Inverse genetic predisposition to colon versus lung carcinogenesis in mouse lines selected based on acute inflammatory responsiveness

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Mouse lines produced by bidirectional selection on the basis of maximum (AIRmax) or minimum (AIRmin) acute inflammatory reactions were examined for the development of chemically induced acute colitis and colon tumors and the development of lung tumors. AIRmax mice were more susceptible than AIRmin to acute colitis induced by ingestion of dextran sodium sulfate showing a 3-fold higher disease activity index and presenting an intense inflammatory infiltrate in the base of colon crypts as well as elevated expression of IL-1β, TNFα, IFNγ and IL-6 mRNA in colon tissue. AIRmax were also more susceptible than AIRmin to colon cancer induced by 2 or 7 weekly doses of 1,2-dimethylhydrazine (DMH), showing significantly higher numbers of colonic aberrant crypt foci (ACF) at 150 days after DMH treatment ($P = 0.01$) and significantly higher numbers of tumors affecting larger intestinal areas at 300–475 days. At the latter time point, however, multiple lung adenomas and large adenocarcinomas were found in AIRmin but not in AIRmax mice. Treatment of mice with nimesulide for 60 days beginning 24 h before the first of two DMH doses almost completely inhibited the appearance of ACF in both lines. Furthermore, ACF numbers and the degree of acute inflammation directly co-segregated in an F2 (AIRmax × AIRmin) intercross population. The results demonstrate that genetic determinants of the inflammatory response differentially influence susceptibility to colon and lung carcinogenesis in the AIRmax and AIRmin mouse model.

Introduction

The colon is the gastrointestinal tract segment mostly affected by neoplasias. About 90% of colon cancers can be clinically classified as non-hereditary or sporadic and only 10% are classified as familial cancers, where highly penetrant mutations in single genes are inherited in a Mendelian manner (1). An example is the mutation in the adenomatous polyposis coli (Apc) suppressor gene present in the autosomal dominant familial adenomatous polyposis (FAP) disease, which is associated with a high predisposition to the development of colon cancer (2). On the other hand, there is increasing evidence that most apparently non-hereditary cancers develop in genetically predisposed individuals (3). This predisposition is largely a polygenic phenomenon involving many low-penetrance genes with small phenotypic effects that additively can be equivalent to the effect of the single genes involved in familial cancers. Linkage or association studies in human populations do not have sufficient resolving power to identify these low-penetrance susceptibility genes. The complexity of the genetic control of tumorigenesis has been demonstrated in laboratory mouse lines that differ largely in susceptibility to tumor induction by several agents in different organs such as the colon, liver, lung, kidney and skin (4–8). These lines are used in crosses to map tumor modifier genes through segregation analysis.

Tumor susceptibility genes are heterogeneous and play different roles in modulating cancer. For example, suppressor genes (APC, p53, Muc2, Dcc), DNA repair genes (Msh2, Mlh1) and oncogenes (k-ras) are mutated in cancer cells and are classified as cell-autonomous (7), while other genes affect cancer progression from outside the transformed cell. The latter genes are frequently polymorphic, influencing specific aspects of the tumorigenic process in a tissue-specific manner. The allele-specific effects of these genes are encoded in the germ-line and several loci linked to susceptibility to colon cancer, named Scc, include these non-cell autonomous genes which modulate colon cancer by paracrine or systemic signals (7–11).

Chronic inflammation in a target organ may provide conditions favorable to tumor development. Bioactive mediators and infiltrating inflammatory cells have a large influence on the tumor microenvironment, as clearly documented by the association between inflammatory intestinal diseases such as ulcerative recto-colitis or Crohn’s disease and colorectal cancer. Individuals with chronic colitis have a higher incidence of colon neoplasia and cancer risk increases progressively with the disease (12–14). Furthermore, genetic polymorphisms in pro-inflammatory cytokine genes are associated with gastrointestinal malignancy susceptibility and severity in humans, with a direct effect of these polymorphisms on individual variations in the level of cytokine production and disease outcome (15).

