Ozone-induced acute lung injury: genetic analysis of F₂ mice generated from A/J and C57BL/6J strains

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Departments of ¹Environmental Health, ²Molecular and Cellular Physiology, and ³Medicine, University of Cincinnati, Cincinnati, Ohio 45267-0056; and 4Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142

Prows, Daniel R., Mark J. Daly, Howard G. Shertzer, and George D. Leikauf. Ozone-induced acute lung injury: genetic analysis of F₂ mice generated from A/J and C57BL/6J strains. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L372–L380, 1999.—Acute lung injury (or acute respiratory distress syndrome) is a devastating and often lethal condition. This complex disease (trait) may be associated with numerous candidate genes. To discern the major gene(s) controlling mortality from acute lung injury, two inbred mouse strains displaying contrasting survival times to 10 parts/million ozone were identified. A/J (A) mice were sensitive (6.6 ± 1 (SE) h) and C57BL/6J (B) were resistant (20.6 ± 1 h). The designation for these phenotypes was 13 h, a point that clearly separated their survival time distributions. Our prior segregation studies suggested that survival time to ozone-induced acute lung injury was a quantitative trait, and genetic analysis identified three linked loci [acute lung injury-1, -2, and -3 (Ali1–3, respectively)]. In this report, acute lung injury in A or B mice was characterized histologically and by measuring lung wet-to-dry weight ratios at death. Ozone produced comparable effects in both strains. To further delineate genetic loci associated with reduced survival, a genomewide scan was performed with F₂ mice generated from the A and B strains. The results strengthen and extend our initial findings and firmly establish that Ali1 on mouse chromosome 11 has significant linkage to this phenotype. Ali3 was suggestive of linkage, supporting previous recombinant inbred analysis, whereas Ali2 showed no linkage. Together, our findings support the fact that several genes, including Ali1 and Ali3, control susceptibility to death after acute lung injury. Identification of these loci should allow a more focused effort to determine the key events leading to mortality after oxidant-induced acute lung injury.

Acute respiratory distress syndrome; oxidative stress; pulmonary edema; quantitative trait; quantitative trait locus

ACUTE LUNG INJURY can result from a variety of direct and indirect insults, yet the pathological end points of these injuries are strikingly similar. Therapeutic intervention has had limited success as evidenced by the fact that mortality from acute respiratory distress syndrome remains around 50% (20, 26, 28). Although the pathophysiological mechanisms of acute lung injury have been described in detail (5, 12), little is known about the genes or gene products linked to this disease and its progression.

More than 60 mediating factors have been associated with acute lung injury (23). These factors include inflammatory mediators (e.g., cytokines, proteolytic enzymes, and reactive oxygen and nitrogen species) that have the potential to damage parenchymal cells and the extracellular matrix. Many of these mediators have been identified through clinical studies designed to test candidate gene products either alone or in combination in pulmonary tissue or bronchoalveolar lavage (BAL) fluid of patients with acute respiratory distress syndrome.

The increasing number of possible mediators of acute lung injury poses a considerable challenge for both clinical and animal research studies to resolve the critical susceptibility factor(s). Clinical trials of affected patients have been confounded by variations in phenotype definition, cause of acute lung injury, and/or severity of underlying illnesses. These difficulties often lead to stratification of the study population into smaller groups with low statistical power or to misclassification of individuals, resulting in equivocal results and weakened interpretations. The use of laboratory animals can circumvent some of these clinical problems and may accelerate studies investigating acute lung injury. In fact, many animal models have been developed to study acute lung injury. But, like clinical studies, the use of animal models to test individual mediating factors has yet to lead to identification of the key events controlling acute lung injury. Because so many factors have been associated with this condition, a candidate-gene approach could require the testing of hundreds of transgenic or knockout mouse models. As an alternative approach, we have utilized a genomewide scan to identify chromosomal regions important in acute lung injury survival. This strategy is not contingent on a priori predictions of important genes or factors but rather identifies genetic loci linked to the phenotype.

