Susceptibility to neoplastic and non-neoplastic pulmonary diseases in mice: genetic similarities

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Bauer, Alison K., Alvin M. Malkinson, and Steven R. Kleeberger. Susceptibility to neoplastic and non-neoplastic pulmonary diseases in mice: genetic similarities. Am J Physiol Lung Cell Mol Physiol 287: L685–L703, 2004; 10.1152/ajplung.00223.2003.—Chronic inflammation predisposes toward many types of cancer. Chronic bronchitis and asthma, for example, heighten the risk of lung cancer. Exactly which inflammatory mediators (e.g., oxidant species and growth factors) and lung wound repair processes (e.g., proangiogenic factors) enhance pulmonary neoplastic development is not clear. One approach to uncover the most relevant biochemical and physiological pathways is to identify genes underlying susceptibilities to inflammation and to cancer development at the same anatomic site. Mice develop lung adenocarcinomas similar in histology, molecular characteristics, and histogenesis to this most common human lung cancer subtype. Over two dozen loci, called Pas or pulmonary adenoma susceptibility, Par or pulmonary adenoma resistance, and Sluc or susceptibility to lung cancer genes, regulate differential lung tumor susceptibility among inbred mouse strains as assigned by QTL (quantitative trait locus) mapping. Chromosomal sites that determine responsiveness to proinflammatory pneumotoxicants such as ozone (O3), particulates, and hyperoxia have also been mapped in mice. For example, susceptibility QTLs have been identified on chromosomes 17 and 11 for O3-induced inflammation (Inf1, Inf2), O3-induced acute lung injury (Aliq3, Aliq1), and sulfate-associated particulates. Sites within the human and mouse genomes for asthma and COPD phenotypes have also been delineated. It is of great interest that several susceptibility loci for mouse lung neoplasia also contain susceptibility genes for toxicant-induced lung injury and inflammation and are homologous to several human asthma loci. These QTLs are described herein, candidate genes are suggested within these sites, and experimental evidence that inflammation enhances lung tumor development is provided.

adenocarcinoma; inflammation; loci; quantitative trait locus; pneumotoxicant

IT IS IMPORTANT TO IDENTIFY intrinsic and extrinsic factors that influence pulmonary responses to airborne pollutants and carcinogens. Extrinsic factors include previous disease history, nutritional factors, and exposure to antigens and pollutants. Intrinsic or “host” factors include age, gender, and genetic background. Genetic susceptibility to environmental agents in humans has been well documented through population studies, familial inheritance patterns, twin studies, and clinical studies. The inbred mouse is an important source for genetic modeling of human diseases because the genetic homogeneity that exists within inbred strains does not occur in human populations. Therefore, the contribution of genetic background to a biological process is more easily determined and partitioned from environmental variance than in humans. Linkage among homologous loci in humans and mice indicates maintenance of significant homology in gene order and chromosomal structure in spite of evolutionary divergence (166). For example, identification of cytokine genes in mice provides the basis for localizing homologous genes in humans (106, 115). The recent mapping of the mouse genome enhances the importance of this animal model for human diseases (19). Complex human disease processes modeled in mice using linkage analyses include Huntington’s disease (168), Duchenne muscular dystrophy (23), insulin-dependent diabetes mellitus (163), Alzheimer’s disease (132), von Willebrand disease (72), chronic granulomatous disease (105), and Niemann-Pick C1 disease (26) among others. The significantly conserved synteny between the two species, the low cost of mouse maintenance compared with other animals, and availability/amenability of transgenic approaches make the inbred mouse the most appropriate model for understanding genetic determinants of susceptibility to neoplastic and non-neoplastic pulmonary diseases.

A complex trait is a phenotype that varies quantitatively when measured among different individuals or strains due to the interactions of genetic (between-strain) and environmental (within-strain) factors as well as by chance (214). To understand the genetic basis of a given phenotype in inbred mice, reproducible interstrain differences in the phenotype of interest
are established to construct a strain distribution pattern (SDP). The SDP may provide important insights about the inheritance of the phenotype, i.e., single (Mendelian) vs. polygenic inheritance.

Segregation and linkage analyses are then performed to localize the genes responsible for differential phenotypic expression to a chromosomal segment. When identified by linkage or linkage disequilibrium analyses, this region is referred to as a quantitative trait locus (QTL). Linkage analyses use segregant backcross and intercross (F2) populations (cohorts) derived from progenitor strains with dissimilar disease endpoint phenotypes. Each mouse from the segregating cohort is genotyped for polymorphic simple sequence length polymorphisms (SSLPs, microsatellites) or single nucleotide polymorphisms (SNPs). The number of markers and their distribution are chosen to provide adequate coverage of the genome within 95% confidence limits. Linkage is inferred when coinheritance of a genetic marker (e.g., an SSLP or SNP) with a gene influencing a specific phenotype occurs more often than expected by independent assortment. This has been used to understand molecular mechanisms of asthma and other genetic disorders (see Refs. 5, 171, and 208). Efficient PCR-based genotyping and computer software (e.g., MAPMAKER-QTL. www.hgmp.mrc.ac.uk/Registered/Option/mapmaker.html and Map Manager QTX, mapmgr.roswellpark.org/mmQTX.html) markedly increase the efficiency and accuracy of these linkage studies and enhance the ability to map quantitative traits.

Additional populations that have been highly successful for linkage analyses include recombinant inbred (223) and recombinant congenic strains (77). With the use of these models to study the inheritance of a disease phenotype, a broad chromosomal region (5-40 cM) may be identified based on recombination events that occur in a specific cross. Linkage disequilibrium relies on recombinations that occurred over generations, starting from the origin of the mutated allele in an ancestral mouse that is fixed to homozygosity during inbreeding, and uses a dense number of markers to narrow that region (150, 240). Haplotype analysis of a particular set of alleles at linked loci found by traditional positional cloning can delineate subregions at which parental strains differ (239). This is useful when the same QTL is identified in several strain combinations. Other methods can narrow the size of this region to facilitate positional cloning or candidate gene analysis. For example, advanced intercross lines allow refined mapping (up to 10-fold reduction) in the size of a QTL; this semirandom (i.e., avoiding brother-sister matings) intercrossing of mice beyond the F2 generation greatly increases the amount of recombination events between any two loci (45).

Recently, an in silico approach using inbred strains whose genome sequences are available through the Celera and public databases has been used to align QTL sequences, identify SNPs between the strains, and suggest putative candidate genes based on physiological function (78, 154). Although this approach requires independent verification by other laboratories, the potential savings in time and resources used to identify QTLs may be considerable.

Chromosomal regions with significant linkage have theoretical minimum logarithm of the likelihood ratio (LOD) scores between 3.4 and 4.3 ($P < 10^{-4}$ to $10^{-5}$), although these LOD scores can be much higher and can differ depending on the cross being tested (i.e., backcross vs. F2 vs. RI). Regions with suggestive linkage have an LOD score between 2.0 and 3.0 ($P < 10^{-3}$ to $10^{-4}$) (126). Although suggestive linkages are worth noting, they sometimes result from false-positive associations (126). After significant linkage has been localized within two chromosomal markers separated by ~20 cM, this chromosomal segment containing the gene of interest is narrowed to <1 cM to pursue physical mapping. A long-range physical map is generated using yeast artificial chromosome libraries and/or genomic restriction enzyme mapping with pulsed-field gel electrophoresis techniques; these are prerequisites to its cloning (214). After minimal genetic and physical intervals have been defined, the corresponding genomic sequences that likely determine the phenotype of interest are identified. The functional role of the candidate genes may be evaluated by using knockout or transgenic mice followed by a microarray approach (131).

Penetrance refers to the degree that animals with a mutant genotype express a mutant phenotype; if 100%, the phenotype is completely penetrant. Low penetrant genes may be modifiers of other genes with larger effects on the phenotype. Epistasis, the interaction that occurs when combined effects of two loci do not equal the sum of individual effects, can be determined using programs such as multiple QTL models (MQM mapping) (60). When an interaction is significant, the effect of one locus depends on the genotype of the other locus.

The remainder of this review is divided into three sections: susceptibility genes responsible for lung inflammation/injury as determined with several mouse models, susceptibility genes responsible for lung cancer in mice, and a comparison of these sets of susceptibility genes.

**Susceptibility Genes for Lung Injury and Inflammation**

**O3-Induced Lung Injury**

O3-induced lung injury is characterized by airway inflammation [increases in polymorphonuclear leukocytes (PMN), alveolar macrophages (AMs), and lymphocytes], hyperpermeability, and epithelial cell damage followed by epithelial proliferation in the terminal bronchioles and alveolar septae. O3 is highly reactive and is largely eliminated by contact with epithelial lining fluid or plasma membranes of resident airway cells (macrophages, epithelial cells) (165). O3 does not penetrate cells yet causes many pulmonary and nonpulmonary events. Therefore, a cascade mechanism has been proposed to account for its toxicity (189). This hypothesis suggests that O3 reacts with unsaturated fatty acids at the air-tissue barrier to form lipid ozonation products (LOPs) including aldehydes, hydroxyhydroperoxides, and Criegee ozonides (see Ref. 189). LOPs activate lipases leading to the production of signal transduction molecules and proinflammatory mediators, such as platelet-activating factor. Together with reactive oxygen species (ROS) generated by O3 exposure, these byproducts of O3 can upregulate transcription factors such as NF-κB (82). Antioxidant enzymes [e.g., superoxide dismutase (SOD) and catalase] and nonenzymatic factors (e.g., ascorbic acid, α-tocopherol) defend against oxidant damage of airway lipid membranes (165, 194) by directly quenching O3 or the ROS byproducts of O3 interaction with biomolecules. Antioxidant levels and activity vary among species and strains (66) and
may contribute to differential responsiveness to O$_3$-induced lung injury.