Here, we investigate the degree of colon inflammation (acute colitis) and colorectal cancer development in two mouse lines selected for high (AIRmax) and low (AIRmin) acute inflammatory response. Both lines originated from an F0 population generated from the balanced intercross of eight inbred strains. The phenotype considered during the bidirectional selection was the degree of local inflammation induced by the s.c. injection of polyacrylamide beads (Biogel),
measured after 24 h by leukocyte counts and protein concentration in the inflammatory exudates (16). At the selection limit, the AIRmax and AIRmin mouse lines differed ∼25-fold in the number of infiltrated leukocytes (mainly neutrophils) and 2.5-fold in protein concentration in the 24 h s.c. inflammatory exudate. The analysis of the heritability of the character, during the several generations of bidirectional selective breedings revealed the involvement of ∼11 quantitative trait loci (QTL) with additive effect, accounting for the phenotypic divergence between AIRmax and AIRmin mice.

Alterations in bone marrow granulopoiesis in response to hematopoietic factors, as well as the production of chemotactic factors by infiltrated or local resident cells contribute to the phenotypic difference between the two lines (17). The differences in the inflammatory responsiveness are reflected in profound changes in resistance/susceptibility of the selected lines to several diseases. AIRmax were found resistant and AIRmin susceptible to intracellular bacterial infections (18) and to Trypanosoma cruzi (Manuscript in preparation), whereas AIRmax were extremely susceptible and AIRmin resistant to pristane-induced arthritis (19). Considering tumorigenesis, AIRmax mice were significantly more resistant than AIRmin mice to the development of skin tumors induced by dimethylbenz[a]anthracene (DMBA) followed by repeated applications of 12-O-tetradecanoylphorbol-13-acetate (TPA) (9), and AIRmax mice were also highly resistant and AIRmin susceptible to lung tumorigenesis induced by several carcinogens (20). With urethane as a carcinogen, AIRmin mice developed a persistent subacute lung inflammation and a 40-fold higher lung tumor multiplicity than did AIRmax mice, which showed a transient lung inflammatory reaction (21). As a consequence of the bidirectional selective process, the alleles of the genes relevant to the ‘maximum’ and ‘minimum’ inflammatory response phenotypes specifically segregated in AIRmax and AIRmin lines, respectively, leading to homozygosity in the loci that controls AIR but maintaining the background genetic heterogeneity in each line. Thus, the selected lines constitute a high-resolution model for the mapping of these QTL by linkage disequilibrium or linkage analysis. Linkage disequilibrium was found at genetic markers inside a 452 kb region in the major pulmonary adenoma susceptibility locus (Pas1); AIRmax and AIRmin mice segregated the resistance and susceptible haplotypes, respectively, implicating Pas1 in inflammatory response regulation (21). The involvement of AIR-regulating QTLs in tumorigenesis was also demonstrated by linkage analysis of skin or lung tumor parameters as a function of the degree of acute inflammatory response in F2 (AIRmax × AIRmin) intercross populations. An inverse correlation of the phenotypes was evidenced in both experiments since high inflammation segregated with resistance or low tumor multiplicity and low AIR, with susceptibility or high tumor multiplicity (9,20).

Here, we show that AIRmax mice are more susceptible than AIRmin to induction of colitis by dextran sodium sulfate (DSS) and to development of aberrant crypt foci (ACF) and colon tumors induced by 1,2-dimethylhydrazine (DMH), indicating a direct correlation between colon cancer susceptibility and acute inflammatory response. We also show that the genetic factors controlling inflammatory responsiveness in these selected mice present an opposite effect on the susceptibility to colon versus lung tumorigenesis.

Materials and methods

Mice

AIRmax and AIRmin mice from the 30th generation of selective breeding and F2 mice produced by F1 × F1 (AIRmax × AIRmin) intercross were produced at the animal facilities of the Laboratory of Immunogenetics at Institute Butantan, Brazil. The genetically heterogeneous founder population (F0) was produced through a three-generation crossing of the following eight inbred mouse lines of distinct origin obtained from The Jackson Laboratory: A/J, BALB/cJ, CBA/J, C57BL6/J, DBA/2J, SJL/J, SWR/J and P/J (16).

