Inheritance of Susceptibility to Bleomycin-induced Pulmonary Fibrosis in the Mouse

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ABSTRACT

Based on the range of patient responses to treatment, and on animal studies, it is hypothesized that individual variation in sensitivity to bleomycin-induced pulmonary fibrosis is controlled genetically. A genetic model has been developed by (a) establishing a distinct difference in bleomycin-induced lung damage in two inbred strains of mice [parental generation: C57BL/6J (fibrosis-prone phenotype) and C3Hf/Kam (fibrosis-resistant phenotype)] and (b) characterizing inheritance of the fibrosing phenotype in the F1 (first filial) and F2 (F1 intercross; second filial) generations derived from the parental strains. Male mice received 100 mg/kg and female mice 125 mg/kg of bleomycin via s.c. osmotic minipump. The animals were sacrificed 8 weeks after treatment or when their breathing rate indicated respiratory distress. The percentage of lung with fibrosis for each mouse was quantified with image analysis of a histological section of the left lung. The mean percentage of fibrosis for the C57BL/6J males was 8.4 ± 0.8% (SE) and 4.4 ± 0.8% for females, and the C3Hf/Kam mice of either sex did not present the fibrosing lesion (mean score, 0%). Significant difference (P = 6 × 10^{-4}) was measured in percentage of fibrosis between the two strains of F1 males, but not F1 females (P = 0.38), suggesting the presence of an X-linked factor associated with the fibrosing phenotype. From an ANOVA the X-linked factor is estimated to contribute 19% of the fibrosis phenotype. A genetic model of two or three loci controlling the fibrosing phenotype is proposed from the data of the parental, F1, and F2 generations. The mouse model demonstrates that susceptibility to bleomycin-induced pulmonary fibrosis is a heritable trait controlled by a few genetic loci.

INTRODUCTION

Almost since its discovery 30 years ago (1), bleomycin has been known to induce pulmonary fibrosis (2). In a review of patient response to bleomycin, Weiss (3) reports that certain individuals are especially sensitive to this side effect of the drug. Hsu et al. (4), based on results from a cytogenetic assay, found interindividual variation in susceptibility of cultured lymphocytes to bleomycin-induced DNA damage. Differences in susceptibility to bleomycin-induced pulmonary fibrosis by mouse strain have also been demonstrated (5–7). On the basis of these observations in humans (2, 3) and animals (5–7), it is hypothesized that susceptibility to bleomycin-induced pulmonary fibrosis is, in part, genetically regulated.

Considerable work has been completed using animal models to study the characteristics of bleomycin-induced pulmonary fibrosis. The lesion caused by bleomycin involves focal collagen deposition (8–10), that may occur after an inflammatory response (11). Based on the varying extent of pulmonary fibrosis in four strains of mice, Schrier et al. (14) proposed that bleomycin-induced fibrosis is under polygenic control, but this genetic basis has not been pursued.

INTRODUCTION

In this study, a mouse model is developed, using inbred strains, to determine the extent of a genetic component in susceptibility to bleomycin-induced pulmonary fibrosis and to characterize the inheritance of this susceptibility. The base of the model consists of two inbred strains of mice that differ in their susceptibility to a fibrosing lesion, C57BL/6J, which is prone (8, 10, 14), and C3Hf/Kam, which is shown here to be resistant. The model developed follows the method for identifying heritable traits outlined by Lander and Botstein (17) and Levitt et al. (22). Initially, a standard protocol for characterizing the fibrosing phenotype, including bleomycin dose, assay of the fibrosing phenotype, and assay time, was developed in parental mice. The inheritance of the fibrosing phenotype was then characterized in F1 and F2 (first and second filial, respectively) generation mice from these progenitor strains after treatment with the bleomycin standard protocol.

MATERIALS AND METHODS

Mice. C57BL/6J mice were purchased from The Jackson Laboratory and housed in microisolator cages in the specific pathogen free room of the animal colony of the Department of Veterinary Medicine. The F1 (C57BL/6J female × C3Hf/Kam male = B6C3F1; C3Hf/Kam female × C57BL/6J male = C3B6F1) and F2 (F1 intercross) generations were bred by us and housed similarly in this colony. The C3Hf/Kam mice were bred and housed in the specific pathogen free animal colony of the Department of Experimental Radiotherapy. At the time of treatment all mice were 8–10 weeks old.

