveolitis characterized by thickened alveolar walls and cells in the airspace. Masson's Trichrome stain. ×63.

(21). The percentage of phenotypic variance for which a specific marker accounts was determined with linear regression.

RESULTS

Of the 900 animals treated, 0.7% were excluded from analysis due to spontaneous tumour development, 10% due to insufficient lung damage, and 20% because they were found dead. There was no difference in the mean time at which animals were found dead \( (P = 0.64) \) and the mean sacrifice time of mice considered in the analyses; therefore, the mice found dead were not a distinct population.

To confirm that the mice received identical treatment in the two animal colonies, the response of 10 C3H males and 10 B6 males treated in each colony was compared. There was no difference in the mean sacrifice time \( (P = 0.65 \text{ for C3H and } P = 0.84 \text{ for B6}) \) or in the mean level of fibrosis \( (\text{zero for all C3H, } P = 0.30 \text{ for B6}) \) of the mice segregated by colony. Additionally, there was no difference in the mean fibrotic score \( (P = 0.63) \) between the F2 mice treated in either colony. Therefore, the data sets of the two colonies were combined.

Strain Differences. The fibrotic lesion in the lungs of B6 mice following a 16-Gy whole-thorax dose is shown in Fig. 1A. This lesion consists of subpleural foci of collapsed alveolar walls and contains a cellular infiltrate and superimposed collagen. The lungs of C3H mice (Fig. 1B) at the same dose do not show fibrosis. Rather, the injury depicted in the C3H lung is alveolitis, evidenced by thickened alveoli, cellular debris in the air spaces, edema, and foamy cells. The lesions are the same following a 14-Gy dose; histology not shown. No control mice showed any evidence of fibrosis.

Sixty-seven of 72 (93%) irradiated B6 mice developed fibrosis, and the mean fibrotic area of 3.6 ± 0.4% (SE) differed \( (P = 8.6 \times 10^{-13}) \).
Inheritance Pattern. The distribution of the fibrosing phenotype in parental, F1, and F2 mice is given in Fig. 2, and those data, along with the sacrifice times, are summarized in Table 1.

The mean fibrotic level of F1 mice was found to be intermediate to the parental strains at 16 Gy and, for the F1 females only, at 14 Gy. After 16 Gy, the fibrotic score of F1 females did not differ from that of F1 males (P = 0.22); for the F1 males and females collectively, the mean area of fibrosis was lower than the B6 fibrosis (P = 2.3 x 10^-7) and greater than the C3H level (P = 0.0001). Similarly, after 14 Gy, the fibrotic level of F1 females was intermediate to the parental strains, lower than B6 fibrosis (P = 8.06 x 10^-5) and greater than the C3H level (P = 0.025). In F1 males at 14 Gy, the mean fibrotic score was lower than that of B6 mice (P = 0.0017) but was not different from that of the C3H mice (P = 0.08).

The level of fibrosis was found to be the same for B6C3F1 mice as for C3B6F1 mice in both males (P = 0.47 for 14 Gy and P = 0.41 for 16 Gy) and females (P = 0.46 for 14 Gy and P = 0.22 for 16 Gy); therefore, there is no evidence for an X-linked or maternal inheritance influence in the data.

In the F2 generation, there was no difference in extent of fibrosis between mice treated with 14 Gy and mice treated with 16 Gy (P = 0.32), and this result did not change when mice were segregated by sex; therefore, analyses were completed of the F2 data set as a whole. The fibrotic response of the F2 generation was not consistent with mitochondrial inheritance as determined by comparing the phenotypes of offspring of a B6C3F1 female with those of a C3B6F1 female, (P = 0.16). ANOVA showed no difference among the four F2 groups (P = 0.49), grouped by the parental F1 cross; therefore, the extent of fibrosis in the F2 generation does not depend on the F1 cross used in derivation. These results did not change when the data were grouped by sex or by dose.

Despite the strain difference in sacrifice time between the fibrosis-prone B6 mice, which lived longer after radiation treatment than the fibrosis-resistant C3H mice, the area of fibrosis in an F2 mouse was not related to sacrifice time (P = 0.43). This result did not change when the data were segregated by sex or dose.

The component of the F2 phenotypic (area of fibrosis) variance attributed to genetic factors, the heritability (H), was determined to be 38 ± 11% for the 14-Gy data set and 38 ± 9% for the 16-Gy data set. The number of genetic factors influencing susceptibility to radiation-induced lung fibrosis was estimated to be 1 for 14 Gy and 2 for 16 Gy.