Population studies. Recent studies have associated O$_3$ exposure with exacerbation of asthma (46, 195), altered lung function in adults and children (22, 47, 125), and mortality (1, 94). That genetic background contributes to O$_3$ susceptibility has been suggested by significant intersubject differences in pulmonary function and inflammatory responses to O$_3$ (see Refs. 15 and 159). Collectively, these studies of healthy human subjects provide strong evidence of a heritable component to pulmonary responses to O$_3$. Further evidence for a genetic contribution to O$_3$ susceptibility is inferred by genetic association studies (12, 179).

Mouse QTL analyses of O$_3$ susceptibility. Significant interstrain variation in the magnitude of inflammatory cell and hyperpermeability responses to O$_3$ exposure in mice provides strong evidence that a genetic component contributes to O$_3$ responsiveness (73, 118, 188). The chromosomal location of the O$_3$ susceptibility genes was determined using susceptible C57BL/6J (B6) and resistant C3H/HeJ (C3) strains (120). A genome-wide search for linkage of pulmonary inflammation phenotypes (PMNs) was performed in intercross animals derived from B6 and C3 progenitors (B6C3F$_2$) using informative SSLPs. This interval mapping identified a significant susceptibility QTL on chromosome 17 (Inf1) and a suggestive QTL on chromosome 11 (Inf2) (Table 1). Inf1 contains the H-2 locus, including major histocompatibility genes and non-major histocompatibility complex (MHC) genes such as the proinflammatory cytokine Tnf (TNF-α). Because Tnf may mediate the inflammatory response to oxidant-related lung injury, this candidate gene was evaluated for differential O$_3$-induced inflammation in B6 and C3 mice (120). Pretreatment of susceptible B6 mice with a monoclonal antibody to TNF-α significantly attenuated the inflammatory response to O$_3$ relative to control mice, supporting the importance of TNF-α in this model. Deletion of TNF-α receptors 1 and 2 also significantly protected against O$_3$-induced inflammation (34).

The hyperpermeability phenotype is not dependent on PMN infiltration (34, 119) and suggests that different genetic mechanisms control these two traits. Therefore, a genome-wide scan for susceptibility QTLs was performed to explain interstrain differences in hyperpermeability induced by O$_3$ exposure using BXH RI strains of mice derived from B6 and C3 progenitors (121). A significant QTL on chromosome 4 and suggestive QTLs on chromosomes 3 and 11 were identified (Table 1). The chromosome 4 QTL contains a candidate gene, Toll-like receptor 4 (Tlr4), which regulates innate immune responses to O$_3$ (34).

Table 1. Lung injury and inflammation susceptibility genes in mice

<table>
<thead>
<tr>
<th>Lung Irritant</th>
<th>Phenotype</th>
<th>Locus ID</th>
<th>Marker (position in cM)</th>
<th>Homologous Human Site</th>
<th>Candidate Genes</th>
<th>Ref. No.</th>
<th>Sig. Level*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone</td>
<td>Inflammation (PMNs)</td>
<td>Inf1</td>
<td>D1Mit120-D1Mit122 (20.0-71.0)</td>
<td>17q11-q21</td>
<td>ScX; Nos2</td>
<td>120</td>
<td>SU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inf2</td>
<td>D1Mit25-D1Mit10 (17.0-24.5)</td>
<td>6p21.3, 21q22.3</td>
<td>Tnf</td>
<td>120</td>
<td>SI</td>
</tr>
<tr>
<td>Hyperpermeability</td>
<td>3</td>
<td>D3Mit19 (87.6)</td>
<td>1q22, 1p31, 2q21-q25</td>
<td>121</td>
<td>SU</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>D4Mit24 (27.8-33.0)</td>
<td>9q32-q33</td>
<td>121</td>
<td>SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>D11Mit34 (46.1)</td>
<td>17q11-q21</td>
<td>C3X; Nos2</td>
<td>121</td>
<td>SU</td>
<td></td>
</tr>
<tr>
<td>Survival times</td>
<td>11</td>
<td>Alij1</td>
<td>D1Mit179 (52.0)</td>
<td>17q11-q21</td>
<td>Nos2; Mpo; ScX</td>
<td>188</td>
<td>SI</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Alij2</td>
<td>D1Mit59 (16.0)</td>
<td>6p21.3</td>
<td>Xdh</td>
<td>188</td>
<td>SI</td>
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<td>Hyperoxia</td>
<td>Inflammation</td>
<td>2</td>
<td>D2Mit271-D2Mit476 (45.0-49.2)</td>
<td>2q31</td>
<td>Nf2</td>
<td>33</td>
<td>SI</td>
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<td>Sulfate-associated particles</td>
<td>Macrophage phagocytic dysfunction</td>
<td>11</td>
<td>D1Mit29 (40.0)</td>
<td>17q11-q21</td>
<td>C3X; Nos2</td>
<td>174</td>
<td>SU</td>
</tr>
<tr>
<td>Nickel sulfate aerosol</td>
<td>Survival times</td>
<td>17</td>
<td>D1Mit125 (20.6)</td>
<td>6p21.3, 16, 21q22.3</td>
<td>Tnf</td>
<td>174</td>
<td>SI</td>
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<tr>
<td>Bleomycin</td>
<td>Pulmonary fibrosis</td>
<td>6</td>
<td>D6Mit183 (26.5)</td>
<td>7q34-q35</td>
<td>Thxas1</td>
<td>187</td>
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<td></td>
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<td>8</td>
<td>D8Mit242-D8Mit271 (47-37)</td>
<td>16q22-q24</td>
<td>88</td>
<td>SU</td>
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<tr>
<td>Radiation</td>
<td>Pulmonary fibrosis</td>
<td>1</td>
<td>D1Mit198 (95.8)</td>
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<td>Tnf56</td>
<td>87, 89</td>
<td>SI</td>
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<tr>
<td></td>
<td></td>
<td>6</td>
<td>D6Mit254 (60.55)</td>
<td>12p13</td>
<td>Tnf56</td>
<td>87, 89</td>
<td>SI</td>
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<td></td>
<td></td>
<td>17</td>
<td>D1Mit179 (16.0)</td>
<td>6p21-21.2</td>
<td>H2</td>
<td>87, 89</td>
<td>SI</td>
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<td>Ovalbumin</td>
<td>Inflammation (eosinophils)</td>
<td>11</td>
<td>D1Mit52-D1Mit53 (2.4-16.0)</td>
<td>5q31</td>
<td>IL-4 gene cluster</td>
<td>254</td>
<td>SI</td>
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<td>BHT</td>
<td>Inflammation (macrophages)</td>
<td>3</td>
<td>D3Mit86-D3Mit19 (76.2-87.6)</td>
<td>1q22, 1p31, 2q21-q25</td>
<td>146</td>
<td>SI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflammation (lymphocytes)</td>
<td>3</td>
<td>D1Mit51 (37.0)</td>
<td>18p11.2</td>
<td>146</td>
<td>SU</td>
<td></td>
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<tr>
<td>Hyperpermeability</td>
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<td>D3Mit19 (87.6)</td>
<td>3q25</td>
<td>146</td>
<td>SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>D8Mit240-D8Mit312 (43.0-45.0)</td>
<td>16q13</td>
<td>146</td>
<td>SI</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>D10Mit61-D10Mit14 (60.0-65.0)</td>
<td>12q14-q21</td>
<td>146</td>
<td>SI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Quantitative trait locus (QTL) significance level. SU, the locus is a suggestive QTL based on Kruglyak and Lander (126) that has been independently verified. SI, the locus is a significant QTL based on Kruglyak and Lander (126). Alij, acute lung injury QTL; Blmhf, bleomycin hydroxylase; Blmhf, bleomycin-induced pulmonary fibrosis; CcX, small inducible cytokine cluster; COX, cyclooxygenase; Chr., chromosome; Gpx, glutathione peroxidase; H2, major histocompatibility complex; Hsl1, hyperoxia susceptibility locus 1; Inf, inflammation; Iki, interleukin-4; Nos2, nitric oxide synthase 2; Nf2, NF-E2-related factor 2; PMN, polymorphonuclear leukocyte; Radpf, radiation-induced pulmonary fibrosis; Sig., significance; ScX, small inducible cytokine cluster; Thxas1, thromboxane A synthase 1; Tlr4, Toll-like receptor 4; Tnf56, fas ligand; Tnf56, TNF receptor R1; Xdh, xanthine dehydrogenase; Mpo, myeloperoxidase.
ceptor 4 (Tlr4), that has been implicated in innate immunity and endotoxin susceptibility (124, 185, 191). Hyperpermeability responses to O3 were compared between C3H/HeOuJ (OuJ) and C3 mice to determine whether Tlr4 has a role in O3 susceptibility; these strains are polymorphic in the Tlr4 coding region such that resistance to endotoxin-induced injury is conferred to C3 mice but not OuJ. Significantly higher bronchoalveolar lavage protein concentrations (a marker of lung hyperpermeability) and lung tissue Tlr4 message were found in OuJ mice after exposure to O3, compared with C3 mice (121). Together, these results indicate that a QTL on chromosome 4 explains a significant portion of the genetic variance in O3-induced hyperpermeability and support Tlr4 as a candidate susceptibility gene.