Oxidative stress (e.g., reactive oxygen and nitrogen species) contributes to the development of acute lung injury (7–9). At concentrations of 4–10 parts/million (ppm), ozone, a well-characterized and powerful oxidant, causes acute lung injury, hemorrhagic pulmonary edema, and death in laboratory animals (25, 27). Goldstein et al. (6) demonstrated an approximately fourfold difference in survival time among 20 inbred strains of mice exposed to ozone. First-generation offspring (F₁) from a sensitive-strain male mated with a resistant-strain female were resistant, suggesting that resistance to ozone was inherited as a dominant trait (or similarly, susceptibility was recessive).
Recently, Prows et al. (24) investigated the genetic factors controlling survival to ozone-induced acute lung injury using recombinant inbred (RI) and backcross mice generated from sensitive A/J (A) and resistant C57BL/6J (B) inbred strains. Employing RI and quantitative trait locus (QTL) analyses, genetic regions (QTLs) significantly linked to survival time were found on mouse chromosomes 11 (acute lung injury-1 (Ali1)) and 17 (Ali3) and a third region suggestive of linkage was identified on chromosome 13 (Ali2). Separate analysis of the two backcross populations revealed significant differences in their mean survival times (MSTs) and phenotype ratios (i.e., the number of resistant to sensitive mice), suggesting that a modifier gene may be imprinted.

The studies reported herein were designed to determine the possible pathological differences between the A and B strains of mice at death after ozone-induced acute lung injury and to further examine the genetic factors responsible for their contrasting survival times. Also, analyses were performed to determine possible interactions between the suggested loci and to establish additional support or refute previous suggestions for an imprinted modifier locus.

**METHODS**

Mice. Male and female A and B control mice and F2 mice (generated from the A and B strains) were bred for this study by The Jackson Laboratory (Bar Harbor, ME). The mice were acclimated for ~1 wk before ozone exposure and maintained in a viral- and pathogen-free, high-efficiency particulate air-filtered environment with a 12:12-h on-off light cycle. Because of our previous evidence (24) suggesting imprinting of a modifier gene (i.e., the phenotype varied depending on which parent the alleles originated), 60 mice (30 males and 30 females) for each of the 4 possible F2 mating schemes were generated (n = 240). By convention, the female strain is listed first in crosses with mice. The four mating groups were group 1, (B × A)F1 (BAF1) female × BAF1 male; group 2, BAF1 female × (A × B)F1 (ABF1) male; group 3, ABF1 female × BAF1 male; and group 4, ABF1 female × ABF1 male.

Ozone generation, analysis, and exposure. Ozone was generated from 100% extra-dry oxygen (Matheson, Columbus, OH) with a model V1-0 ultraviolet ozonator (OREC, Phoenix, AZ) and analyzed continually with a Dasibi (Glendale, CA) model 1008-PC direct-reading instrument. This instrument has an internal calibration system and was routinely checked and calibrated against a US Environmental Protection Agency transfer standard. Mice were placed in a 0.32-m3 stainless steel inhalation chamber (capable of complete air exchange every 2 min) and exposed continuously to filtered room air containing 10 ppm ozone. The status of the mice and concentration of ozone were checked hourly throughout the exposure periods. The University of Cincinnati (Cincinnati, OH) Institutional Animal Care and Use Committee approved the exposure protocols, and the published guidelines for laboratory animal use were followed.

Exposures were performed on mice ~1 wk after receipt from The Jackson Laboratory. Group 1 mice required only a single additional mating cycle for generation because the BAF1 mice needed for these matings are commonly stocked at The Jackson Laboratory. On the other hand, groups 2–4 all required an initial generation of at least one of the F1 groups (i.e., ABF1) for the second mating cycle to produce the respective F2 generations. A total of 7 exposures were performed between mid-August to early November, with groups ranging from 35 to 44 mice in the first 6 exposures and 22 mice in the final exposure. All exposures contained at least one control A and B mouse (either sex) and equal numbers of males and females of each group. Five of the exposures contained mice from only one mating group; the other two exposures contained mice from two mating groups. These groupings were necessary to expose all mice at similar ages and depended on when and how they were received from the vendor. Mean (± SE) ozone concentrations for all exposures were significantly <1%.

