Genetic Analysis of Macrophage Characteristics as a Tool to Identify Tumor Susceptibility Genes: Mapping of Three Macrophage-Associated Risk Inflammatory Factors, Marif1, Marif2, and Marif3

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ABSTRACT

Genetic predisposition to cancer is influenced by allelic variation in tumor susceptibility genes (TSGs) as present in the germline. We previously demonstrated in the mouse that TSGs frequently participate in genetic interactions, indicating that they represent molecular networks. Inflammation may constitute one of the molecular networks underlying susceptibility to cancer by influencing the tumor microenvironment. Because macrophages play a key role in inflammation and are often associated with tumors, we argue that a subset of TSGs can be identified by examining the genetics of macrophage characteristics. A panel of inflammation-related assays was established to phenotype mouse bone marrow-derived macrophages, which included stimulation with lipopolysaccharides followed by measurement of secretion of tumor necrosis factor α and the p40 chain of interleukin-12 and of expression of inducible nitric oxide synthase and cyclooxygenase-2. This panel of assays was used for linkage analysis and applied to bone marrow-derived macrophages derived from individual mice of segregating crosses between inbred strain O20 and the highly related strains NTX-10 and NTX-20, which differed from O20 in only 10% of their genome, to reduce genetic complexity. Three macrophage-associated risk inflammatory factors were mapped—Marif1, Marif2, and Marif3—that each affected several inflammation-related assays, confirming that they function within molecular networks. Moreover, Marif1 and Marif2 were localized in regions with established linkage for both quantitative and qualitative aspects of lung cancer susceptibility. These studies provide a novel approach to investigate the genetics of microenvironmental influence on predisposition to tumorigenesis, thereby contributing to development of new strategies that aim to prevent or treat cancer.

INTRODUCTION

Cancer is a complex disease that is influenced by both genetic and environmental factors. Identification of tumor susceptibility genes (TSGs) is crucial to identify the origin of variation in genetic predisposition to cancer and provides essential information to understand the molecular basis of tumor development. Several TSGs have been identified from families that exhibited Mendelian inheritance patterns of tumor incidence due to germline transmittance of one cancer-predisposing allele with very high penetrance toward the tumor phenotype (1). Although these high-penetrance TSGs have revolutionized clinical practice, their incidence among the human population is low, and collectively, familial forms of cancer account for less than 10% of all cancer cases (2, 3). Importantly, predisposition to the 90% of nonfamilial, sporadic forms of cancer is also strongly influenced by hereditary factors (4). However, identification of this group of TSGs from the human population is hampered by their low penetrance toward the cancer phenotype and their multiplicity. Reduction of genetic and environmental heterogeneity by using inbred strains of mice as a model for tumorigenesis in humans has proven to be an excellent tool to unravel the complexity of tumor susceptibility. By now, more than 100 TSGs have been mapped onto the mouse genome and several TSGs have been identified (5, 6). We previously mapped a total of 30 susceptibility to lung cancer loci, Sluc1–Sluc30, by making use of recombinant congenic strains, a system of mouse inbred strains specifically developed to dissect multigenic traits in which each recombinant congenic strain shares 12.5% of its genes from a common donor strain onto a background of 87.5% genes derived from a common background strain. Importantly, this reduction in genetic complexity allowed us to incorporate a systematic search for pair-wise epistatic interactions between TSGs, which revealed that 29 out of 30 Sluc loci were involved in one or more genetic interactions with other Sluc loci or sex of the mice (7–9). Similar results were obtained when this strategy was applied to studies of susceptibility to colon cancer (10–12). These studies clearly demonstrated that each TSG can have considerable main effects despite their multiplicity, however, these effects were masked by their participation in genetic interactions. The multitude of genetic interactions implies that they represent complex molecular networks underlying cancer predisposition.