Linkage analysis of susceptibility to death induced by exposure to high concentrations of O3 in susceptible AJ and resistant B6 mice as well as in AXB and BXA RI strains, identified significant QTLs on chromosomes 11, 13, and 17 (188; Table 1). The significant QTL on chromosome 11 is similar to that for susceptibility to inflammation induced by exposure to 0.3 ppm O3 (120).

Hyperoxic Lung Injury

Because the lung directly interfaces with the environment and its vast vasculature, the pulmonary epithelium is a major target for injury caused by exogenous oxidants such as environmental pollutants and hyperoxia, as well as by endogenous ROS generated by inflammatory cells (24, 212). Many pulmonary diseases [e.g., acute respiratory distress syndrome (ARDS), emphysema] require oxygen therapy to maintain lung function, further increasing the oxidant burden to the lung. Hyperoxia (>95% oxygen) causes extensive pulmonary damage characterized by inflammation (increases in PMNs and macrophages) and the death of capillary endothelial and alveolar epithelial cells in mice, and this results in pulmonary edema and severe respiratory impairment (83). The toxicities generated by oxygen exposure are mediated in part by superoxide, hydroxyl radicals, and H2O2 formed by the incomplete reduction of oxygen. Increased cellular ROS formation occurs during hyperoxia, and this is deleterious to cellular macromolecules. The pulmonary response to hyperoxia includes altered expression of antioxidant enzymes that protect against oxidant damage such as manganese SOD, copper zinc SOD, catalase, glutathione peroxidase, and heme oxygenase-1. Hyperoxia can also induce tissue inhibitor of metalloproteinases, pulmonary surfactant apoprotein A, and metallothionein (97).

Population studies. Hyperoxia can lead to the production of ROS that are implicated in the pathogenesis of several lung disorders, including ARDS, bronchopulmonary dysplasia (BPD), ischemia-reperfusion injury, and cancer (83). Infant respiratory distress syndrome (IRDS) occurs secondary to surfactant deficiency and lung immaturity and is common in premature infants. The mechanical ventilation and oxygen treatments for IRDS patients cause many infants to develop chronic BPD. Although development of BPD is multifactorial, pulmonary barotraumas (imbalances between ambient pressure and the endogenous pressure within the lung) and exposure to high concentrations of inspired oxygen are the most accepted causative factors (234). African-American infants, compared with Caucasian infants matched for gestational age and birth weight, have a decreased incidence of BPD. Males develop more BPD than gestational age- and sex-matched female infants (31). Although these findings include maturational differences, they also suggest genetic background as a determinant to the pathogenesis of hyperoxic lung injury and its repair. Identifying factors that influence individual susceptibility is important because of the potential impact of oxidants on lung function.

Mouse QTL analyses of hyperoxia susceptibility. Inbred strains of mice and rats vary in their survival with continuous hyperoxia exposure (74, 151). A genome-wide screen for hyperoxia susceptibility QTLs was performed using a B6C3F2 cohort derived from B6 (hyperoxia-susceptible) and C3 (hyperoxia-resistant) mice exposed to hyperoxia for 72 h (33). Selective genotyping identified a significant QTL on chromosome 2 (188; Table 1). Interval mapping of 12 BXH RI strains independently confirmed a significant susceptibility locus for inflammation on chromosome 2.

A potential candidate gene within the chromosome 2 QTL is the nuclear transcription factor Nrf2 [NF-E2-related factor 2, or Nfe2l2 nuclear factor (NF), erythroid-derived 2, like 2]. Nrf2 is an essential regulator of antioxidant and phase 2 enzymes (103, 108, 236) and thus has a key role in protecting cells against carcinogenicity, mutagenicity, and oxidative stress (247). A functional role of Nrf2 in the pathogenesis of hyperoxic lung injury was evaluated by comparing responses to hyperoxia in a strain deficient in Nrf2 (ICR/Sv129-Nrf2−/−) to wild-type (ICR/Sv129-Nrf2+/+) mice. Significantly greater inflammatory and hyperpermeability responses after 72-h hyperoxia exposure were induced in Nrf2−/− mice compared with Nrf2+/+ mice, consistent with the hypothesis that loss of Nrf2 function enhances susceptibility to hyperoxia-induced lung injury (32). A T→C substitution at the −336 position in the Nrf2 promoter in B6 mice cosegregated with hyperoxia susceptibility in B6C3F2 mice, supporting Nrf2 as a candidate hyperoxia susceptibility gene (32).

Particle-Induced Lung Injury

Fossil fuel combustion produces sulfur oxides (SOx) and particles as well as secondary products such as acid aerosols arising when combustion products interact with atmospheric constituents (e.g., high humidity). Although the particle components may be more important than SO2, their interaction produces an acid component to the particle mixture that may be lethal. Although the epidemiology of the health effects of combustion pollutants is well studied, the underlying physiological/toxicological mechanisms have not been identified.

Population studies. Increased particulate exposure has caused acute and chronic respiratory effects throughout the industrialized world (186, 192). Susceptible subpopulations include the elderly and patients with chronic heart disease, chronic obstructive pulmonary disease (COPD), asthma, and compromised immune systems (206, 220, 235). In addition to reducing lung function, these exposures enhance respiratory illness (e.g., chronic cough, bronchitis, pneumonia) in children and other sensitive subpopulations (40). Epidemiological studies have not yet focused on the contribution of genetic background as a host factor for susceptibility to particle effects, but results from several rodent studies support this hypothesis (174, 175, 187).
Overview of the mouse model. Acid-coated particles induced strain-specific recruitment of inflammatory cells but no increased lung hyperpermeability in mice (175). AM Fc receptor-mediated phagocytosis (an indicator of innate immune defense) was the major phenotype that varied between strains exposed to particles; B6 mice were susceptible to suppression of phagocytosis and C3 mice were resistant (175). The phagocytic function of AMs protects the airways from injury and represents the “first line” of immune host defense. The integrated activities of the pulmonary phagocytic and immune systems protect against bacteria and other environmental pathogens. Phagocytosis of the invading pathogen is coordinated with intracellular killing (oxidative burst) to maintain sterility of the respiratory tract. Loss of AM phagocytic function is an indicator of pulmonary toxicity in many mouse models (71, 252). Disruption of any step in intracellular killing, such as phagocytosis, compromises host defense.

QTL analyses for particle susceptibility. A genome-wide scan with B6 and C3 mice was performed (174) using suppression of AM Fc receptor-mediated phagocytosis as the response phenotype. Linkage analyses identified a significant susceptibility QTL at the H-2 region on chromosome 17 and a suggestive QTL on chromosome 11; these sites overlapped QTLs for O3 susceptibility. Common linkage assignments suggest that similar genetic mechanisms may control pulmonary responses to O3-induced inflammation and macrophage phagocytic dysfunction induced by acid-coated particles. Another linkage study measured survival time after nickel sulfate administration induced acute lung injury. Significant linkage among backcross progeny derived from A/J (sensitive) to B6 (resistant) mice was found on chromosome 6 (Table 1; 187).

Radiation and Bleomycin-Induced Lung Injury

Population studies. Epidemiological studies suggest that a genetic component affects susceptibility to radiation- or bleomycin-induced pulmonary fibrosis (70, 243). Thoracic radiotherapy is currently used to ablate tumors, including small cell lung cancer (SCLC) (70). Although few individuals develop fibrosis in response to radiation, the dosages used in sensitive patients are used to set the maximum amounts of radiation administered to all patients receiving radiation therapy. Bleomycin, an antibiotic derived from Streptomyces verticillus, is a chemotherapeutic agent for tumors including squamous cell carcinomas (SCC) and lymphomas (17). In the lungs of susceptible individuals, bleomycin can induce pulmonary fibrosis and lead to death (198). The fibrotic lesions caused by radiotherapy and bleomycin are similar (28).

Overview of the mouse model. Radiation induces lung injury and fibrosis in susceptible strains of mice, and the resultant collagen-rich areas contain fragments of hyaline material and inflammatory infiltrates (209). The lung injury caused by bleomycin in sensitive strains is characterized by alveolitis with loss of alveolar architecture, focal collagen deposition, and inflammatory infiltrates (2, 85). Radiation- and bleomycin-induced cytotoxicities include DNA fragmentation and altered macromolecular synthesis, particularly in alveolar type II cells. Disordered epithelial repair is also important in the development of fibrosis (18). The extent of bleomycin cytotoxicity is inversely related to bleomycin hydrolase activity, the enzyme that degrades bleomycin (205). Altered concentrations of transforming growth factor-β and interleukin (IL)-1β occur in lungs of mice treated with radiation or bleomycin (98, 109). Increased deposition of procollagens and extracellular matrix proteins, such as fibronectins, also occurs (85).