Lung histology. The lungs from A and B mice (n = 6–8/time point), either control (anesthetized with 50 mg/kg of pentobarbital sodium) or exposed to 10 ppm ozone until death, were prepared for histological examination. After anesthesia (control mice) or death (exposed mice), the posterior descending aorta was severed and the trachea was exposed. A silt in the larynx was made, and the trachea was cannulated with 0.58-mm-ID polyethylene tubing (Cly Adams, Parsippany, NJ). Before cannulation, a 23-gauge needle attached to a 3-ml syringe was connected to the tubing. Before the chest wall was opened, the lung was infused with 1 ml of 10% phosphate-buffered Formalin (Fisher Scientific, Pittsburgh, PA) at a fluid height of 25–30 cm for 1 min. After infusion, the trachea was ligated, and the lung was removed from the thoracic cavity and immersed in cold fixative for at least 24 h. To determine lung injury induced by ozone, a 0.25-inch cross section of the left midlobe and distal tip of the left lobe was paraffin embedded, sectioned (5 μm), stained with hematoxylin and eosin, and examined by light microscopy. Histopathological changes, including peribronchiale and perivascular edema, alveolar capillary congestion and hemorrhage, inflammatory cell infiltration, thickening of the alveolar epithelium and septa, and epithelium separation from its basement membrane, were assessed in lung specimens from both strains.

Lung wet-to-dry weight ratios. To assess the extent of pulmonary edema, lung wet-to-dry weight ratios were determined. After anesthesia (control mice) or death (exposed mice), the posterior descending aorta was severed and the trachea was exposed and ligated just distal to the larynx. The heart and lung were removed intact with the trachea severed at the larynx (i.e., just above the suture) to prevent possible loss of edema fluid. The heart, esophagus, and excess fat and tissue were removed, and the lung and trachea wet weights were obtained. The tissues were placed in a desiccator containing silica gel (Sargent-Welch) for 96 h to determine the dry weights. In preliminary tests, samples reweighed at 192 h had weights equal to those obtained at 96 h.

Phenotyping. The mice were exposed continuously to 10 ppm ozone, and the survival time was recorded to the nearest next hour for each animal. For quantitative trait analyses, the individual survival times were used. For studies examining imprinting, in addition to using the quantitative measure of MST, individual F2 mice were also phenotyped qualitatively as sensitive or resistant. “Resistant” in this context is a relative term based on survival time compared with more “sensitive” strains. The criteria for designation as the sensitive phenotype were previously established and defined as those mice surviving ≤13 h and resistant mice were those surviving >13 h (24). This time point clearly separated the phenotypes of the parental strains.

DNA preparation. After death, the liver was removed from each mouse, flash-frozen in liquid nitrogen, and stored at −40 °C for subsequent genotype studies. Genomic DNA was isolated with a DNA extraction kit (Wizard DNA, Promega).
Samples were analyzed for purity (260- to 280-nm absorbance ratio), and DNA concentrations (absorbance at 260 nm) were quantitated with a Beckman DU-64 spectrophotometer.

Genotype analysis. PCRs were performed to genotype F₂ progeny for microsatellite markers located throughout the mouse genome. Primer pairs were chosen based on known polymorphisms between the A and B strains and were purchased from Research Genetics. PCR was performed in 15-μl reaction volumes in 96-well plates (Polyfiltronics) with a 4-block thermocycler (Mead Johnson model PTC-225). The final concentration for each reaction was 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM each deoxynucleotide 5′-triphosphate (Promega), 1× RediLoad (Research Genetics), and 0.132 μM each microsatellite primer. This reaction mixture was added to 100 ng of genomic DNA and 0.6125 U of Taq DNA polymerase (GIBCO BRL). The final mixtures were initially denatured at 94°C for 3 min followed by 36 cycles at 94°C for 30 s, 55°C for 45 s, and 72°C for 30 s. A final elongation step at 72°C for 7 min was followed by refrigeration at 4°C until gel analysis. The PCR products were differentiated on a MetaPhor agarose gel (FMC BioProducts) and visualized by ethidium bromide staining. The agarose concentration used (2.5–4%) was dependent on the size of the allele variants and had a resolution in size of ~5% difference between A and B alleles.