Linkage Analysis. Markers at each of the loci that influence susceptibility to bleomycin-induced pulmonary fibrosis were used to genotype the radiation treated F2 mice and the linkage data are presented in Table 2. For the 14-Gy data set, 88 of 127 (69%) F2 mice

Table 2. Effect of genotype on phenotype of susceptibility to radiation-induced pulmonary fibrosis

<table>
<thead>
<tr>
<th></th>
<th>B6/B6</th>
<th>C3H/C3H</th>
<th>B6 + C3H</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 Gy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D17Mit13</td>
<td>2.3^a (0.6)^b</td>
<td>0.4 (0.8)</td>
<td>1.1 (5.5)</td>
<td>88</td>
<td>0.005</td>
</tr>
<tr>
<td>D11Mit5</td>
<td>0.6 (1.4)</td>
<td>1.0 (6.4)</td>
<td>1.4 (6.3)</td>
<td>77</td>
<td>0.32</td>
</tr>
<tr>
<td>D11Mit320</td>
<td>0.9 (1.5)</td>
<td>0.95 (5.2)</td>
<td>1.7 (8.4)</td>
<td>79</td>
<td>0.47</td>
</tr>
<tr>
<td>16 Gy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D17Mit13</td>
<td>2.2 (8.7)</td>
<td>0.3 (0.4)</td>
<td>1.4 (6.4)</td>
<td>180</td>
<td>7.6 x 10^-6</td>
</tr>
<tr>
<td>D11Mit5</td>
<td>1.3 (5.7)</td>
<td>1.1 (4.8)</td>
<td>1.3 (4.9)</td>
<td>178</td>
<td>0.32</td>
</tr>
<tr>
<td>D11Mit320</td>
<td>1.3 (5.2)</td>
<td>1.1 (5.7)</td>
<td>1.3 (4.8)</td>
<td>178</td>
<td>0.31</td>
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<td>All</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D17Mit13</td>
<td>2.0 (8.2)</td>
<td>0.3 (0.4)</td>
<td>1.2 (5.2)</td>
<td>264</td>
<td>3.0 x 10^-6</td>
</tr>
<tr>
<td>D11Mit5</td>
<td>1.2 (5.0)</td>
<td>1.0 (5.2)</td>
<td>1.4 (5.3)</td>
<td>255</td>
<td>0.37</td>
</tr>
<tr>
<td>D11Mit320</td>
<td>1.1 (3.2)</td>
<td>1.0 (5.4)</td>
<td>1.4 (7.0)</td>
<td>257</td>
<td>0.38</td>
</tr>
</tbody>
</table>

^a Mean area of fibrosis.
^b Variance.
were used in genotyping, as were 180 of 220 (82%) F2 mice treated with 16 Gy. The remaining mice were sacrificed at the end of the experiment and were excluded from this analysis due to insufficient lung damage (see "Materials and Methods"). There was no difference in the mean fibrotic levels of mice of the 14-Gy and 16-Gy data sets selected for genotyping ($P = 0.40$).

The marker D17Mit13, on chromosome 17, is implicated in radiation-induced lung fibrosis because the determined $P (P = 3.0 \times 10^{-6})$ exceeded the linkage standard ($P = 5.2 \times 10^{-5}$) for this type of cross (21). The linkage standard is also exceeded for the 16-Gy data set alone ($P = 7.6 \times 10^{-6}$) but not for the 14-Gy data set alone, as shown in Table 2. This genetic factor is consistent with additive inheritance because each B6 allele of marker D17Mit13 increased the mean fibrotic score of F2 mice by approximately 0.9% (area of fibrosis units) from the level of fibrosis in mice with C3H/C3H genotype. Furthermore, the level of fibrosis in F2 mice heterozygous for marker D17Mit13 was 104% of the midlevel between mice homozygous C3H and mice homozygous B6, which is consistent with an additive mode of inheritance. This locus is estimated to account for 6.6% of the phenotypic variance of the F2 generation. The markers of the bleomycin chromosome 11 QTL were not linked to susceptibility to radiation-induced lung fibrosis (see Table 2). This result did not change when males and females were considered separately.

DISCUSSION

The inheritance studies of the fibrotic phenotype in the parental, F1, and F2 generations suggest that susceptibility to radiation-induced lung fibrosis is a heritable trait and is controlled by two autosomal factors. Through genomic mapping, a QTL at the MHC has been implicated as one of the factors.