QTL analysis of radiation and bleomycin susceptibility. Inbred strains vary in their susceptibilities to radiation- (49, 67, 209) and bleomycin- (86, 203) induced lung injury. A genome-wide screen for radiation-induced pulmonary fibrosis in B6C3F2 mice found significant linkage on chromosomes 1, 6, and 17 and suggestive linkage on chromosome 18 (Table 1) (87, 89); interestingly, the chromosome 6 QTL was only observed in females. To confirm the chromosome 17 QTL, congenic mice on a fibrosis-resistant background were made that contained the chromosome 17 region from a sensitive strain (C3H.C57Bl/6J.D17Mit175-D17Mit47) (89); these congenic mice are fibrosis prone.

Two significant QTLs for pulmonary fibrosis resulting from bleomycin exposure of B6C3F2 mice are on chromosomes 17 (at H-2) and 11 (88) (Table 1). The chromosome 11 site is significant only in male mice and is epistatic with H-2; in females, H-2 is the major susceptibility locus. A suggestive QTL is mapped to chromosome 8 (see Table 1). Use of mice congenic for H-2 narrowed the region within H-2 to 2.7 cM (88). Male B6 mice consomic (i.e., mice in which a chromosome from one strain is substituted in another strain) for chromosome 11 derived from fibrosis-resistant C3H/Kam mice developed significantly less bleomycin-induced fibrosis. Bleomycin hydrolase, a candidate gene on chromosome 11, functions as an MHC class 1 epitope-processing protease; this may be the mechanism by which H-2 interacts with the chromosome 11 QTL in male mice. The chromosome 11 and 17 loci map to the same sites as those for O3 and particle-induced lung injury, and the chromosome 8 QTL is in the same region as susceptibility to butylated hydroxytoluene (BHT)-induced elevations in cyclooxygenase (COX)-2 content (Table 1).

Allergen-Induced Lung Injury

Population studies. Asthma is a worldwide epidemic affecting 155 million people of all ages (38). It is characterized by airway narrowing caused by inflammation (eosinophilia) and mucous hypersecretion and is exacerbated when smooth muscle contraction is hyperresponsive to nonspecific stimuli. This airway constriction leads to symptoms of coughing, wheezing, chest tightness, and shortness of breath. Several studies indicate a genetic component to asthma. Familial clustering of the main phenotypic characteristics of asthma (increased IgE, eosinophilia) occurs in relatives of asthmatic patients (113). In twin studies, concordance rates for asthma are significantly higher in monozygotic than dizygotic twins (53), suggesting heritability as high as 75%. (See Ref. 171 for more in-depth review of the genetic contribution to asthma and allergy.)

Overview of the mouse model. A chronic inflammation mouse model commonly used to study pulmonary allergy involves sensitization of mice to allergen [usually ovalbumin (OVA)] over 14 days followed by multiple allergen challenges at days 41, 44, and 47 (16). The resulting inflammation is characterized by eosinophil, lymphocyte, and macrophage infiltration and thickening of the airway epithelium. IgE released by B lymphocytes binds to its high affinity receptor (FceR1) on
both mast cells and eosinophils, leading to release of products that elicit inflammation (39). IL-4 and IL-13 are important mediators of IgE synthesis (64). The airway hyperresponsiveness component of asthma may be induced by several mechanisms, including airway remodeling (95).

**QTL analysis of asthma/allergen-induced lung injury.** F₂ progeny derived from BP2 (sensitive) and BALB (intermediate) progenitors were used in an OVA model to identify a significant QTL responsible for asthma-associated eosinophil infiltration on chromosome 11 (254) (Table 1). This chromosome 11 site is homologous to a major site linked to IgE responsiveness and eosinophil levels on human chromosome 5 (5q31–33) (153, 155). Major candidate genes at this site are IL-4, IL-9, and IL-13. The MHC region on human chromosome 6p is another major QTL for asthma and hyperresponsiveness (43, 93), and a homologous site was found in several mouse lung inflammation/injury models. Human chromosome 7 contains a QTL for atopy (a type 1 allergic reaction), asthma, and eosinophilia. Additional human QTLs are on chromosome 12q and 13q14 for atopy and asthma, and a site on chromosome 16p21 for IgE (172).

**BHT-Induced Lung Injury**

*Overview of the mouse model.* 3,5-Di-tert-butyl-4 hydroxyltoluene, BHT, the most widely used synthetic food preservative, causes lung injury and inflammation in mice and selectively promotes tumorogenesis when administered to certain inbred strains after protooncogene activation. It is not BHT itself, but oxidative metabolites of BHT produced in high concentrations in mouse lung, that are responsible for these pneumotoxicities (141, 226–229). BHTOH, the tert-butyl hydroxylated metabolite, is more potent than BHT at causing pneumotoxicity (158) and promoting lung tumor formation (229). Chronic administration of BHT causes alveolar type 1 cell death followed by regenerative hyperplasia of type II cells and changes in the contents and activities of several enzymes, including PKC (protein kinase C)-α, calpain II, COX-1 and -2, and 5-lipoxygenase (10, 117, 160). In sensitive BALE mice, AM infiltration and decreased PKC-α and calpain II contents contribute to BHT-induced tumor promotion (10, 160). Deletion of Nrf2 increased BHT-induced lethality (29), implying that detoxification of oxidative BHT metabolites limits BHT toxicity. BHT induces the activity of pulmonary NQO1, a phase 2 enzyme regulated by NRF2 (213). Aspirin and celecoxib each inhibit BHT-induced inflammation, supporting a role for eicosanoid pathways in BHT-induced lung inflammation (10, 117).

Although no epidemiological studies have been done to determine whether BHT causes or exacerbates lung inflammation in humans, the United States Food and Drug Administration assigned an “interim regulation” where BHT could not be added to foods in which it was not already used before 1977 (64a).

**QTL analysis for BHT susceptibility.** Strains of mice differ in BHT-induced inflammation (9, 160); CBX RI strains derived from BALB/cBy (sensitive) and C57BL/6J (resistant) progenitors were used to perform a QTL analysis. These RI strains differed in susceptibility to BHT-induced promotion and inflammation, including macrophage and lymphocyte infiltration, hyperpermeability, and COX-2 content (146). A chromosome 3 QTL modulated sensitivity to macrophage infiltration and hyperpermeability (Table 1). Other QTLs that significantly affected the extent of inflammation were on chromosome 18 for macrophage infiltration, chromosome 3 for lymphocyte infiltration, and chromosomes 8 and 10 for COX-2 induction. The chromosome 3 QTL that modulates BHT-induced macrophage infiltration and hyperpermeability also influences hyperpermeability induced by O₃. This chromosome 3 QTL includes a gene responsible for PKC-α activity (D3Mit19), an enzyme perturbed during BHT-induced lung injury (55).

**Comparison of Susceptibility Genes Identified For Inflammation/Injury Models**

Three large clusters of QTLs for the models described above appear on chromosomes 3, 11, and 17, with smaller clusters on chromosomes 8 and 18 (Fig. 1). Candidate genes within the H-2 locus on chromosome 17 include Tnf. Several of these QTLs are similar to susceptibility loci for extrapulmonary inflammation in mice, rats, or humans. For example, the hyperoxia-induced susceptibility gene on chromosome 2 is located near Dscc2 (dextran-sulfate sodium-induced colitis; 47 cM), a susceptibility gene for inflammatory bowel syndrome in mice (137). In a human COPD linkage study, 12p was identified as a QTL, and this is homologous to the region surrounding the chromosome 6 site (54–74 cM) for radiation-induced pulmonary fibrosis in mice (215). Bhr5 (57), bronchhoxygenative activity, is also located near this chromosome 6 locus (50.5 cM). A QTL for airway hyperreactivity (52 cM) falls within the chromosome 11 QTL that affects pulmonary sensitivities to O₃, hyperoxia, particles, and BHT (Table 1) (91). Many other inflammation models map within H-2, including mouse Mmu17 (a resistance gene for trypanosomiasis) (101) and human Bhr3 (bronchohyperreactivity 3, 14 cM) (91).

**SUSCEPTIBILITY GENES FOR LUNG CANCER**

*Human Lung Adenocarcinoma.*

More people die from lung cancer than from the combined deaths caused by solid tumors at all the next most frequent organ sites: breast, colorectal, prostate, and pancreas (128). Human lung cancer is divided into two clinical classes, SCLC and non-small cell lung cancer (NSCLC). NSCLC accounts for 20% of lung cancer cases and is almost exclusively associated with a smoking etiology (202). NSCLC tumors are centrally located in bronchi and express neuroendocrine markers such as neural specific enolase and synaptophysin. NSCLC tumors are usually too widespread after diagnosis for surgical resection but shrink upon chemotherapy and radiotherapy; unfortunately, drug-resistant cells emerge that grow rapidly and lead to death.