QTL mapping and analyses for linkage. Initially, all 240 F₂ mice were genotyped for 61 microsatellite markers distributed at 20- to 30-centimorgan (cM) intervals across the genome. All phenotype (i.e., survival times) and genotype data were then analyzed for linkage with the MAPMAKER/EXP V3.0b (15, 22) and MAPMAKER/QTL V1.1b (17, 18, 21) computer packages. Log of the odds ratios (LOD scores) of ≥2.8 and ≥4.3 were used as threshold levels for statistically suggestive and significant linkage, respectively, in a genome-wide search of F₂ mice (16). The data closely fit a normal distribution and were used directly in QTL analysis. Regions on chromosomes 4–6, 11, and 17 had LOD scores ≥1.6 and were subsequently typed for an additional 27 markers. MSTS and phenotype ratios (resistant to sensitive mice) for the separate F₂ mating groups suggested that imprinting may be involved in the overall susceptibility to ozone-induced lethal-lity. Therefore, in this experimental design, PCRs were performed from all four F₂ mating schemes with the cumulative search function of MAPMAKER/QTL) with QTL Cartographer (1, 2). Interval mapping results from MAPMAKER/QTL were performed with QTL Cartographer to establish an agreement between the two programs before conditional linkage analysis.

Statistical analysis. Lung wet weights, dry weights, and wet-to-dry weight ratios were analyzed for differences with a two-tailed paired Student’s t-test. MSTS of males, females, and mating groups were compared for differences with a one-way analysis of variance, with Student-Newman-Keuls test for multiple comparisons. In addition to MST comparisons, imprinting was examined by comparing the resistant-to-sensitive phenotype ratios among the mating groups with a Yates-corrected χ²-test for a 2 × 2 contingency table. Values are reported as means ± SE, and significance was accepted for values of P ≤ 0.05.

RESULTS

Lung injury. To assess lung injury immediately after death in A and B mice exposed to ozone, histology and lung wet-to-dry weight ratios were examined. The histopathology associated with this injury did not vary between A and B mice. Lungs removed immediately after death were dark red and often remained inflated after puncture of the diaphragm. Microscopic examination of the lungs from these mice revealed enlargement of the perivascular space, capillary bed congestion, and focal regions of hemorrhage (Fig. 1). Sporadic areas of airway epithelial damage with basement membrane separation, alveolar septal thickening, and alveolar proteins were also noted.

Accompanying these histological changes were increases in lung wet-to-dry weight ratios that were comparably elevated in the A and B mice immediately after death (Fig. 2). Exposed A mice had more than a 3-fold increase in wet weight (389 ± 17 mg) and a 1.5-fold increase in dry weight (48 ± 2 mg) compared with those in control A mice (120 ± 4 and 31 ± 1 mg, respectively). Similarly, exposed B mice presented with lung wet (352 ± 23 mg) and dry (47 ± 4 mg) weights significantly greater than those in control mice (121 ± 6 and 30 ± 6 mg, respectively).

Phenotype analysis. The MST of the total F₂ population was 19 ± 1 h and not significantly different from that of control resistant B mice (21 ± 1 h). In fact, all mating groups of the F₂ mice and, therefore, all combinations of mating groups had a resistant-like phenotype (i.e., MST > 13 h). The MST for group 1 mice (21 ± 1 h) was significantly greater than that of group 4 mice (18 ± 1 h). Although the MST for total males (18 ± 1 h) did not differ from that of total females (20 ± 1 h), the MSTs of group 3 males (17 ± 1 h) and females (22 ± 1 h) did differ significantly from each other. Similarly, the MST of male mice differed from that of female mice when generated from either ABF₁ females (i.e., groups 3 and 4; males 17 ± 1 h; females 20 ± 1 h) or crosses with a BAF₁ male (i.e., groups 1 and 3; males 18 ± 1 h; females 22 ± 1 h).