Bleomycin Treatment. Lung damage was elicited by administering bleomycin (clinical-grade Blenoxane; gift of Sal Lucania and of Dr. Terry Dugan, Bristol-Myers Squibb, Evansville, IN) through osmotic minipumps implanted s.c. Bleomycin was dissolved in 0.9% NaCl solution and loaded into 7-day minipumps (model 2001; ALZA Corp., Palo Alto, CA). Each mouse was anesthetized with Nembutal (5.6 mg/mL sodium solution) at a dose of 0.01 ml/g mouse body weight given i.p. The pump was implanted through a small incision in the mouse's back, and this wound was sealed with wound clips.

Breathing Rate Assay. The mice were checked daily for signs of distress. To identify mice in respiratory distress, breathing rates were collected at 4 weeks after the implantation of the pumps, and then weekly to 8 weeks, which

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

3 The abbreviations used are: QTL, quantitative trait loci; ROI, region of interest; HP, hydroxyproline.
was the end of the experiment. Respiratory distress was defined as a breathing rate more than 130% of the control value. The plethysmograph breathing rate assay developed in this laboratory (23) and recently amended (24) was used.

**Histology and Fibrosis Scoring.** At autopsy, the lungs were removed; the right four lobes were archived, and the left lobe was submitted for histology. The single left lobe of each mouse was perfused with 10% neutral buffered formalin and fixed for at least 24 h in formalin. The tissue sections were stained with H&E and with Masson’s trichrome to identify the site(s) of collagen deposition in the lung, and examined by light microscopy. Each lung section was scored for both the fibrosis phenotype and the extent of inflammation.

A quantitative endpoint of the fibrotic phenotype was necessary to permit genetic modeling based on the variance of the amount of fibrosis in parental, F1, and F2 mice. The area of the fibrosing phenotype for each mouse was scored by image analysis of a single left lobe section. The fibrosing phenotype was defined as the fibrotic scar appearing in lungs of C57BL/6J mice after bleomycin treatment and is shown in Fig. 1. This lesion consisted of subpleural foci of collapsed alveolar walls with collagen superimposed.

The fibrotic lesion was quantified by collecting a 24-bit color image (frame grabber; Vision plus AT, Bedford, MA) of each section with a color video camera (model DXC-960MD, Sony Medical Systems, Montvale, NJ) attached to a stereomicroscope and interfaced to a computer. Two ROIs were defined on the image using OPTIMAS software (Bioscan, Edmunds, WA), as illustrated in Fig. 2. The total area (Lung ROI) of the left lobe was determined by the computer using a threshold pixel value for tissue. A user-selected ROI was then drawn on the image to enclose the fibrosis (Fibrosis ROI). The percentage of fibrosis was calculated as:

\[
\% \text{ Fibrosis} = \frac{\text{Fibrosis ROI}}{\text{Lung ROI}} \times 100
\]

To determine whether scoring one section per mouse would ensure accurate classification of the fibrosing phenotype, 10 sections of lung tissue from each of 6 fibrosing mice and 20 mice with zero fibrosis were analyzed.

The extent of inflammation was also assessed for each lung section to present the total lung response to bleomycin treatment. A graded system was used to score inflammation, because the diffuse inflammatory lesion lacks the clear boundary that was used to define the area of fibrosis. Inflammation was scored by assigning a score of 0 to 3 to each left lobe section. A score of 0 indicates no inflammation; a score of 1 indicates a single limited area of inflammation characterized by the presence of a few foamy cells and cells in the air space; a score of 2 represents a moderate inflammation that involves more than one area in the lung; and a score of 3 indicates a severe inflammatory response involving more than half of the lung.