NSCLC develops more peripherally in bronchioles and alveoli in adenocarcinoma (AC) and in central bronchi in SCC. Over the past few decades, AC has become the most frequently diagnosed form of lung cancer, accounting for one-third of lung cancer cases (202). This is due in part to changes in smoking habits, such as a deeper inhalation of filtered cigarettes that brings noxious gases to more peripheral locations. Like SCLC, AC metastasizes before clinical symptoms appear, and premalignant lesions, called atypical alveolar hyperplasias, are often noted upon biopsy along with the primary tumor.
Squamous metaplasia, a precursor lesion of SCC, can be detected in cells exfoliated into the sputum by their abnormal morphology and immunocytochemical features. Because AC grows more deeply within the lungs, however, it is difficult to expectorate alveolar and bronchiolar cells for sputum diagnosis, decreasing the likelihood of finding early sputum markers.

Large cell carcinoma, the least frequent NSCLC subtype, ultrastructurally resembles poorly differentiated AC or SCC. Although smoking is the major cause of lung cancer, only 10–15% of smokers develop this disease, suggesting that genetic predisposition accounts for the differential etiology. This is supported by a similar incidence of lung cancer among close relatives. AC is the form of lung cancer most highly associated with clustering in twin and family studies (204). AC is also the predominant form of lung cancer in women (178), patients with an early age of onset (210), and those lung cancer patients who have never smoked.

Polymorphisms in genes for cytochrome P-450 and glutathione-S-transferase enzymes that metabolize and detoxify carcinogens in cigarettes, respectively, as well as DNA repair genes (7), are associated with increased lung cancer risk. Enhanced predisposition has also been ascribed to certain \( \alpha_1 \)-anti-trypsin polymorphisms, which suggests a relationship with COPD (248). The KRAS protooncogene is mutated in one-third to one-half of AC tumors (35), and a rare KRAS allele is more frequent in Italian and Japanese AC patients than in healthy cohorts (51).

Genes also underlie variations in the strength of addictive smoking behavior, such as determining the density of central nervous system nicotinic receptors (197). The multiplicity of low-penetrance susceptibility genes, and the occurrence of gene-gene and gene-environment interactions, have thus made it difficult to study the genetics of human lung cancer (245). The rarity of lung cancer families and the high fatality rate dictate that only archival specimens are available for molecular analysis. This impairs collecting detailed smoking data for all family members to assess this environmental variable in interpreting familial clustering.

Mouse Lung Tumor Model of AC

Mice develop lung tumors spontaneously and in response to carcinogens, radiation, and viruses, and resemble human AC in their anatomy, histogenesis, and molecular features (140). Because tumors can be experimentally induced in susceptible inbred strains, the entire sequence of early changes that generates a neoplastic phenotype from a previously normal cell can be elucidated (142). For example, within days after carcinogen administration, \( \text{Kras} \) becomes mutated and permanently activated in alveolar type II pneumocytes (11) and bronchiolar Clara cells (50), the putative cells of lung tumor origin (139). Decreased expression of the tumor suppressor gene \( \text{Cdkn2a} \) occurs in response to enhanced DNA methylation at CpG islands (or alternating cytosine and guanine nucleotide bases) in its promoter region (181) and at later stages of progression by gene deletion (90). \( \text{Cdkn2a} \) encodes the p16INK4 and p19ARF inhibitors of cyclin-dependent protein kinases, products obtained from the same gene by alternative splicing (190), and their reduced concentrations hasten cell cycle transit. Hyperplastic foci appear within a few weeks after carcinogen exposure that expand in size to become benign adenomas (211). These adenomas display elevated expression of proteins that mediate cell proliferation, such as cyclins and proliferating cell nuclear antigen, and decreased expression of differentiation markers, such as the anti-proliferative Clara cell secretory protein, CC10, and phase 1 and 2 metabolic enzymes (140). After several months, the tumors display malignant behavior, such as invasion of the surrounding normal tissue, morphological abnormalities like nuclear atypia, and continued biochemical differentiation away from an adult phenotype and
toward an earlier ontogenic stage, e.g., inability to induce cytochrome \(P-450\) enzymes in response to xenobiotics (65). Although metastasis is seldom studied in this primary tumor model, these tumors can metastasize (138).

Pulmonary adenomas grow in solid or papillary patterns. Solid adenomas express features of type II pneumocytes, such as lamellar bodies, and are probably derived from this cell type (217). Papillary tumors may display bronchiolar Clara cell features, such as pleiomorphic nuclei, dense granules, and high succinic dehydrogenase activity (112), and type II cell features such as surfactant protein \(C\) expression (156). Hyperplastic Clara cell foci are observed soon after carcinogen administration (65), and the frequency of \(Kras\) mutations in Clara cells is higher than in type II cells (50). It is controversial whether papillary tumors emanate from Clara cells, type II cells, or a developmental precursor cell common to these two cell types (104). The relative proportion of solid vs. papillary tumors in a mouse with multiple tumors depends on the carcinogen used to induce tumorigenesis (80), the age of the animal at the time of carcinogen application (which presumably affects the status of parenchymal cellular differentiation) (21), the strain of mice (225), and the duration after carcinogen exposure (224). Papillary tumors have a longer latency than solid tumors and are associated with a greater likelihood for malignant conversion (111).

**Lung AC Susceptibility Genes**

**Urethane-induced lung AC.** Inbred strains vary in tumor multiplicity (the number of tumors per mouse), tumor incidence (the number of mice with tumors), and tumor size (the sum of all measured tumor-bearing surfaces in step sections corresponding to tumor volume) that arise from both spontaneous (135) and carcinogen-induced lung tumorigenesis (92, 143). For example, in a urethane model, \(A/J\) and SWR mice developed many tumors and were classified as having a sensitive phenotype (143). Strains such as BALB/cJ, with a high tumor incidence but low tumor multiplicity, were referred to as intermediate. Most strains are resistant (e.g.,\(B6, DBA, and C3\)). \((A/J \times B6)F1\) hybrids developed a number of tumors that is the arithmetic mean to their parental strains, i.e., \(A/J\) developed 30 tumors/mouse, \(B6\) developed 0 tumors/mouse, and the \(F1\) hybrids developed 15 tumors/mouse (143). When AXB and BXA RI (derived from parental \(A/J\) and \(B6\) mice) strains were injected with urethane, tumor multiplicity varied continuously between the strains, demonstrating that more than a single gene regulated pulmonary adenoma susceptibility (\(Pas\) or pulmonary adenoma susceptibility) (145).

The appearance of a restriction fragment-length polymorphism of the \(Kras\) protooncogene correlates with sensitivity or resistance to lung tumor development among AXB and BXA RI strains (199). Festing et al. (59) used AXB RI strains and a \(AB6f2\) cross (derived from \(A/J\)olaHsd and \(C57BL/6\)olaHsd) to genotype low and high responders at polymorphic microsatellites throughout the genome. Significant QTLs responsible for strain variations in tumor multiplicity were mapped to chromosomes 9 and 17, and a suggestive QTL was mapped to chromosome 19 (\(Pas4\), 2, and 3, respectively; see Table 2). Gender also contributed to susceptibility, since males developed more tumors than females. These QTLs only affected tumor multiplicity when at least one susceptible \(Pas1\) (\(Kras\)) allele was present, and their effects were additive. Festing et al. (58) used \(A/J\) Balb/oLaHsd\(F2\) mice to map the QTLs responsible for lung tumor induction. When both parental strains contain the susceptible \(Pas1\) allele, the loci that contribute to the intermediate phenotype of the \(BALB/c\) strain in the absence of any contribution of the \(Kras\) polymorphism may be discriminated. Significant QTLs were identified on chromosomes 4, 11, and 18 (\(Pas9, 5, and 7, respectively; Table 2\)), and sexual dimorphism was observed. AXB and BXA RI strains confirmed the \(Pas1\) site and other suggestive QTLs on chromosomes 4, 10, 17, and 19 that act as modifiers of \(Pas1\) (133) (Table 2). With the use of CXB RI strains, additional QTLs on chromosomes 4, 14, and 15 were found (146) (Table 2). The chromosome 4 locus also contributes to BHT-induced tumor promotion.

The \(Pas1\) QTL was mapped to chromosome 6 in \((A/J \times C3)F2\) mice (69) (Table 2). Additional linkage studies with \((A/J Mus spreitus)F1\) crossed to B6 produced an interspecific strain called \(ASBF1\) (147), and a significant QTL on chromosome 11 governed lung tumor resistance (\(Par1\) or pulmonary adenoma resistance). The \(Pas1\) site was common among mouse strains with sensitive and intermediate phenotypes for tumor multiplicity and size ( BALB/c\(\times SWR/J\)F2 and (BALB/c\(\times C3H/He\)F2) cohorts (149) (Table 2). This study was the first to demonstrate a QTL responsible for tumor size using urethane as the carcinogen. A genome-wide scan with (\(SWR/J \times BALB/c\)F2) mice identified additional significant QTLs on chromosomes 6 and 18 that strongly reduced tumor multiplicity (\(Par2 = Pas7\) and \(Par4\); Table 2) (148) and demonstrated a significant QTL on chromosome 4 responsible for tumor size, designated pulmonary adenoma progression 1 (\(Papg1\)) (Table 2).