The survival times of individual mice are shown in Fig. 3. In all F₂ mating groups, the range of survival times extended above and below that of the F₁ range. Although the survival times of the F₂ mice expanded the resistant range seen in F₁ mice, it did not span the entire sensitive range. Thus the overall range of the A and B progenitor strains (2–24 h) was shifted toward more resistance in the F₂ population (8–32 h).

Comparing the percentage of sensitive mice (≤13 h) among the four different F₂ mating groups revealed a significant difference (χ²-test) between groups 1 (13%) and 4 (25%). This difference in phenotype designation concurs with the difference in MSTs of these mating groups. The percentage of sensitive mice in the group 2 and 3 subpopulations were identical to each other (13%) and intermediate to that of the group 1 and 4 mice. Comparing groups 1 and 4 also showed a significant difference in percentage of mice surviving > 25 h (i.e., longer than all B parents tested). Eighteen percent...
(11 of 60) of group 1 mice survived >25 h, whereas only 3% (2 of 60) of group 4 mice survived this long. Again, mice in groups 2 and 3 were intermediate (see Fig. 3).

QTL analysis. After microsatellite marker genotyping and genetic linkage mapping, MAPMAKER/QTL analysis of the 240 F2 mice identified an interval on chromosome 11 significantly linked to ozone-induced acute lung injury (Fig. 4). This region had a peak LOD score of 6.8 at 4 cM distal to D11Mit179. This peak coincided with that previously suggested in the analysis of backcross mice (A3 BAF1 and BAF1 A), and the interval overlapped much of the Ali1 QTL (24). QTL analysis with QTL Cartographer confirmed the MAPMAKER/QTL results.

Because phenotype ratios of groups 1 and 4 differed, all subpopulations of F2 mice were also analyzed separately and in various combinations in an attempt to distinguish modifying QTL(s) that may be imprinted or sex linked (Table 1). Ali1 was the only QTL reaching significant (LOD score ≥ 4.3) or suggestive (LOD score ≥ 2.8) linkage in the total F2 population (16), but analysis of separate F2 subpopulations revealed suggestive linkages to other putative QTLs modifying the overall phenotype (Table 1). No significant or suggestive linkages were found in analysis of the F2 males, although a peak LOD score of 2.3 in the genomewide search occurred at Ali1. In contrast, F2 females showed significant linkage to Ali1 (LOD score 6.1) and suggestive linkage to a QTL on distal chromosome 5 (D5Mit240 + 10 cM). This QTL yielded an LOD score of 3.1 and had a negative weight in the MAPMAKER/QTL analysis, suggesting that it was opposing Ali1 (e.g., a modifying susceptibility locus in the B strain). The highest X-chromosome LOD score in F2 females was 1.3, arguing against a sex-linked QTL. Separate mating group analyses also identified a QTL on chromosome 6 (D6Mit183 + 10 cM) in group 3 mice and the F2 offspring generated from matings with an ABF1 mother (i.e., groups 3 and 4 combined; Table 1).

A QTL on chromosome 17 (peak at D17Mit139 + 6 cM) was suggestive of linkage in group 1 mice and reached significant linkage in offspring produced with a
BAF1 female (i.e., groups 1 and 2 combined; Table 1). None of the analyses suggested linkage to Ali2 on chromosome 13, a putative QTL reported in the analysis of backcross mice generated from the A and B strains or to previously suggested QTLs on chromosomes 3, 7, and 12 (24).

Analysis for QTL interactions. Results from the cumulative search (MAPMAKER/QTL) are given in Table 2. After fixing Ali1 in the analysis of all F2 mice, D17Mit139 + 2 cM and D5Mit233 + 4 cM were sequentially identified as possible modifying QTLs, and together, the three loci explained 21% of the genetic variance of the F2 mice. After confirmation of MAPMAKER/QTL with the QTL Cartographer program, the total F2 data set was analyzed for conditional linkage. Only Ali1 was identified as significant in conditional linkage analysis, so further analysis for significant epistatic interactions was not warranted for this data set.