**Experimental Design:** Parental Studies. Dose-response studies were completed in the C57BL/6J (43 males, 42 females) strains to identify the dose of bleomycin that maximizes the difference in the development of the fibrosis between these two strains while minimizing acute toxicity. The acute toxicity phase was defined as death occurring 14–20 days after pump implantation. The mice received a dose of bleomycin (100 mg/kg of body weight), which has been shown to induce significant fibrosis in the lungs of C57BL/6J mice, over 6 weeks of study (8), and also 125 and 150 mg/kg. The animals were sacrificed by cervical dislocation at 8 weeks or when in respiratory distress as indicated by an elevated breathing rate. To determine whether the C57BL/6J strain develops fibrosis at a later time, 10 C57BL/6J males were given 100 mg/kg bleomycin and 10 C57BL/6J females were given 125 mg/kg bleomycin and sacrificed at 12 weeks. Five male mice of each of the parental strains were given 125 mg/kg bleomycin and sacrificed at 18 days after pump implantation to examine whether lung damage was associated with the acute toxicity phase. Ten of each of the parental C57BL/6J and C3H/HeJ mice were chosen for the study, with saline-filled pumps.

**Inheritance Studies.** The standard treatment dose of 100 mg/kg for males and 125 mg/kg for females, as determined in the parental study, was delivered to the B6C3F1 (28 males, 18 females) and C3B6F1 (30 males, 20 females) strains and to F1 mice (140 males, 99 females) representing all four possible F1 intercrosses. The mice were sacrificed at 8 weeks or when in respiratory distress as indicated by an elevated breathing rate.

**Data Analysis.** Tests for differences in fibrotic area between groups were done with single-factor ANOVA. The male and female data sets were considered separately, because male mice were treated with a dose of 100 mg/kg and female mice with a dose of 125 mg/kg.

**Analyses of the area of fibrosis data set (the phenotype) were completed to evaluate the possibility of an X-linked factor or genomic imprinting or mitochondrial inheritance associated with lung fibrosis. To investigate the influence of X linkage or genomic imprinting in the F1 generation, the level of fibrosis in the two strains of F1 males was compared as was the level of fibrosis in F1 females. To test for consistency with X linkage in the F2 generation, the F2
parental mice. Fourteen to 20 days after pump implantation, all mice exhibited an acute toxicity characterized by wet, slick fur, but with no increases in breathing rate. Histological examination of the lungs from five male C57BL/6J mice treated with 125 mg/kg bleomycin and sacrificed during this acute phase indicates the lungs were free of fibrosis. Three of these five mice sacrificed during the acute toxicity phase, however, exhibited mild inflammation (scores of 1), and the other two scored 0. The lungs from five male C3Hf/Kam mice sacrificed at the same time contained no histological injury. The mice sham treated with the saline pumps exhibited no acute toxicity.

To verify that fibrosis in one histological section per lung was representative of the level of fibrosis in the entire lobe, serial sections from fibrosing and nonfibrosing mice were analyzed. No fibrosis was evident in any of the 10 sections from each of the 20 nonfibrosing mice. Fibrosis was present in all sections from the lungs of six fibrosing mice. The amount of fibrosis varied less between sections from the same lung (the maximum coefficient of variation of these six mice was 15.2%) than the intrastrain coefficient of variation of 44% for C3HfBL/6J females and 81% for C3HfBL/6J males.

The fibrotic lesion in the lungs of male C3HfBL/6J mice after a 7-day s.c. exposure to 100 mg/kg bleomycin is shown in Fig. 1A. This lesion consists of foci of collapsed alveolar walls located in the subpleura and contains a cellular infiltrate and superimposed collagen. The lungs of C3Hf/Kam mice (Fig. 1B) at the same dose do not show fibrosis. The fibrotic lesion was always localized to the subpleura and was often multifocal, as shown in Fig. 2.

Because of the high incidence of toxicity after a dose of 150 mg/kg, this dose was discontinued, and the area of the fibrosis was scored in the 100- and 125-mg/kg dose groups only. At the dose of 100 mg/kg, all C57BL/6J male mice expressed the fibrosing phenotype, whereas it was absent from all C3Hf/Kam male mice, including those sacrificed at 12 weeks. The higher dose did not increase the mean fibrotic score in C57BL/6J mice (8.6 versus 8.4% at 100 mg/kg; P = 0.90) and 4 of 10 C57Hf/Kam male mice developed minimal fibrosis at a dose of 125 mg/kg, with a mean area of 0.1%. Thus, the dose of bleomycin to discriminate C57BL/6J male mice as susceptible to the fibrosing phenotype and C3Hf/Kam male mice as resistant, with minimal acute toxicity, was determined to be 100 mg/kg.