Tumorigenesis is characterized by accumulation of genetic changes in the genome of cancer cells. Somatically acquired mutations in oncogenes and tumor suppressor genes result in dominant gain-of-function and recessive loss-of-function alterations, respectively, providing cancer cells with enhanced proliferation and survival rates compared with normal cells. Therefore, allelic variants of oncogenes and tumor suppressor genes form one group of candidate genes for TSGs. However, cancer cells in vivo are embedded within a microenvironment containing normal cells, and it has become increasingly clear that the interaction between transformed malignant cells and nontransformed cells like fibroblasts, endothelial cells, and immune cells is critical to cancer pathogenesis (13). The observation that many cancers arise from sites of infection, chronic irritation, and inflammation supports the notion that inflammatory cells contribute significantly to the neoplastic process (14, 15). Evidence that genetic variation in hematopoietic cells affects susceptibility to tumors of nonhematopoietic origin was provided by elegant experiments in which skin tumor initiation and progression was examined upon transplantation of bone marrow obtained from matrix metalloproteinase-9-proficient and -deficient mice (16). Moreover, aspirin and other nonsteroidal anti-inflammatory drugs reduce the number of intestinal tumors and lung tumors in mice (17, 18), whereas in humans, nonsteroidal anti-inflammatory drugs have been demonstrated to reduce the number of colorectal tumors (19, 20). These data illustrate that inflammation constitutes one of the molecular networks underlying susceptibility to cancer and indicate that genes expressed by hematopoietic cells to mediate inflammatory responses form another group of candidate genes for TSGs. Macrophages play a key role in both inflammation and tumorigenesis. Their phenotypic plasticity allows...
them to initiate inflammatory responses by functioning as sensors for pathogens as well as to suppress inflammatory responses by functioning as professional phagocytes that efficiently remove apoptotic cells (21, 22). Moreover, macrophages are often present within the tumor microenvironment where they are both a source and a target of many cytokines, chemokines, and other signaling molecules, thereby contributing both positively and negatively to various stages of tumorigenesis by influencing processes like angiogenesis, tissue remodeling, and subversion of antitumor immunity (23).

We applied a novel strategy to identify a subset of TSGs that affect susceptibility to tumors of nonhematopoietic origin through their function in hematopoietic cells. Instead of investigating the heterogeneous process of tumorigenesis in vivo, we predicted that TSGs that influence susceptibility to cancer through their function in macrophages can be revealed by analysis of the genetics of inflammation-related macrophage characteristics in vitro. Primary bone marrow-derived macrophages (BMMF) were obtained from mice of segregating crosses between inbred strains that were known to differ from each other in several Sluc loci and exposed to a panel of inflammation-related assays. Here, we report the mapping of three macrophage-associated risk inflammatory factors, Marif1, Marif2, and Marif3.

**MATERIALS AND METHODS**

**Mice, Crosses, and Genotyping.** Mouse inbred strains O20/A, B10.O20/Dem, NTX-10/Dem, and NTX-20/Dem (henceforth referred to as O20, B10.O20, NTX-10, and NTX-20, respectively) were originally obtained from Dr. P. Demant and maintained at the VU University Medical Center animal facilities. All experiments were approved by the animal experimentation ethics committee, according to local and governmental regulations. B10.O20 is an H2 congenic strain that carries the H2w congenic background (N8). O20 and B10.O20 are the parental strains of the O20-congenic-B10.O20 (Obc) strains, a series of recombinant congenic strains that each carry 12.5% of the B10.O20 genome onto an O20 background (6, 24). NTX-10 and NTX-20 were derived from strain Obc9-9 by backcrossing to O20. F2 hybrid mice were obtained from crosses between the common background strain O20 and each of the strains NTX-10 and NTX-20 and used for experiments between 10 and 20 weeks of age. Mice were sacrificed by asphyxiation in CO2, and DNA was isolated from their tails followed by genotyping with simple sequence length polymorphism markers according to standard procedures (7). Marker positions were derived from the mouse genome database (MGI 2.97). All F2 mice were typed for the markers D2Mit156, D4Mit54, D4Mit126, D6Mit36, D8Mit155, D8Mit3, D8Mit125, and D19Mit60. In addition, (O20 × NTX-10)F2 mice were typed for D4Mit112, D16Mit9, D19Mit61, and D19Mit3, and (NTX-20 × O20)F2 mice were typed for D2Mit56, D6Mit52, D7Nds2, and D11Mit15. On average, the B10.O20-derived genic segments covered 145 cM of the NTX-10 genome distributed over seven chromosomes and 131 cM of the NTX-20 genome distributed over seven chromosomes.