The cumulative search strategy was also used in the analysis of the 45 F2 mice surviving the shortest or longest times in ozone. Analysis of these individuals at the phenotypic extremes, i.e., the most sensitive and most resistant mice, should eliminate intermediate phenotypes that may be hiding weaker QTLs. Results of this analysis suggested similar QTLs on chromosomes 5 and 11 to those of the total analysis. In addition, this analysis suggested linkage with D6Mit8, a marker near the locus suggested in group 3 mice and the F2 offspring generated from matings with an ABF1 mother (i.e., groups 3 and 4 combined). In total, the QTLs on chromosomes 5, 6, and 11 reached a cumulative LOD score of 9.5 and explained 33% of the genetic variance in the 45 phenotypic extreme F2 mice (Table 2).

DISCUSSION

Mortality from acute lung injury remains high, and the lack of recent progress to increase survival compels alternative research and therapeutic methods. As a different strategy to the standard candidate-gene approach, we have systematically scanned the mouse genome to identify genes involved in acute lung injury susceptibility. To derive an animal model to determine the genes involved, several strains of inbred mice were exposed to 10 ppm ozone continuously to induce acute pulmonary edema and death. The survival time phenotype differed significantly between sensitive A and resistant B mice, allowing genetic analysis to investigate the factors governing the differential response (24). From results obtained by RI and QTL analyses of backcross mice, we proposed that 3 QTLs (i.e., Ali1, Ali2, and Ali3) were important in controlling survival time to ozone-induced acute lung injury.
The present study was initiated to investigate similarities or differences in ozone-induced acute lung injury and pulmonary edema in sensitive A and resistant B strains of mice and to further delineate the genetic factors in a separate population of mice. Two common methods were used to verify acute lung injury in the two strains after death from ozone exposure. First, light microscopy revealed similar lung histopathology in the A and B mice, showing marked enlargement of the perivascular space, alveolar congestion, and focal regions of hemorrhage. Other lung indexes, including thickening of the alveolar epithelium and septa and epithelium separation from its basement membrane, were similarly noted in both strains and varied among mice within the strains. Second, wet weights, dry weights, and wet-to-dry weight ratios of lungs from A and B mice after ozone-induced mortality demonstrated an acute pulmonary edema, consistent with that previously described in the literature (6, 25, 27). No differences were found in the wet-to-dry weight ratios of lungs from A and B mice under control conditions and immediately after death from ozone exposure, although the time to reach end-stage pulmonary edema in the two strains differed three- to four-fold.

Differences in survival time between the A and B strains may be due to a decreased ability of the sensitive A strain to protect against oxidant injury, and once this protective mechanism is overwhelmed in the resistant B strain, a similar progression to pulmonary edema ensues. This would suggest that the rate of pulmonary edema development is similar in the two strains, but the lag time differs. On the other hand, it is also possible that the rate of pulmonary edema differs between the two strains, with a faster progression in the sensitive strain. Similar wet-to-dry weight ratios at an intermediate exposure time point for each strain suggest that this is not the case (data not shown).

Regardless, it is apparent that once the lung wet weight approaches three times that of its control wet weight (or wet-to-dry weight ratios reach twofold those of control values), survival of both strains is jeopardized. Therefore, although the time to end-stage pulmonary edema differs significantly, the extent of edema fluid...
accumulation and its progression to subsequent death appears to be similar in the two strains.

To further delineate the results of our backcross analysis (24), an F2 population derived from A and B mice was generated and tested. Because previous findings suggested that one of the modifying QTLs might be imprinted (24), equal numbers of mice from all four possible F2 breeding schemes were generated. The MSTs and phenotype ratios of individual and combinations of mating groups seem to reduce to a common theme; groups 1 and 4 significantly differ from each other, but groups 2 and 3 are similar. The significant differences in MSTs and phenotype ratios between F2 mice from groups 1 and 4 support the previous findings that a QTL involved in ozone-induced acute lung injury and mortality may be imprinted. Although the survival time differences are small, the putative imprinted locus has consistently resulted in 2- to 3-h differences in MSTs among related groups. For example, group 1 mice survived an average of 3 h longer than group 4 mice. Similarly, the BAF1 × A backcross mice survived an average of 2–3 h longer than the A × BAF1 backcross mice, and the BAF1 × B backcross mice survived ~2 h longer than B × BAF1 backcross mice (24). In addition, BAF1 mice survived ~2 h longer than ABF1 mice. A complicating factor in determining a possible mode of inheritance for the imprinted gene is that females survived ~2 h longer than males, yet QTL analysis found no evidence of a sex-linked modifier gene in either the F2 or backcross analyses. At this time, no mode of inheritance or pattern of imprinting can be offered to consistently explain these data. Nonetheless, imprinting of a QTL that modifies the survival time in ozone an average of 2–3 h is supported by these studies.