The mean percentage of fibrosis in the lungs of C3HfBL/6J females at a dose of 100 mg/kg was not significantly different from that at 125 mg/kg (2.7 versus 4.4%; P = 0.10), but at the lower dose, 10 of 32 mice did not develop fibrosis. At a dose of 125 mg/kg, all C3HfBL/6J female mice developed the fibrosing phenotype. In C3Hf/Kam females, only 1 of 29 mice developed any fibrosis at 8 weeks at the higher dose of 125 mg/kg, and 1 of 10 developed fibrosis (area = 0.4%) by 12 weeks. Thus, the dose of bleomycin to distinguish C3HfBL/6J female mice as susceptible to the fibrosing phenotype and C3Hf/Kam female mice as resistant, with minimal acute toxicity, was determined to be 125 mg/kg. No control or saline-treated mice showed any evidence of fibrosis.

The scores of inflammation in the lungs are presented by mouse strain in Table 1. The mice sacrificed before the end of the experiment

### Table 1 Left lung inflammation scores

<table>
<thead>
<tr>
<th></th>
<th>Male inflammation scores</th>
<th>Female inflammation scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>0</td>
</tr>
<tr>
<td>C3Hf/Kam</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>B6C3F1</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>C3Hf/Kam</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>F2</td>
<td>136</td>
<td>33</td>
</tr>
</tbody>
</table>

* Number of mice with zero inflammation.
Table 2: Acute toxicity, respiratory distress, and mean percentage of fibrosis in parental and inheritance studies

<table>
<thead>
<tr>
<th></th>
<th>Males (100 mg/kg)</th>
<th>Females (125 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Respiratory distress</td>
</tr>
<tr>
<td>Acute toxicity</td>
<td>0 (0)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>C3Hf/Kam</td>
<td>20</td>
<td>32 (11%)</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>28</td>
<td>16 (76%)</td>
</tr>
<tr>
<td>B6C3F1 (C57 X C3H)</td>
<td>28</td>
<td>13 (46%)</td>
</tr>
<tr>
<td>C3B6F1 (C3H X C57)</td>
<td>30</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>F2</td>
<td>140</td>
<td>23 (20%)</td>
</tr>
</tbody>
</table>

Fig. 4. Final breathing rate of male mice after 100 mg/kg bleomycin.

Fig. 5. Distribution of percentage of fibrosis in males (top) and females (bottom). Bar, mean score.

all had inflammation scores of 2 or 3. Mice of the C3Hf/Kam strain always presented moderate to severe inflammation (scores of 2 or 3), and most of the C3Hf/Kam mice did not develop inflammation. The incidence of respiratory distress (Table 2) also indicates the strain difference in response to bleomycin. The B6C3F1 males were the only F1 type relatively sensitive to the bleomycin, as indicated by respiratory distress and inflammation. As shown in Table 2, a similar fraction of F1 males and F2 females were sacrificed because of functional difficulty. Minimal acute toxicity was measured in the inheritance study as indicated in Table 2. Mice exhibiting lethal acute toxicity were excluded from the analyses.

Fig. 4 shows the distribution of breathing rates at sacrifice for male mice of the parental, F1, and F2 generations after treatment with 100 mg/kg of bleomycin, and includes mice sacrificed when sick and at the end of the experiment. There is a clear separation of breathing rate data for the two parental strains, as the C3Hf/Kam mice have breathing rates near 4–5 breaths/s, and the C57BL/6J breathe at 6–9 breaths/s. Mean breathing rates for control mice range from 4–5 breaths/s for C3Hf/Kam and 5–6 breaths/s for C57BL/6J mice (data not shown). The final breathing rate of the B6C3F1 males was significantly higher than that of the C3B6F1 males [6.5 ± 0.2 (SE) versus 5.8 ± 0.1 breaths/s; P = 0.002], and this difference also occurred between the F1 females (B6C3F1, 5.8 ± 0.1; C3B6F1, 5.5 ± 0.1; P = 0.0005; data not shown).