DSS-treated mice were maintained under SPF (specific pathogen free) conditions while long-term DMH-treated groups were maintained in conventional conditions. Under both conditions, mice were free of intestinal parasites. Equivalent numbers of male and female mice were used in the experiments. The studies were approved by the Animal Experimentation Ethics Committee of Institute Butantan.

Induction of acute colitis

Mice were given sterile, filtered water containing 2.5% (w/v) DSS (40 kDa; Sigma) ad libitum for 5 days, followed by 2 days of regular drinking water. A disease activity index (DAI) was derived from three major clinical signs, i.e. weight loss, diarrhea and rectal bleeding (22), monitored daily. The DAI was calculated as the sum of weight loss, diarrhea and rectal bleeding scores. The presence or absence of diarrhea or of rectal bleeding (defined as diarrhea containing visible blood/mucus or gross rectal bleeding) was scored as 0 or 1, respectively.

Acute inflammatory reaction (AIR) to Biogel P100

Biogel P100 (Bio-Rad, 67% suspension in phosphate-buffered saline (PBS)) was injected s.c. in the back. After 24 h, pouches were rinsed twice with 1 ml PBS–20 U/ml heparin and local exudate was collected. Nucleated cells were counted in Malassez chambers in an aliquot of the supernatant diluted in 1% acetic acid (16).

Induction of ACF and colon tumors

Groups of 45 AIRmax and AIRmin mice were injected i.p. with two or seven weekly doses of the carcinogen DMH (Sigma Aldrich Chemical) for total doses of 60 and 185 mg/kg body wt, respectively (23), and were killed in groups of ∼10 of each line at 60–475 days after the last injection.

Colonos were removed, gently flushed with ice-cold PBS, opened longitudinally from anus to cecum and fixed flat in 10% buffered formalin. After fixation, colon sections were stained with 0.2% methylene blue for 5 min and tumors and ACF were visualized with a stereo microscope (×32). The area (A = πr²) of colon tumors was calculated based on the mean diameter (in mm) of the lesion. For each mouse the total area of the colon covered by tumors was calculated by the sum of the area of the several lesions. Colonos were embedded in paraffin, cut into 5 µm longitudinal sections and stained with hematoxylin and eosin for histological analysis.

Lung tumors

Lungs were infused with Carney solution for macroscopic tumor count. The incidence (number of tumor-bearing mice) and the total volume of lung tumors for each mouse were calculated as described previously (21). Lungs were immersed for 24 h in Carnoy and fixed with 10% formalin. Paraffin-embedded sections were stained with hematoxylin and eosin for histology.

Nimesulide treatment

Nimesulide (4-nitro-2-phenoxymethanesulfonanilide) (Asta Médica, Brasil) was added at 400 p.p.m. to the powdered food. Mice were fed ad libitum from 1 day before the first dose of the carcinogen and until killing at 60 days after the second DMH dose (24).

RNA isolation and RT–PCR analysis of gene expression

Total cellular RNA was isolated from 100 mg of frozen colon tissue using Trizol reagent (Invitrogen) according to the manufacturer’s instructions. To generate cDNA, 1 µg of total RNA was reverse-transcribed at 42°C for 50 min in a 20 µl reaction volume containing 50 mM Tris (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 3 mM dithiothreitol, 10 mM dNTP mix and 0.5 µg of oligo(dT), plus 200 U Superscript Reverse Transcriptase III (Amersham Pharmacia Biotech). PCR amplification was routinely performed with 1 µl cDNA and carried out in a 24 µl reaction volume [10 mM Tris (pH 9), 50 mM KCl, 1.5 mM MgCl₂, 200 µM of each dNTP and 20 pmol of specific 5’ and 3’ primers], plus 0.5 U Platinum Gold Taq polymerase on an Eppendorf Master Gradient Thermocycler with the primers described in Table I.
The amplification protocol consisted of 15 min at 42°C, 5 min at 95°C and 35 cycles of denaturation at 95°C for 60 s, annealing at 58°C for 60 s and extension at 72°C for 60 s followed by 10 min at 72°C. PCR products were separated on a 2% agarose gel, visualized by ethidium bromide staining and band intensities were quantified by densitometry (Image Master Amersham). IL-1β, IL-6 and TNF-α mRNA expression was normalized to that of β-actin, and expression of IL-4, IL-10, IL-12, IFN-γ and TGF-β to that of hydroxymethylbilane synthase (HMBS) with respect to amplicon size and amplification efficiency.