QTL analysis of the total F2 population with MAPMAKER/QTL and QTL Cartographer confirmed that Ali1 is contained in a region significantly linked to acute lung injury survival time. Ali1 had a peak LOD score of 4.3 in the BAF1 × A backcross population at 2 cM distal to D11Mit179 and explained 42% of the genetic variance (24). In the F2 population, a QTL peak at nearly the identical chromosomal position yielded an LOD score of 6.8 and explained 13% of the genetic variance between the A and B mice. Together, these findings support the existence of a major gene controlling resistance to ozone-induced acute lung injury on chromosome 11, within a QTL interval spanning ~20–25 cM from D11Mit245 to D11Mit10.

The difference in the ability of Ali1 to explain the genetic variance in the backcross and intercross data (i.e., 42% in the backcross but 13% in the F2) can be attributed to several reasons. 1) The F2 generation has a wider selection of genotypes at relevant QTLs (e.g., with N loci involved, there are 2^N different combinations of genotypes in the backcrosses but 3^N genotypes in the F2 intercrosses). 2) Each specific locus will show a wider range of effect in the F2 generation because there are three genotypic classes segregating compared with only two in the backcrosses. 3) The F2 may include additional QTLs of a more recessive nature, which could not have been observed in the backcrosses. Thus the variance explained by a QTL in a backcross and F2 analysis may differ substantially from each other, even though they are of the same importance with respect to the trait.

A QTL suggested by F2 subpopulation analyses in the present study was found on chromosome 17 (peak at 2–6 cM distal to D17Mit139). Because multiple combinations of data were analyzed, the threshold for significant linkage should be adjusted upward to account for the multiple testing. Therefore, the LOD score of 4.3 may not be significant, although it is certainly highly suggestive of linkage. The analysis of the total F2 population yielded an LOD score of 2.0, making it difficult to assess the importance of the chromosome 17 QTL. Additional support for this QTL came from RI data. Analysis of 27 A × B and B × A RI strains with the Map Manager computer program (19) suggested significant linkage to D17Mit2 (LOD score 3.5), previously proposed as Ali3 (24). The Ali3 peak is ~15 cM distal to D17Mit139. Also of note, the Inf2 locus, which has been linked to ozone-induced inflammation (31), is located ~10 cM proximal (centromeric) to D17Mit139. Therefore, the chromosome 17 QTL suggested in this study is located between Ali3 (D17Mit2) and Inf2 (peak at D17Mit10), with an overall distance of ~25 cM separating these two loci. Whether the Ali3 and Inf2 QTLs represent the same or distinct loci will require additional study.

Table 2. MAPMAKER/QTL analysis of ozone-induced acute lung injury in total and extreme responding F2 populations