The distribution of the fibrosing phenotype in F1 and F2 male and female mice is given in Fig. 5, and these data are summarized in Table 2. The F2 data set includes 30 (32%) of 95 female mice and 46 (40%) of 116 male mice that did not develop fibrosis. Using Wright’s formula (Eq. B) and the data presented in Table 2, the number of QTL for the male data set is 3.0, and for the female data set it is 0.3.

The mice of the F1 generation displayed minimal fibrosis in response to bleomycin, with the exception of the higher fibrotic response of the B6C3F1 males. The mean area of fibrosis in B6C3F1 males is significantly lower than that of C3Hf/Kam males (4.2 versus 8.4%; P = 0.0004). To determine whether the response of the F1 generation was due in part to X linkage or genomic imprinting, the percentage of fibrosis data were compared by parental strain dam. The amount of fibrosis in the B6C3F1 (C57BL/6J dam) mice was greater than that in C3B6F1 (C3Hf/Kam dam) mice for the males (P = 6 × 10^-6) but not the females (P = 0.38), which is consistent with X linkage. There was no evidence of X linkage in the F2 female mice (P = 0.63).

The contribution of an X-linked factor was estimated as the relative interstrain variance in the F1 compared to the parental (F0) generation. This approach considers the between-strain variation in the parental generation, where the strains differ in all the factors that constitute susceptibility to fibrosis, and the between-strain variation in the F1 generation, because the F1 males only differ in their sex chromosomes. The relative variance between the strains of F1 males is an estimate of the contribution of the X factor to the total genetic variance. Using the following equation and the phenotypic data set of the males, the contribution of an X-linked factor is estimated to be 19% of total genetic variance.

\[
\frac{\sigma^2_{F1}}{\sigma^2_{F0}} = \left( \frac{\hat{X}_{B6C3F1} - \hat{X}_{F1}}{\hat{X}_{C3B6F1} - \hat{X}_{F1}} \right)^2 + \left( \frac{\hat{X}_{C3B6F1} - \hat{X}_{F1}}{\hat{X}_{C3Hf/Kam} - \hat{X}_{F1}} \right)^2 \times 100\%
\]

The F2 data are not consistent with mitochondrial inheritance as determined by comparing the results in offspring of a B6C3F1 dam
with those of a C₃B₆F₁ dam for either the males (P = 0.69) or the females (P = 0.11). To investigate the effect of an F₁ cross (F₁ dam and sire) on the percentage of fibrosis data, the data of the F₂ generation were grouped by F₁ cross as presented in Table 3. ANOVA showed no difference among the four F₂ groups for males (P = 0.94) or females (P = 0.34); therefore, the extent of fibrosis in the F₂ generation does not depend on the F₁ cross.

**DISCUSSION**

The foundation of this genetic model of bleomycin-induced lung fibrosis is the presence of the distinctive fibrosing phenotype in the lungs of C₅B₁/6J mice and the lack of fibrotic response in lungs from C₃Hf/Kam mice after the same dose of bleomycin. The inheritance studies of this clear fibrosing phenotype in parental, F₁, and F₂ generations suggest that susceptibility to bleomycin-induced lung fibrosis is a heritable trait controlled by two or three genetic factors, one of which may be X-linked.

The susceptibility to bleomycin-induced lung damage is unequivocally presented in the histology of each mouse. The imaging assay of percentage of fibrosis was developed so that these histological data could be quantified, and the assay has been used by others (10, 25, 26). Quantifying fibrosis in one section per lung was determined to be representative of fibrosis in the whole lung, in agreement with Van Rongen et al. (25).