The semiquantitative analysis was performed in the colon of untreated or 5 day DSS-treated mice. Animals were maintained in SPF conditions and received water for 2 days before killing.

**Phospholipase A2s (PLA2g2a) gene polymorphism analysis**

Genomic DNA was amplified by PCR with the following primers: sense- CGC AGT TTG GGG AAA TGA TTC and antisense- TCC AGG CTC TTG TAG CAA CAC GAT TTT C. The annealing temperature was 58°C and the product was digested with BamHI (Invitrogen) for 3 h at 37°C. Products were visualized in ethidium bromide-stained 2% agarose gels (25,26).

**Statistical analysis**

The non-parametric Mann–Whitney rank-test was applied to analyze differences between AIRmax and AIRmin mice in DAI scores and tumor numbers and size. For linear regression analysis, ACF numbers were square root-transformed and infiltrated cell numbers were natural log (ln)-transformed to approximate a Gaussian distribution. Differences were considered significant at \( P < 0.05 \).

**Results**

**Acute colitis**

AIRmax mice presented weight loss and diarrhea with bleeding on Day 4 of treatment with 2.5% DSS in drinking water, whereas AIRmin mice presented rectal bleeding without diarrhea on Day 5. During the experiment, the DAI in AIRmax mice was 3-fold that of AIRmin mice (Figure 1A). Histological analysis revealed an intense inflammatory infiltrate around and in the base of colon crypts in AIRmax but not in AIRmin mouse preparations at 5 days after DSS treatment (Figure 1B). Experiments repeated twice with 6 mice in each group evidenced no differences between males and females of both lines.

**Expression of pro- and anti-inflammatory cytokine mRNA**

Semiquantitative analysis of mRNA expression of inflammatory cytokines (IL-1β, IL-6, IL-12, IFNγ and TNF-α) and of anti-inflammatory cytokines (TGF-β, IL-10 and IL-4) in colon tissue from mice treated with 2.5% DSS for 5 days or untreated (Figure 2) revealed higher expression of IL-1β and IL-6 in DSS-treated AIRmax compared with AIRmin mouse colon, with expression of IFNγ only in the colon of AIRmax-treated mice, whereas TNF-α, IL-12 and TGF-β