Another linkage study that used urethane to induce lung tumors in \((A/J \times BALB)F1 \times A/J\) backcross mice mapped a significant tumor resistance QTL to chromosome 18 (\(Par2\) (170). \(Par3\) was identified on chromosome 12 after interval mapping using SMXA RI strains (derived from SM/J and A/J progenitors) crossed to A/J mice (SMXA \(\times A/J\)F1; in these mice, \(Par1\) and \(Par3\) acted synergistically (180).

Linkage disequilibrium was applied in three studies to map QTLs responsible for lung tumorigenesis with urethane as the carcinogen. Manenti et al. (150) refined the \(Pas1\) site to a region <2 megabases (Mb), and Wang et al. (240) found major (significant) QTLs on chromosomes 4, 6, and 8. Twenty-seven minor (suggestive) QTLs were also identified using this technique on chromosomes 4, 5, 7, 9, 10, 13, 14, and 19, most of which are of low penetrance. Several of these minor QTLs were identified previously in linkage analysis studies and only those sites that were verified by other mapping studies were included in Table 2. In these studies, the strains used ranged from sensitive to resistant to lung tumorigenesis. The third study (152) used two outbred mouse strains selected for high and low acute inflammation response and found, by linkage disequilibrium, that \(Pas1\) is the major site responsible for urethane-induced lung inflammation and lung tumorigenesis.

Overlap of several QTLs identified by different groups using urethane as the lung carcinogen strengthens the concept that these are lung tumor susceptibility QTLs (see Table 2).

**N-ethyl-N-nitrosourea-induced lung AC.** Strains vary in lung tumor formation in response to \(N\)-ethyl-\(N\)-nitrosourea (207).
Table 2. Lung tumor susceptibility genes in mice

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Carcinogen Used</th>
<th>Phenotype</th>
<th>Named Locus</th>
<th>Marker position (cM)</th>
<th>Homologous Human Site</th>
<th>Candidate Genes</th>
<th>Ref. No.</th>
<th>Sig. Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ENU TS</td>
<td>Sluc5</td>
<td>Sluc5</td>
<td>1q22–q23</td>
<td>Rxrg</td>
<td>62, 232 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ENU TS</td>
<td>Sluc2</td>
<td>Sluc2 (41)</td>
<td>2q24–q31</td>
<td></td>
<td>60, 232 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Urethane TM</td>
<td>PAS9</td>
<td>D4Mit77 (42)</td>
<td>9p21–p22; 1p31–p32</td>
<td>Cdkn2a,b; Ifa; Jun</td>
<td>58 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Urethane TM</td>
<td>Par4</td>
<td>D6Mit50 (3)</td>
<td>7q31</td>
<td></td>
<td>148 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>ENU TS</td>
<td>Sluc7</td>
<td>Sluc7</td>
<td>7q31–q32.2</td>
<td></td>
<td>62, 232 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>ENU TS</td>
<td>Sluc3</td>
<td>Sluc3</td>
<td>10q11.2</td>
<td></td>
<td>60, 232 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Urethane TM</td>
<td>Par1</td>
<td>Kras (74)</td>
<td>12p12.1</td>
<td></td>
<td>48, 62 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Urethane TM</td>
<td>D10Mit86</td>
<td>D10Mit86, 126 (17–21); D10Mit282; 2 (12–16)</td>
<td>6q22–q23, 6q22–25</td>
<td>133, 240 SU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Urethane TM</td>
<td>Par1</td>
<td>D1Mit54 (56)</td>
<td>17q21.1–q23</td>
<td>Mpo; Rasgrf; Rbp2</td>
<td>147, 180 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Urethane TM</td>
<td>Par3</td>
<td>D1Mit5 (38)</td>
<td>14q11–q13; 31q11–q12</td>
<td>Pkcn; Gpx2; Fos</td>
<td>180 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Urethane TM</td>
<td>Par2</td>
<td>D1Mit56 (56)</td>
<td>6q24–q25.2</td>
<td></td>
<td>232 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Urethane TM</td>
<td>Par5</td>
<td>D1Mit139 (32)</td>
<td>17p13.1–p12</td>
<td></td>
<td>58 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Urethane TM</td>
<td>Par4</td>
<td>D6Mit50 (3)</td>
<td>6q24–q25.2</td>
<td></td>
<td>232 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Urethane TM</td>
<td>Par2</td>
<td>D2Mit5 (38)</td>
<td>14q11–q13; 31q11–q12</td>
<td>Pkcn; Gpx2; Fos</td>
<td>180 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Urethane TM</td>
<td>Par2</td>
<td>D2Mit5 (38)</td>
<td>14q11–q13; 31q11–q12</td>
<td>Pkcn; Gpx2; Fos</td>
<td>180 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Urethane TM</td>
<td>Par2; Par7</td>
<td>D1Mit52 (80)</td>
<td>17q21.1–q23</td>
<td>Mpo; Rasgrf; Rbp2</td>
<td>147, 180 SI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Urethane TM</td>
<td>Par3</td>
<td>D1Mit139 (32)</td>
<td>17p13.1–p12</td>
<td></td>
<td>58 SI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bolded sections include sites in common with inflammation/injury susceptibility genes. *QTL significance level. SU, the locus is a suggestive QTL based on Kruglyak and Lander (126) that has been independently verified; SI, the locus is a significant QTL (126); Adrb2, adrenergic receptor β-2; Broca1, breast cancer 1; Cdkn2a,b; cyclin- dependent kinase inhibitor 2A, 2B; Cyp17, cytochrome P-450 17; ENU, N-ethyl-N-nitrosourea; Fos, FBJ osteosarcoma oncogene; Gna1, guanine nucleotide binding protein α; Gpx2, glutathione peroxidase 2; Gsta, glutathione-S-transferase α; Gsta, glutathione-S-transferase β; Ifa, interferon-α gene family; Jun, Jun oncogene; Kras, Kirsten rat sarcoma oncogene 2; MCA, 3-methyl-cholanthrene; Met, met protooncogene; Pas, pulmonary adenoma susceptibility; Par, pulmonary adenoma resistance; Pparg, peroxisome proliferator-activated receptor γ; Rara, retinoic acid receptor α; Rbp2, retinol binding protein 2; Rarb, retinoid X receptor-β; Rsx, retinoid X receptor-γ; Pkcn, protein kinase C-γ; Sluc, susceptibility to lung carcinogenesis; TM, tumor multiplicity; TS, tumor size; Tnf, tumor necrosis factor α.
Interval mapping in (AB6F1 × B6) backcross mice identified a suggestive QTL responsible for tumor multiplicity on chromosome 19 (Pas3) and confirmed the Pas1 site on chromosome 6 (48). An (OcB9 × O20)F2 cohort was used to map QTLs responsible for overall tumor burden by combining multiplicity and tumor size (called susceptibility to lung cancer, Sluc) (60). QTLs were identified on chromosomes 2, 6, 11, and 19 (Sluc2, 3, 4, 1, respectively). Five OcB strains were used to make F2 crosses with O20 (OcB-3, OcB-6, OcB-9, and OcB-16), although these strains did not differ significantly in tumor size from O20 (62). OcB-4 is more susceptible than the original donor strain (B10.O20). Linkage analysis yielded 10 additional significant Sluc loci on chromosomes 1, 4, 6, 7, 8, 9, 12, 14, and 18 (Sluc5–14; see Table 2). Sluc6, 8, 13, and Kras on chromosomes 4, 7, 14, and 6 were associated with differences in tumor multiplicity, whereas the other loci were linked to tumor size. In a much larger study, crosses with these five OcB strains determined 16 new significant and suggestive Sluc loci (Sluc15–29; Table 2), all with linkage to tumor size (232). The Sluc genes are of low penetrance and display complex epistatic interactions (232). Only those sites found either by other mapping studies within the same group or independently by other groups were included in Table 2 (therefore, excluding Sluc15, 17, 19, 20, 22, 24, 27, and 30).

3-Methyl-cholanthrene-induced lung AC. Strain differences in AC formation were observed using 3-methyl-cholanthrene (MCA) as the carcinogen (9). Interval mapping in CXB RI strains identified QTLs responsible for susceptibility to lung tumorigenesis induced by MCA alone and lung tumor promotion using an MCA/BHT protocol (146). Significant QTLs at H-2 (the Pas2 site) and chromosome 18 were at least partly responsible for tumor promotion induced by BHT. This is the only study that has identified a locus responsible for two-stage pulmonary carcinogenesis.

Comparison of susceptibility genes. Multiple chromosomes contain overlapping QTLs (see Fig. 2). Some genes that regulate tumorigenesis are independent of the carcinogen used to start the process. It is difficult to determine whether the Pas, Par, and Sluc genes are identical, since different strains, carcinogens, and phenotypes were used. Unique QTLs may be expected since the different carcinogens require metabolic activation and detoxification by different enzymes. Carcinogens do not cause identical Kras mutations (250). For example, urethane induces codon 61 Kras mutations, MCA causes codon 12 mutations, and the age of the mice at the time of carcinogen exposure determined the particular base substitution within codon 12 mutated by MCA (75). Another set of genes is responsible for pulmonary adenoma histological type (Pah). If a single time period following carcinogen treatment is selected when examining benign tumor histology, strains vary in the proportion of papillary and solid tumors (225). When tumor multiplicity was compared with histological type, no correlation was observed between these phenotypes, suggesting that different sets of genes are responsible.