The fibrotic phenotype is the same lesion Harrison et al. (8) produced in the lungs of C₅B₁/6J mice with bleomycin administered through osmotic minipumps. In that study (8) and others (6, 9, 11, 12, 14, 25), HP content was used to assay tissue collagen as a measure of lung fibrosis. The HP assay has been used to discern differences in susceptibility to bleomycin-induced fibrosis among mouse strains (6, 8, 14) but not to categorize individual mice. In our preliminary studies to develop the quantitative assay of fibrosis, the amount of HP in the right lungs of mice from both parental strains was quantified using a standard technique (27). Higher levels of HP per lung from C₅B₁/6J mice than from C₃Hf/Kam mice were recorded, but the amount of lung tissue HP did not correlate with the severity of the fibrosing lesion measured by quantitative histology (data not shown). In a review of the pathology of pulmonary fibrosis, Burkhardt (28) also observed that in some studies in which tissue sections showed obvious fibrosis, the HP assay demonstrated normal amounts of collagen. The HP measurement, therefore, is not a suitable assay to represent the fibrosing phenotype in each F₂ mouse, as would be necessary to develop a genetic model.

The C₅B₁/6J fibrosis-prone phenotype and the C₃Hf/Kam fibrosis-resistant phenotype after bleomycin treatment reported here are consistent with what others have reported in these two strains in response to other cytotoxic insults, including radiation (29), ozone (30), and hyperoxia (31). In addition, the present study agrees well with the many investigations (6, 7, 10, 14) that have classified C₅B₁/6J as highly fibrosing after bleomycin treatment, although one study (5) has described C₃H/He mice as fibrosing in response to bleomycin.

Bleomycin administration produced differences in pulmonary function between the parental strains and between the F₁ strains. An elevated breathing rate was found to be an accurate determinant of functional difficulty, in good agreement with Jaeger et al. (32), because all animals sacrificed due to respiratory distress in the parental and inheritance studies had histological evidence of pulmonary inflammation and/or fibrosis. The use of breathing rate measures, a functional assay, in this study allowed the identification of mice with severe respiratory insufficiency such that fewer than 10% of C₅B₁/6J mice died compared to 25% in a study by Harrison et al. (8). Breathing rate measures did not predict fibrosis, however, because mice presented fibrotic areas of up to 6% of the lung at the end of the experiment, without any indications of functional difficulty. Changes in breathing rate after bleomycin were due to all lung damage, including both fibrosis, a focal lesion, and inflammation, a more diffuse lesion. The effect of a diffuse versus a focal lesion on breathing rate was demonstrated most clearly in F₁ females. B₃C₅F₁ females had higher breathing rates at sacrifice, and more mice had higher inflammation scores than B₃C₅F₁ females, but there was no difference in the level of fibrosis measured.

The area of fibrosis was measured in three generations of mice to identify the inheritance of this phenotype. A number of programs are available to analyze phenotypic data. None of these programs, however, are appropriate to evaluate the data set of this study. The lung fibrosis measured is an induced response for which there is no baseline level. The C₃Hf/Kam mice did not develop fibrosis, and this null response and associated zero variance, along with X-linkage, has not been addressed in statistical packages. The BCROSS program of SAGE (33) permits the evaluation of genetic models, but this program is not suitable for the present phenotypic data, because BCROSS analysis requires normality in the data set, nonzero variance measures, and autosomal inheritance, all of which are inconsistent with the present data.

Despite statistical limitations, a model of inheritance can be proposed from the data set presented in Fig. 5 and Table 2. First, the male mice of the two F₁ strains developed significantly different levels of fibrosis in response to bleomycin, which is suggestive of either an X-linked factor or genomic imprinting associated with the phenotype. An X-linked factor is indicated when offspring display different sensitivity to treatment and can be categorized by the X chromosome they receive. Alternatively, genome imprinting arises when the phenotype produced by a gene differs when that gene is paternally inherited from when that gene is maternally inherited (34). The genomic imprinting interpretation of the F₁ data was rejected as the two strains of F₁ females developed the same amount of bleomycin-induced pulmonary fibrosis. The contribution of an X-linked factor is estimated to be 19% of the total genetic variance using the equation presented in the results. Because the level of fibrosis is low in F₁ females, the X-linked factor is assumed to be recessive. No effects of X linkage were identified in the F₁ females, which might indicate that epistatic interactions are required for the expression of this factor.