The estimated two factors that influence this phenotype agree with the fibrotic pathway proposed by Franko et al. (5) in which two autosomal genes function additively to determine the extent of the principle type of fibrosis in C57LJ mice compared to CBA/J mice. There is strong evidence for the genetics of this proposed fibrotic pathway because data from the present study and the Franko study, using two different fibrosis-prone (B6 and C57LJ) and two different fibrosis-resistant (C3H and CBA/J) mouse strains, and a similar histological assay of fibrosis indicate that susceptibility to pulmonary fibrosis is influenced by the same number of autosomal factors (2), with no evidence of sex linkage.

In this study, the marker D17Mit13, which identifies a QTL influencing susceptibility to bleomycin-induced pulmonary fibrosis in B6 compared to C3H mice, was used to implicate the same locus in susceptibility to radiation-induced lung fibrosis. The identified QTL on chromosome 17 is near the MHC, which has been implicated, through allelic association, in susceptibility to silica-induced pulmonary fibrosis in humans (22). Further evidence of a common genetic factor in the fibrosis-susceptible response of B6 compared to C3H mice comes from other genetic models of lung fibrosis using these progenitor strains. In addition to radiation and bleomycin, B6 mice have been shown to be fibrosis prone in response to ozone (12), hyperoxia (13), and silica (14) exposure, and C3H mice are the fibrosis-resistant strain in these studies.

The chromosome 17 QTL was demonstrated to be linked to susceptibility to radiation-induced lung fibrosis in mice treated with 14 and 16 Gy. Using the 14-Gy data set only, however, the marker D17Mit13 could not be established as being linked to the phenotype following the linkage standard derived by Lander and Kruglyak (21). Because the fibrotic response of mice treated with 14 Gy follows that of mice treated with 16 Gy in parental differences, heritability, and phenotypic differences by marker D17Mit13 genotype, the lack of significant linkage demonstrated at 14 Gy was most likely due to insufficient animal numbers. In the 14-Gy data set, only 88 F2 mice were informative for a mapping study, compared to the 180 mice genotyped at 16 Gy. The similarities between the 14-Gy and 16-Gy data sets further indicates that the inheritance of susceptibility to radiation-induced pulmonary fibrosis is not a function of dose.

There is evidence for the effect of a second genetic factor in influencing susceptibility to radiation-induced lung fibrosis. In addition to the estimate of two factors from Wright's formula, the QTL on chromosome 17 accounted for 6.6% of phenotypic variance, which is a fraction of the estimated heritability (38%). Furthermore, the mean fibrotic score of F2 mice, which are homozygous B6 at D17Mit13, was 2.0% whereas the mean B6 level of fibrosis was 3.6%. This second factor was not, however, the bleomycin QTL of chromosome 11. In the bleomycin study (10), the QTL on chromosome 11 was identified in male mice only, but in the present work, this QTL was not confirmed in a data set of males only or in the whole radiation data set. Although there are no obvious candidate genes for this second factor, differences in the radiation-induced expression of cytokine genes have been measured between the parental strains used in this study (23, 24).

Following a whole-thorax radiation dose, the C3H pulmonary response was massive alveolitis leading to sacrifice within 16 weeks or resolution of the lung damage with no fibrosis at the end of the experiment, depending on the dose. This is in contrast to the B6 pulmonary response of alveolitis and fibrosis leading to later sacrifice in almost all (93%) the mice of this strain. The differences in the radiation response of the parental strains, in the level of fibrosis, and in time to sacrifice may indicate that these two measures are related. The level of fibrosis of an F2 mouse, however, was shown to be independent of its sacrifice time. Fibrosis may, therefore, be the response to insufficient resolution of alveolitis in mice with the genetic susceptibility to fibrose, and not a time specific response.

Approximately 30% of the treated mice were excluded from the analyses. The fraction of animals lost to the analyses as found dead or sacrificed with insufficient lung damage was due to the subjective nature by which animals were judged to be in distress. This fraction lost, however, does not affect the results and conclusions derived from the data set of mice confirmed to have significant lung damage.

In this study, it was demonstrated that a factor which influences genetic susceptibility to bleomycin-induced lung fibrosis also influences susceptibility to radiation-induced lung fibrosis. The existence of a QTL can be confirmed by achieving highly significant linkage (21), which would require many more mice in the study, or by a separate study confirming the result. Evidence to evaluate this QTL for a pulmonary radiation-induced fibrotic response specifically could be derivable using the model of Franko et al. (5).

REFERENCES

Murine Susceptibility to Radiation-induced Pulmonary Fibrosis Is Influenced by a Genetic Factor Implicated in Susceptibility to Bleomycin-induced Pulmonary Fibrosis

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