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<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tr>
<td>β-actin</td>
<td>GTG GGT CGC TCT AGG CAC CAA</td>
<td>CTC TTT GAT GTC ACG CAC GAT TTT C</td>
</tr>
<tr>
<td>IL1-β</td>
<td>GAG ATT GAG CTG TCT GCT CA</td>
<td>AAG GAG AACCAA GCA AGC AC</td>
</tr>
<tr>
<td>IL6</td>
<td>GTA CTC CAG AAG AAC AGA GG</td>
<td>TGC TGG TGA CAA CCA CCG CC</td>
</tr>
<tr>
<td>TNF-α</td>
<td>TTG ACC TCA GCG CTG AGT TG</td>
<td>CCT GTA GCC CAC GTC GTA GC</td>
</tr>
<tr>
<td>HMBS</td>
<td>AAA GTG CCG TGG GAA CCA G</td>
<td>GAG GCG GTG GTT GAG GTT TC</td>
</tr>
<tr>
<td>IFNγ</td>
<td>GCT CTG AGA CAA TGA ACG CT</td>
<td>AAA GAG ATA ATC TGG CTC TGC</td>
</tr>
<tr>
<td>IL12p40</td>
<td>CAG TAC ACC TGC CAC AAA GGA</td>
<td>GTG TGA CCT TCT CTD CAG ACA</td>
</tr>
<tr>
<td>IL-4</td>
<td>TCG GCA TTT TGA ACG AGG TC</td>
<td>GAA AAG CCC GAA AGA GTC</td>
</tr>
<tr>
<td>IL-10</td>
<td>TCA AAC AAA GGA CCA GCT GGA CAA CAT ACT G</td>
<td>CTG TCT AOG TCC TGG AGT CCA GCA GAC TCA A</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>ACC GCA ACA ACG CCA TCT AT</td>
<td>GTA ACG CCA GGA ATT GTT GC</td>
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Fig. 1. Acute colitis in DSS-treated AIRmax and AIRmin. (A) Kinetics of DAI during 7 days of DSS treatment. Data are given as mean ± SD. *P < 0.05. (B) Histological analysis of DSS-treated colon segments from AIRmax and AIRmin mice. Note inflammation at the base of the crypts in AIRmax tissue.

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**Table I.** Sequence of primers in 5’→3’ orientation for RT–PCR
mRNA were expressed in control and treated mice; IL-4 and IL-10 were induced by DSS in both mouse lines.

Colon carcinogenesis

ACF, defined as putative pre-neoplastic lesions (27), were quantitated in DMH-treated AIRmax and AIRmin mice. In both lines, ACF were distributed all along the colon with a tendency to decrease in the cecum and to increase in proximal and medial colon at later times after DMH treatment. Histological analysis of the lesions revealed hyperplasia and varied degrees of dysplasia (Figure 3A and B). After two carcinogen doses, ACF were more numerous in AIRmax than in AIRmin mice at all time points examined (Figure 3C). With seven DMH doses, the difference between both lines was significant only at 150 days after the last dose of carcinogen (Figure 3D). There were no differences between males and females in ACF numbers.

Effect of Nimesulide on DMH-induced ACF. When mice were treated with 400 p.p.m. of nimesulide in the food for 60 days, the incidence and multiplicity of ACF induced by two doses of DMH decreased significantly both in AIRmax and AIRmin animals (Table II), pointing to the role of inflammation in the development of these pre-neoplastic lesions.

Correlation between AIR and DMH-induced ACF in the F2 intercross between AIRmax and AIRmin mice. One hundred F2 mice were injected with two doses of DMH with a 1 week interval (total of 60 mg/kg body wt). After 60 days, mice were injected s.c. with 0.75 ml Biogel P100 suspension and local AIR was measured 24 h later by leukocyte counts in the inflammatory exudates. At this time mice were killed for the determination of ACF numbers. Phenotype analysis of individual F2 mice revealed a linear progression of ACF numbers with inflammatory response to Biogel ($r = 0.97, P < 0.001$) (Figure 4), consistent with the results of the nimesulide experiments and further suggesting that common genetic factors influence the differential patterns of colon tumorigenesis and inflammatory responsiveness observed in AIRmax and AIRmin mice.

Colorectal tumors

Lesions suggestive of neoplasia, evidenced by the presence of dysplastic cells with characteristics of carcinoma (adenocarcinoma), were first detected in the colon at 300 days after DMH treatment. Tumors ~5–10 mm in diameter affecting large areas of colon were observed in AIRmax mice treated with seven doses of DMH (Figure 5A and E and Table III), whereas small lesions were found in the colon of one AIRmin mouse (Figure 5C). With two doses of DMH, tumor diameters did not exceed on average, 0.5–1.5 mm in either mouse line. Nevertheless, the area of the colon affected with tumors was significantly larger in the AIRmax-treated groups (not shown). No differences were found between males and females.