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<tr>
<th>QTL Analysis</th>
<th>QTL Analysis With Cumulative Search</th>
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<tr>
<td>F2 Population</td>
<td>LOD score</td>
</tr>
<tr>
<td>D11Mit179 + 4 cM (Ali1)</td>
<td>6.8</td>
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<tr>
<td>D5Mit240 + 10 cM</td>
<td>2.3</td>
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<tr>
<td>D17Mit139 + 6 cM</td>
<td>2.0</td>
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<tr>
<td>D6Mit10 + 5 cM</td>
<td>3.5</td>
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<tr>
<td>D11Mit263 (Ali1)</td>
<td>3.4</td>
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<tr>
<td>D5Mit240 + 10 cM</td>
<td>9.5 (+2.6)</td>
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\( \Delta \text{LOD} \), change in LOD score (nos. in parentheses); MST, mean survival time; cM, centimorgan. MAPMAKER/QTL was used to scan genome of F2 mice for putative QTLs linked to ozone susceptibility phenotype. For cumulative search analyses, loci in brackets were fixed to remove variance explained by prominent QTL(s), and genome was rescanned to determine QTLs that could explain more of the overall genetic variance. Ali1 is the prominent QTL in F2 mice, and suggested modifier QTLs include loci on chromosomes 5, 6, and 17.
Two other QTLs located on chromosomes 5 (peak at D5Mit240 + 10 cM) and 6 (peak at D6Mit8 + 5 cM) were also suggestive of linkage in analyses of F2 subpopulations. The chromosome 5 locus indicated a QTL opposing the resistance trait (e.g., a susceptibility-modifying locus in the resistant B strain). The prospect that B mice may have a sensitivity locus for ozone-induced acute lung injury suggests that they do not carry the best combination of alleles for resistance. A better admixture of alleles among the A and B mice could explain why several F2 mice had an increased survival time (i.e., a shift toward more resistance) compared with their B progenitor strain. Given this scenario, however, one would also expect a small number of mice with a worse allelic combination, making them more prone to death, but these were not identified.

The chromosome 6 locus is of interest because it is near the gene for transforming growth factor-α (TGF-α), a growth factor involved in lung remodeling, wound healing, and fluid transport after lung injury (4, 23). TGF-α has been found in the pulmonary edema fluid of patients with acute lung injury (3). As a preliminary test of this candidate gene, Korfhagen et al. (14) have recently exposed transgenic mice overexpressing TGF-α and their littermate controls to an inhalation insult inducing an oxidative stress in the lung. Survival times of selected transgenic lines suggested that TGF-α expression has a protective role in the lung. Accordingly, an in vivo protective function of TGF-α has been previously shown in the lung (29) and intestine (10, 13) after acute injury and stress.

Although the QTLs on chromosomes 5 and 6 may be important in the overall phenotype, neither of their LOD scores approaches the significance threshold in an F2 analysis (i.e., an LOD score ≥ 4.3). Although they might indicate the presence of weak QTLs, given the number of different subclasses of the data examined, these LOD scores also could have arisen by chance. Based on the recent recommendation (16), these QTLs will not be named at this time.

Several candidate genes for Ali1 have been previously suggested (24) and include macrophage inducible nitric oxide synthase (Nos2), myeloperoxidase (Mpo), and the small soluble cytokines (Scyas) such as monocyte chemoattractant protein-1 (Scya2) and macrophage inflammatory protein-1α (Scya3). Numerous other genes also map within or near the Ali1 QTL interval and could play a role in acute lung injury susceptibility, including corticotropin-releasing hormone receptor (Crhr), granulocyte colony-stimulating factor (Csf3), and nerve growth factor receptor (Ngrf) (see Fig. 4).

Candidate genes for the other putative QTLs can also be suggested. Ali2 was not supported in the F2 analysis, so its importance to acute lung injury awaits further analysis. Candidates for Ali3 include Inf2 (11), xanthine oxidase/dehydrogenase (Xdh), tumor necrosis factor-α (Tnfa), and 70-kDa heat shock protein (Hsp70). As mentioned above, Tgfa is a candidate for the QTL on chromosome 6 as is surfactant protein B (Sftp3). No obvious candidates can be suggested for the putative QTL on chromosome 5.

In summary, F2 analysis has identified and further delineated Ali1 as a region on chromosome 11 containing a major gene controlling resistance to ozone-induced acute lung injury. Additional evidence for an imprinted modifier gene was suggested. Furthermore, Ali3 and single QTLs on chromosomes 5 and 6 may play modifying roles in the overall phenotype, whereas Ali2 was not supported in this analysis. No evidence was found for significant epistatic interactions between putative loci, although possible important interactions between Ali1 and one or more of the minor QTLs were not examined in this report. Candidate genes for these QTLs were suggested, allowing a more focused candidate-gene approach to determine the genes involved in oxidant-induced acute lung injury development.

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