Several QTLs are at the same or similar locations as susceptibility genes for other types of cancer, such as liver, skin, and mammary cancer. For example, the distal region of chromosome 1 between 80–90 cM contains Sluc5 and Hcs7, a hepatocarcinogenesis susceptibility gene (68). This region of chromosome 1 was frequently amplified in mouse lung AC cell
lines, and the homologous sites in human AC (1q32-41) were also frequently amplified (200). Chromosome 4 contains Pas9 and Papg1 near Pctr1 (43 cM), a plasmacytoma resistance gene (253). In addition, Sluc6, Sluc21, and an unnamed site at 60 cM are all near Pla2g2a (phospholipase A2, group II A, a colon tumor susceptibility gene, 68 cM) (164) and Ssic1 (susceptibility to small intestinal cancer, 67 cM) (61). These chromosome 4 regions are frequently deleted in human AC cell lines, the homologous region of rat chromosome 5 in lung AC, chromosome 14 regions are frequently deleted in human AC cell (susceptibility to small intestinal cancer, 67 cM) (61). These colon tumor susceptibility gene, 68 cM) (164) and Spr1 (chromosome 12 is located near chromosome 18 (37 cM) identified by Wang et al. (240). This chromosome 7 region is frequently deleted in mouse AC cell lines and at homologous sites in human AC (1p15.5, 1p13. Hcs3 (59 cM) (68) on chromosome 12 is located near Sluc12 and Par3 and is commonly amplified in the mouse AC cell lines (200). Last, chromosome 14 rearrangements commonly occur during later stages of neoplasia in mice, the homologous region in rats, and the homologous region in human (13q) lung AC, chronic lymphocytic leukemia, mammary cancer, and liver cancer (110, 201, 222).

The major candidate gene for Pas1 is Kras. In mouse strains sensitive to lung carcinogens, only one copy of a 37-bp intronic sequence in Kras is present vs. resistant strains that have two tandemly arranged copies (251). The 37-bp deletion in susceptible strains could alter transcriptional regulation, because there is less binding of a nuclear protein present in susceptible strains but not in resistant strains (30). Human studies have shown a negative correlation between elevated Kras expression and survival in patients with lung AC (169). In addition, inhibitors of ras (e.g., farnesyltransferase inhibitors perillyl alcohol and FTI-276) reduce mouse lung tumorigenesis (129), suggesting that ras plays a critical role in mouse lung tumor formation. Spectral karotyping in mice demonstrated a duplicated region of chromosome 6 that contained Pas1 and Sluc3 (200); in human AC, the homologous region (12p12) is also amplified (183, 219).

Table 3. Epidemiological studies associating lung inflammatory diseases with an increased risk of lung cancer development

<table>
<thead>
<tr>
<th>Year</th>
<th>Disease Associated With Increased Risk</th>
<th>Gender</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>COPD</td>
<td>Men and women</td>
<td>37</td>
</tr>
<tr>
<td>1986</td>
<td>COPD</td>
<td>Men</td>
<td>216</td>
</tr>
<tr>
<td>1987</td>
<td>Smoking-induced airway obstruction</td>
<td>Men</td>
<td>230</td>
</tr>
<tr>
<td>1991</td>
<td>Bronchitis, pneumonia, emphysema</td>
<td>Women</td>
<td>178</td>
</tr>
<tr>
<td>1993</td>
<td>Asthma</td>
<td>Men and women</td>
<td>237</td>
</tr>
<tr>
<td>1994</td>
<td>Chronic cough, phlegm, increased FEV1</td>
<td>Men and women</td>
<td>102</td>
</tr>
<tr>
<td>1995</td>
<td>Asthma, chronic bronchitis, pneumonia, tuberculosis</td>
<td>Women</td>
<td>246</td>
</tr>
<tr>
<td>1999</td>
<td>Chronic bronchitis, emphysema</td>
<td>Men and women</td>
<td>158</td>
</tr>
<tr>
<td>2000</td>
<td>Silicosis</td>
<td>Men</td>
<td>36</td>
</tr>
<tr>
<td>2000</td>
<td>Cryptogenic fibrosing alveolitis</td>
<td>Men and women</td>
<td>99</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume.

COMPARISON OF SUSCEPTIBILITY GENES FOR LUNG INFLAMMATION/INJURY MODELS AND LUNG CANCER

Epidemiological Studies

Relationships between lung inflammation/injury and lung cancer in humans are summarized in Table 3. For example, genetic predisposition to COPD was associated with increased risk of developing lung cancer (37). However, a difficulty with human studies is sorting out genetic vs. environmental contributions to disease (e.g., cigarette smoking is a risk factor for both emphysema and cancer). An illustration of this difficulty is the occurrence of bronchoalveolar carcinoma (BAC), a subtype of AC, in nonsmoking women living in the Yunnan Province, China (127). Twenty percent of human AC cases in women are not due to smoking. The etiology of this high BAC in Chinese women is unknown, although cooking practices in which carcinogenic, polycyclic, aromatic hydrocarbons are produced in soft coal ovens and acrolein is generated upon heating rapeseed oil at high temperatures in unventilated kitchen may be causal. Yunnan has an ethnically diverse population, and unfortunate interactions between carcinogen exposure and susceptibility alleles are possible.

Comparison of Lung Inflammation/Injury Susceptibility Genes and Lung Tumorigenesis Susceptibility Genes

Genes responsible for susceptibility to both tumor promotion and chronic inflammation caused by BHT have been mapped (146). With the use of the MCA/BHT promotion model in which a single low dose of MCA is followed by six weekly doses of BHT, one QTL for tumor promotion was assigned to chromosome 18 (37–41 cM), and this same site regulated predisposition to macrophage infiltration. Among the mapping studies for susceptibility to lung injury/inflammation and to lung carcinogenesis in mice, eight susceptibility QTLs are common to both (Fig. 3). Although the overlapping of susceptibilities at these sites may be coincidental, this is certainly consistent with the hypothesis that common molecular/genetic mechanisms are important to both lung injury/inflammation and carcinogenesis. For example, the chromosome 11 and 17 sites include gene clusters that affect several inflammation and carcinogenic responses (Fig. 3). Candidate genes Tnf and Nos2 (nitric oxide synthase 2) are involved in inflammation sequelae. Lemon et al. (130) recently used a microarray approach to identify additional candidate genes for the Pas and Par sites, several of which are related to inflammation, again supporting a connection between inflammation and tumorigenesis. For example, a candidate gene for Par1 (Pas5) on chromosome 11 is 12-lipoxygenase, an enzyme important in the generation of eicosanoid mediators.

Pharmacological and Genetic Perturbations of Inflammation Affect Mouse Lung Tumorigenesis

Several pharmacological studies support involvement of inflammatory mediators in mouse lung tumorigenesis (Table 4). Aerosolized budesonide, an anti-inflammatory steroid, inhibited lung tumor formation by >90% when administered with myoinositol (242). Nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit COX activity decreased susceptibility to lung tumorigenesis by ~30%, whereas sulindac sulfone, a metabolite of sulindac, inhibited tumorigenesis by >90%
Aspirin BP A/HeJ
— Naproxen NNK (chr) A/J
— Sulindac NNK (chr) A/J
2
2
Indomethacin NNK (chr) A/J
Budesonide
‡
1
H9252
noted in italics.

Fig. 3. Combined susceptibility QTLs for lung inflammation/injury and lung tumorigenesis. Chromosomes are numbered, and the scale (cM) is at left. Positions of loci are at right (cM). The location noted for each locus is approximate based on literature. The candidate genes for some of the loci are noted in italics. Adrb, adrenergic receptor β1; Ccl, small inducible cytokine cluster; Gna1, guanine nucleotide binding protein; Kras, Kirsten ras; Nos2, inducible nitric oxide synthase; Nrf2, nuclear factor erythroid-derived 2, like 2; Tlr4, Toll-like receptor 4; Tnfa, tumor necrosis factor-α; Tnfsf6, fas ligand.