Lung tumors

Starting at 300 days after DMH treatment, lung lesions characterized histologically as adenomas and adenocarcinomas were found in the majority of AIRmin mice. In AIRmax and AIRmin mice treated with seven doses of DMH and killed 475 days later, lung adenomas and adenocarcinomas were present in 9 of 12 AIRmin mice (Figure 5D and F and

Fig. 2. Effect of DSS treatment on cytokine expression in colonic mucosa. (A) RT–PCR analysis of cytokine gene expression in the colon of DSS-treated or untreated AIRmax and AIRmin mice. (B) and (C) Quantification of cytokine expression in AIRmax and AIRmin colon, respectively, based on band intensity normalized to the intensity of the corresponding β-actin or HMBS control band and expressed as relative density (%). Data are from two independent experiments for each mouse line.
Table III), whereas only 2 of 9 AIRmax mice developed one small lung adenoma \((P < 0.001)\) (Figure 5B and Table III). In control groups, three of seven age-matched untreated AIRmin mice showed one small lung adenoma. Despite the considerable difference between the DMH-treated versus untreated AIRmin mice in lung tumor incidence and size, the spontaneous appearance of lung adenomas in AIRmin mice might be related to the effect of the susceptible allele at the \(\text{Pass1}\) locus which is found in this line. The results in DMH-treated AIRmin mice are in agreement with those obtained in a previous study, in which AIRmin were more susceptible than AIRmax to the development of lung tumors induced by other carcinogens \((20)\). Thus, an inverse susceptibility to lung and colon carcinogenesis is suggested in these selected mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mice</th>
<th>ACF incidence</th>
<th>ACF numbers (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMH AIRmax</td>
<td>11/11*</td>
<td></td>
<td>12.18 ± 1.96*</td>
</tr>
<tr>
<td>DMH AIRmin</td>
<td>8/12*</td>
<td></td>
<td>2.92 ± 0.91*</td>
</tr>
<tr>
<td>DMH + nimesulide</td>
<td>3/13</td>
<td>0.46 ± 0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/16</td>
<td>0.06 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

\*Mice were killed at 60 days after two doses of DMH (65 mg/kg body wt).

\*Nimesulide (400 p.p.m.) in powdered food for 60 days.

Table II. Effect of nimesulide treatment on DMH-induced ACF formation in AIRmax and AIRmin mice
Analysis of the phospholipase A2 (Pla2g2a) gene polymorphism

The calcium-dependent non-pancreatic group IIA secretory phospholipase A2 (Pla2g2a) is a proposed candidate gene for the Mom1 (modifier of Min1) locus, which decreases tumor multiplicity in the multiple intestinal neoplasia (Min) mouse model (28). Certain mouse lines such as A/J carry a naturally mutated gene that confers sensitivity to tumorigenesis, while Pla2g2a is wild-type in resistant strains. This polymorphism is found within a BamHI endonuclease restriction site and the product of the mutated gene is a non-functional enzyme (25). Data further suggest a tumor modifier role for Pla2g2a in chemically induced colon tumorigenesis that is equivalent to its role in the Min model (26).

Since the susceptible A/J line is one of the eight isogenic mouse lines that constituted the founder population for the selection of AIRmax and AIRmin, we analyzed this polymorphism in the two lines. The mutated (Pla2g2a<sup>Mom1-s</sup>)
alleles of A/J susceptible mice was found in AIRmin only, whereas the wild-type (Pla2g2aMom1r/r) allele was fixed in AIRmax, resulting in a highly significant ($P = 0.0002$) allele frequency disequilibrium between the two lines (Table IV).

### Table IV. Pla2g2aMom1 genotypes of AIRmax and AIRmin mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>DMH$^a$</th>
<th>No. of mice with Pla2g2aMom1$^b$ genotype</th>
<th>Colon tumors</th>
<th>Lung tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Incidence (%)</td>
<td>Tumor area$^b$ (mean ± SE)</td>
</tr>
<tr>
<td>AIRmax</td>
<td>-$^c$</td>
<td>0/10</td>
<td>0/10</td>
<td>97.8 ± 35.9</td>
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<tr>
<td></td>
<td>+</td>
<td>9/9 (100)</td>
<td>3/7 (43)</td>
<td>13.3</td>
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<tr>
<td>AIRmin</td>
<td>-</td>
<td>0/7</td>
<td>0/7</td>
<td>1/12 (8.3)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>10/10</td>
<td>3/7 (43)</td>
<td>13.3</td>
</tr>
</tbody>
</table>

$^a$DMH was administered in seven weekly doses (total dose 185 mg/kg body wt). Mice were killed at 475 days after the last DMH injection.  