Secondly, both F₁ strains in this study developed fibrosis, indicating that one of the proposed loci must be autosomal and associated with a dominant factor (see Table 2). The level of fibrosis in male mice of the B₃C₅F₁ strain is significantly lower than that of males of the C₅B₁/6J strain, although both strains have the proposed X-linked and autosomal dominant factors. This difference could be due to a second autosomal factor (recessive) contributing to the fibrotic phenotype in the C₅B₁/6J mice or due to incomplete dominance of the first autosomal factor in B₃C₅F₁ males.

The confirmation and identity of the proposed factors can only be

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<th>Females</th>
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<tr>
<td></td>
<td>n</td>
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<tr>
<td>B₃C₅F₁ × B₆C₃F₁</td>
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<td>1.8 (16)</td>
</tr>
<tr>
<td>B₃C₅F₁ × B₆C₃F₂</td>
<td>25</td>
<td>2.0 (21)</td>
</tr>
<tr>
<td>C₅B₁/6J × B₆C₃F₂</td>
<td>27</td>
<td>1.8 (12)</td>
</tr>
<tr>
<td>B₆C₃F₁ × B₃C₅F₂</td>
<td>27</td>
<td>1.4 (8.9)</td>
</tr>
<tr>
<td>Total F₂</td>
<td>116</td>
<td>1.7 (14)</td>
</tr>
</tbody>
</table>

* Female of cross listed first.

Avg. mean percentage of fibrosis; var, variance.
achieved with genomic mapping. With Wright's formula (Eq. B), which doesn't allow for sex-linked effects, the number of factors achieved with genomic mapping. With Wright's formula (Eq. B),

Possible genes associated with the loci controlling bleomycin-induced pulmonary fibrosis include those of the MHC (5, 7), those involved in DNA repair (16), and bleomycin hydrolase (15). Ekimoto et al. (5) have proposed the MHC role based on differences they observed in the level of pulmonary fibrosis in two congenic mouse strains differing only in h-2 haplotype. The response of the parental strains used in the present study is consistent with MHC influences as the strains displayed different levels of bleomycin-induced lung fibrosis and differ in h-2 haplotype; C57Bl/6J mice are h-2b, and C3H/Hf/Kam mice are h-2a.

Bleomycin is known to produce DNA strand breaks (4, 36); thus, sensitivity to bleomycin, measured as lung damage, could involve a deficient response in repairing or minimizing this DNA damage. Harrison et al. (16) reported a DNA repair deficiency in C57Bl/6J mice, a fibrosing strain, as compared to BALB/c mice, a nonfibrosing strain, after a dose of bleomycin that induced the same initial DNA damage in both strains.

A third possibility for the difference in susceptibility to bleomycin-induced pulmonary fibrosis is a difference in the level of bleomycin hydrolase, which inactivates bleomycin, between the parental strains. The lung tissue level of bleomycin hydrolase has been shown to be inversely correlated with the development of bleomycin-induced pulmonary fibrosis in three mouse strains, including the fibrosing C57Bl/6J and nonfibrosing BALB/c (15). Although the level of bleomycin hydrolase in C3H/Hf/Kam mice was not determined, this strain has been shown to be nonfibrosing in response to bleomycin in a manner similar to the BALB/c mice, which have a high level of bleomycin hydrolase in the lung (15).

Approximately 10% of mice were lost early in these studies because of acute toxicity. In a study administering bleomycin by different routes, Harrison et al. (8) reported acute toxicity associated with i.v. and s.c. injections but not with the osmotic minipump. Death in the acute toxicity phase, in this investigation, was attributed to severe dehydration and is not related to the development of fibrosis, as death occurred 1 week before even the earliest fibrotic responders, and the lungs of mice sacrificed while in this phase showed no fibrotic injury histologically.

In summary, this three-generation mouse model demonstrates that susceptibility to bleomycin-induced pulmonary fibrosis is a heritable trait that can be quantified by image analysis of histological sections. The model suggested from the data is two or three loci, one of which may be X linked, associated with susceptibility to the fibrosing phenotype.

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Inheritance of Susceptibility to Bleomycin-induced Pulmonary Fibrosis in the Mouse

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