Table 4. Summary of anti-inflammatory drug effects on mouse lung tumorigenesis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Carcinogen</th>
<th>Strain</th>
<th>Effect</th>
<th>Reference No.</th>
</tr>
</thead>
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<tr>
<td><strong>Glucocorticoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosterone</td>
<td>Urethane</td>
<td>A/J</td>
<td>↓</td>
<td>52</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>ENU†</td>
<td>C57BL/10</td>
<td>↓</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>BP</td>
<td>A/J</td>
<td>↓</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>NNK</td>
<td>A/J</td>
<td>↓</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>Smoke</td>
<td>A/J</td>
<td>↓</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>A/J</td>
<td>↓</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>NNK</td>
<td>FVB</td>
<td>↓</td>
<td>134</td>
</tr>
<tr>
<td>Budesonide‡</td>
<td>BP</td>
<td>A/HeJ</td>
<td>—</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>A/J</td>
<td>↓</td>
<td>182</td>
</tr>
<tr>
<td><strong>Nonsteroidal anti-inflammatory drugs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>BP</td>
<td>A/HeJ</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>NNK</td>
<td>A/J</td>
<td>—</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>Smoke</td>
<td>A/J</td>
<td>—</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>MCA + BHT</td>
<td>BALB/cBy</td>
<td>—</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>NNK (chr)§</td>
<td>A/J</td>
<td>↓</td>
<td>54, 193, 249</td>
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<tr>
<td>Indomethacin</td>
<td>NNK (chr)</td>
<td>A/J</td>
<td>↓</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>Urethane</td>
<td></td>
<td>↓</td>
<td>162</td>
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<tr>
<td>Sulindac</td>
<td>NNK (chr)</td>
<td>A/J</td>
<td>↓</td>
<td>75, 144</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td>A/J</td>
<td>↓</td>
<td>81</td>
</tr>
<tr>
<td>Naproxen</td>
<td>NNK (chr)</td>
<td>A/J</td>
<td>↓</td>
<td>27</td>
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<tr>
<td><strong>COX-2 specific inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS398</td>
<td>NNK (chr)</td>
<td>A/J</td>
<td>↓</td>
<td>193</td>
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<tr>
<td>Celecoxib</td>
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<td>BALB/cBy</td>
<td>—</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>Urethane</td>
<td></td>
<td>—</td>
<td>117</td>
</tr>
<tr>
<td><strong>Lipoxygenase inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDGA</td>
<td>Urethane</td>
<td>A/J</td>
<td>↓</td>
<td>162</td>
</tr>
<tr>
<td>A79175</td>
<td>NNK (chr)</td>
<td>A/J</td>
<td>↓</td>
<td>27</td>
</tr>
</tbody>
</table>

BHT, butylated hydroxytoluene; BP, benzo(a)pyrene; NDGA, nodihydroguaiacetic acid; NNK, 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butaneone; VC, vinyl carbamate. *1, Decreased tumor multiplicity; —, no effect. †ENU was applied transplacentally. ‡Budesonide was applied by aerosolization. §NNK was administered chronically in the drinking water. [Modified from Kisley et al. (117).]

family (107) and inhibit NF-κB (76). Depletion of AMs by including chlorine in the drinking water of mice given MCA/BHT significantly decreased tumorigenesis, implying a role of these inflammatory cells in lung tumorigenesis (9).

Table 4. Summary of anti-inflammatory drug effects on mouse lung tumorigenesis

Studies have assessed the significance of inflammation and lung disease with respect to tumorigenesis using transgenic and knockout mice in which genes affecting inflammation have been altered. Prostacyclin (PGI2) is a potent pulmonary anti-inflammatory mediator (233); in mice, PGI2 overexpression significantly decreased lung tumorigenesis (114). Mice deficient in granulocyte/macrophage colony-stimulating factor (GM-CSF, a proinflammatory mediator) have pulmonary alveolar proteinosis and reduced surfactant catabolism, cell adhesion, and phagocytosis by AMs (231). GM-CSF overexpression enhances lung growth and causes alveolar type II epithelial cell hyperplasia in transgenic mice and supports a role for inflammation in carcinogenesis (100). Nos2-deficient mice have significantly less lung inflammation (122) and reduced tumor formation (116) than wild-type mice. Mice deficient in TNF-α or TNF receptors have significantly less inflammation (34) and tumors (13) than the wild-type mice. Finally, female mice deficient in IL-10, an anti-inflammatory cytokine mediator, developed more tumors than the wild-type mice (13). These studies with gene-targeted mice are consistent with a modulatory role for inflammation in lung tumorigenesis.

Mechanistic Speculations: How Does Inflammation Influence Carcinogenesis?

Chronic inflammation at or near a hyperplastic site predisposes humans to the development of many types of cancer, including breast, liver, large bowel, urinary bladder, prostate, pancreas, gastric mucosa, ovary, and skin (41, 42). Infectious agents (bacterial, viral, or parasitic) cause chronic inflammation and inflammation disorders and can predispose humans to the development of cancer; for example, bladder cancer has been associated with schistosomiasis, liver cancer with hepatitis B and C, and stomach cancer with Helicobacter pylori (173). Growing evidence supports the hypothesis that 15% of malignancies are initiated by infections (41). We propose a working model of how inflammation encourages multistage lung tumorigenesis (Fig. 4). In the initiation stage, DNA damage can be induced by
leukocytes and other phagocytic cells by their production of ROS and/or reactive nitrogen species (RNS) (136). 12-O-tetradecanoylphorbol-13-acetate (TPA), a skin tumor promoter, induces inflammation that stimulates interactions between epithelial and mesenchymal cells that influence epidermal growth (14, 176). Angiogenesis, a necessary component for the benign to malignant transition from adenoma to AC and a key component of metastasis, is correlated with inflammation, as is seen in chronic inflammatory diseases such as psoriasis and rheumatoid arthritis (84).

Inflammatory cells adjacent to a developing tumor include macrophages, neutrophils, eosinophils, and mast cells, each of which produces cytokines, oxidants (e.g., ROS), proteases, and soluble mediators of cell death such as TNF-α and IFN (42). Mast cells and macrophages, for example, release factors that enhance angiogenesis and promote lung inflammation. Release of ROS and RNS by macrophages and neutrophils causes tissue injury, lipid peroxidation, protein degradation, stimulation of the arachidonate pathway, and DNA strand breaks (25). Tissue injury occurs when elevated elastase and protease levels destroy the extracellular matrix (ECM), altering gas exchange and the epithelial barrier to water transport (25). Proteins (including protective antioxidant enzymes) polymerize and misfold after interacting with peroxidized lipids or ROS and lose function. Thromboxane A2 and leukotrienes produced when ROS stimulate inflammatory and epithelial cells can encourage several different stages of tumor development via multiple pathways (25).

Chronic exposure to TPA increases the percentage of lymphocytes, macrophages, mast cells, and capillaries in the dermis (4). Epithelial and stromal interactions provide regulatory signals necessary to maintain homeostasis. If signaling is disrupted by neoplastic conversion, cross talk between epithelial and stromal cells is modulated (176). Bronchiolar Clara cells and alveolar type II cells secrete ECM material that influences the signaling between the lung stroma and these AC precursor cells, similar to ovarian surface epithelium (157, 184, 196). In the lungs, BHT promotes tumors via proliferation and inflammation. To allow initiated Kras-mutated epithelial cells to overgrow their normal neighboring cells, oxidative BHT metabolites responsible for promotion and injury/inflammation/repair selectively induce apoptosis in non-tumorigenic cell lines (56), decrease gap junctional intracellular communication (79), and depress signal transduction (160). Prostanoid and cytokine mediators may influence each of these actions. Epithelial cells secrete prostaglandins and chemokines to recruit macrophages to a site of injury where they release proangiogenic stimuli (96). Primary rabbit (20) and bovine (221) AMs in culture released mediators that stimulated type II cells to proliferate. Specific chemokines (CXCL1/GROα, CXCL2/ GROβ, CXCL3/GROγ, and CXCL8/IL8) promote thepreneoplastic to neoplastic transformation in melanoma (238), whereas TNF-α-deficient mice were resistant to skin carcinogenesis induced by TPA or okadaic acid promotion protocols (8).

Evidence supports a role for inflammation in progression from adenoma to AC. CXC chemokines with the ELR (Glu-Leu-Arg) motif are important in ligand/receptor interactions in neutrophils as potent angiogenic factors (218). IL-8 (CXCL8) and epithelial neutrophil activating peptide (ENA-78) are angiogenic for human NSCLC (6). CXC chemokines without the ELR motif, such as IFN-γ-inducible protein (IP-10/CXCL10), inhibit NSCLC angiogenesis (218). Macrophage proinflammatory human cytokine 3α, a ligand for CCR6, is a chemokine that directs cell migration and modulates angiogenesis in pancreatic cancer (123). Other inflammatory mediators involved in angiogenesis include TNF-α and nitric oxide. TNF-α can induce several angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and thymidine phosphorylase (8). In breast cancer, TNF-α produced by infiltrating macrophages may regulate thymidine phosphorylase, a key enzyme in angiogenesis (8). Nos2 null mice had 54% less VEGF protein expression in the few lung tumors that developed compared with their wild-type counterparts (116). These data support a role of inflammation in regulation of angiogenesis and, as a consequence, lung tumor development.

In summary, substantial evidence supports the hypothesis that inflammation stimulates, and may be necessary for, many types of cancer (lung, colon, stomach, liver, skin, and mammary tissues, among others). Some genes that affect the predisposition to pulmonary inflammatory diseases are also likely responsible for susceptibility to lung cancer. Thus the micro-environment surrounding AC precursor cells and more mature tumors is critical to the tumorigenic process and suggests...
additional pathways to explore for novel approaches to prevent or abolish lung tumors.

ACKNOWLEDGMENTS

We thank Theodora Devereux for critically reviewing the manuscript.

GRANTS

This work was supported in part by National Institutes of Health Grants CA-33497 and CA-96133 (to A. M. Malkinson) and HL-66109 and HL-57142 (to S. R. Kleeberger).

REFERENCES

Invited Review

L700

COMMON LUNG DISEASE SUSCEPTIBILITY GENES