$^b$Area (mm$^2$) and volume (mm$^3$) were calculated based on the mean diameter of tumors. For each mouse the total area of the colon covered by tumors or the total volume of lung tumors were calculated by the sum of the several lesions.  

$^c$1.5-year-old mice.  

**Discussion**

AIRmax mice genetically selected based on their strong acute inflammatory reaction were found to be significantly more susceptible than their low responder AIRmin counterparts to the development of acute colitis induced by ingestion of DSS. Colitis scored as DAI based on body weight loss, diarrhea and rectal bleeding was 3-fold higher in AIRmax than in AIRmin mice; the disease was also characterized by the presence of a vast cell infiltrate in the base of colon crypts, represented mainly by monocytes in AIRmax mice. Although TNF-$\alpha$, IL-12 and TGF-$\beta$ were expressed in the colon of control mice, expression of pro-inflammatory cytokines such as IL-1$\beta$, IL-6 and IFN-$\gamma$ was elevated in colon of DSS-treated AIRmax mice compared with AIRmin mice. These cytokines sharply distinguish between inflamed and non-inflamed intestinal mucosa, as demonstrated in different models by the use of inhibitors of the cytokines or their receptors and by the study of intestinal inflammation in cytokine-deficient animals (29–32). Although showing some variation between the two colon preparations in each group, the anti-inflammatory IL-10 and the TH2 cytokine IL-4 were induced by DSS in AIRmax and in AIRmin mice. These cytokines might contribute to the inhibition of the inflammatory TH1 response or to the blockade in the inflammatory cascade and recovery of colon homeostasis. Treg subsets, which control intestinal inflammation via IL-10 and TGF-dependent mechanisms, might be relevant to this process (33).

A strong genetic influence is found in human inflammatory intestinal diseases (34). In the mouse, the genetic control of susceptibility to colitis is evidenced by the differential susceptibility of inbred lines (35). The pattern of inheritance is complex and likely multigenic. Crosses between resistant and susceptible strains have revealed colitogenic linkages on chromosomes 1, 2, 3, 5, 11 and 18 and several attractive candidate genes that control inflammatory reactions map to these regions (36,37). Interestingly, Dssc2 (dextran sulfate sodium-induced colitis) at 47 cM in chromosome 2 is located near a significant locus for susceptibility to lung inflammation and hyperpermeability by hyperoxia as well as a locus, Scc1, associated with susceptibility to DMH-induced colorectal cancer. Other common loci such as Scc15 in chromosome 11 and Scc5 in chromosome 18 modulate susceptibility to chemically induced colon cancers and to DSS-induced colitis (7,38). The concordance in map location of QTLs for lung and colon inflammation and cancer in independent experimental models suggests that common genetic factors influence inflammation and cancer development.

In the present study, we found strain differences in responsiveness to acute inflammatory stress in the large intestine of the two mouse lines genetically selected on the basis of acute inflammation induced by s.c. injection of Biogel beads. Consistent with this different phenotype, AIRmax mice developed large numbers of ACF and colon adenocarcinomas involving large intestinal areas after repeated injections of the carcinogen DMH. Unlike AIRmax mice, the AIRmin line exhibited no increase in ACF number from 60 to 150 days after treatment with seven doses of DMH. The number of ACF in a colon varies with time, indicating that they are in a dynamic state and may remodel or regress (27). AIRmin mice might express a protective or resistance factor in the colon that impedes progression of carcinogen-induced foci and that might underlie the reduced numbers of colon tumors found in AIRmin compared with AIRmax mice at 300–475 days after DMH injection.

On the other hand, AIRmin mice showed multiple adenomas and adenocarcinomas in the lungs late after DMH treatment, whereas ACF- and colon tumor-bearing AIRmax mice developed very few lung tumors. Previous studies using the carcinogen urethane (ethyl carbamate) (20) or DMBA (21) also revealed induction of multiple lung lesions only in AIRmin mice. Together, the data indicate an organ-specific pattern of cancer susceptibility in AIRmax and AIRmin mice. Different patterns of tumor susceptibility in different organs have been widely described in several mouse strains (39,40) and current hypotheses to explain these phenotypes include (i) different susceptibility genes might
The continuous administration of the selective cyclooxygenase (COX)-2 inhibitor, nimesulide, during DMH treatment almost completely abolished the early appearance of ACF in AIRmax and AIRmin mice. The effect was observed for incidence as well as for ACF multiplicity. In contrast, we showed previously that nimesulide as well as aspirin rendered the resistant AIRmax mice significantly more susceptible to urethane-induced lung tumorigenesis and to the metastatic spread of implanted melanomas (20,41). However, our present results are consistent with those of Fukutake et al. (24) who demonstrated that nimesulide inhibited azoxymethane-induced late colon tumors in ICR mice without suppressing tumor development in the liver or lungs; indeed, nimesulide-treated mice showed a higher incidence of lung tumors. Overall, the available data suggest that COX-derived eicosanoids have distinct and perhaps opposite effects on tumorigenesis depending on the target organ (42–44). Our results support the notion that COX-2 inhibitors, currently under investigation as cancer chemopreventive agents, differentially modulate lung and colorectal cancer.

In co-segregation tests in a F2 intercross (AIRmax×AIRmin) population, susceptibility to colon carcinogenesis increased in mice with increasing inflammatory response (P < 0.001). In a previous study using another F2 population, we found an inverse correlation between multiplicity of urethane-induced lung tumors and acute inflammatory reactivity to Biogel (20). Together, the results support the hypothesis that genetic determinants of acute inflammatory responsiveness segregated in AIRmax and AIRmin mice have tissue-specific effects, modulating lung and colon carcinogenesis in an opposite manner.

Polymorphisms in inflammatory genes have been implicated in the risk of colon cancer. In the present study, we detected polymorphism between AIRmax and AIRmin mice in the Pla2g2a gene. Studies of the potential role of Pla2g2a as a candidate gene for Momi1 in colon chemical carcinogenesis using azoxymethane, in susceptible and resistant mouse lines (25,26,45) have established a link between the normal Pla2g2aMomi1r allele and resistance and between the mutated allele Pla2g2aMomi1s and susceptibility. Nevertheless, contrasting activities for the Pla2g2a enzyme in colon cancer have been described: Pla2g2a catalyzes the release of a series of pro-inflammatory and pro-carcinogenic products from fatty acids such as arachidonic acid, the substrate for COX-1 and -2. COX-2 has been implicated in the enhancement of intestinal tumorigenesis, since it is upregulated in human and mouse colon tumors. However, other data suggest that Pla2g2a is not associated with COX-dependent tumorigenesis (46). In our model, the mutated form of the Pla2g2a gene was found only in AIRmin mice, whereas AIRmax were all homozygous for the normal allele. It is likely that the enzyme participates in the expansion of chronic tissue disorders in the colon of AIRmax mice, promoting tumor progression, although other genetic factors certainly contribute to the different pattern of colon tumorigenesis observed in the two lines, since the normal Pla2g2a allele is also present among AIRmin mice.

Together our results suggest that genetic components of the high responder AIRmax mice might condition both the propensity to colon cancer and the resistance to lung cancer development, while the opposite is observed in AIRmin mice. Thus, a dual role for effectors of the inflammatory reaction seems likely, which can predispose or prevent tumor formation depending on the target organ.

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Conflict of Interest statement. None declared.

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