**BMMF Cultures, Lipopolysaccharide (LPS) Treatment, and Detection of Tumor Necrosis Factor α (TNF-α).** The p40 Chain of Interleukin-12 (IL-12p40), and Nitric Oxide (NO). Femurs and tibiae of adult mice were flushed with PBS. Bone marrow cells were plated in 145-mm bacteriological plastic Petri dishes (Greiner, Frickenhausen, Germany) and cultured in a 37°C incubator containing 5% CO2. BMMF were obtained by differentiation of bone marrow cells in RPMI 1640 (Life Technologies, Paisley, United Kingdom) containing 10% heat-inactivated FCS (HyClone, Logan, UT), 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM t-glutamine (BioWhittaker, Ver- viaers, Belgium), and 20 mM 5-[2-hydroxyethyl]-1-piperazineethanesulfonic acid (Life Technologies), supplemented with 15% (v/v) L929-conditioned medium as a source of colony-stimulating factor-1 (25). After 8 days of culture, individual mice yielded an average of about 40 × 106 mature macrophages, conforming to classification by Leenen et al. (26), based on flow cytometric analysis using a FACScan (Becton Dickinson, San Jose, CA). Monoclonal Abs were used directed against the markers F4/80, Mac-1a (M1/70), and Mac-2 (M3/88), all acquired as supernatants from hybridomas cultured in our laboratory. At 8 days, BMMF were harvested using PBS-dissolved lidocaine (Sigma, St. Louis, MO) and distributed into 24-well tissue culture plates (Greiner) in 500-µl aliquots at a concentration of 1 × 106 cells/ml. Cells were harvested in PBS supplemented with 1% (w/v) BSA (fraction V; Sigma), 2% formaldehyde, and methanol at −20°C for 20 min, and blocked in PBS supplemented with 0.1% (w/v) BSA containing 2.5% normal goat serum (Life Technologies). Cells were incubated with primary antibodies directed against iNOS (rabbit polyclonal) and COX-2 (mouse monoclonal) obtained from Transduction Laboratories (Lexington, KY) diluted in blocking solution for 1 h, followed by incubation with FITC-conjugated goat-antirabbit IgG (Jackson ImmunoResearch, West Grove, PA) and Alexa594-conjugated goat-antimouse IgG (Molecular Probes, Leiden, the Netherlands) secondary antibodies together with 0.5 µg/ml Hoechst dye 33258 (bisbenzimide; Sigma) to stain the nuclei. Samples were mounted in vinyloil mounting media (27, and results were viewed using a Nikon Eclipse E800 microscope equipped with epi-fluorescence optics and appropriate filters that allowed detection of FITC, Alexa594, and Hoechst dye. Filters were used to capture separate digital images for iNOS, COX-2, and nuclei from identical, randomly selected microscopical fields (×20 objective) using a DXM1200 Nikon digital camera. Each image was saved as a TIFF file with a resolution of 1280 × 1024 pixels. Staining for iNOS and COX-2 was quantified using Scion Image software (version β 4.0.2). Each image was converted to grayscale and inverted, resulting in pixel values ranging from 0 to 255 that represented staining intensity. Areas that stained positive were automatically selected for measurements. Total staining intensity per selected area was calculated by multiplying the number of pixels/area with the area mean intensity. Addition of these values for all areas/microscopical field for iNOS (COX-2, respectively) divided by the number of nuclei present in the corresponding field revealed values representing relative mean iNOS (COX-2, respectively) protein levels per cell. More than 500 cells obtained from three separate microscopical fields were analyzed for each experimental condition.

**Statistical Analysis.** Cultures of BMMF were initiated in “experimental groups” of five F2 mice at a time together with one age-matched female O20 mouse that was taken along as a common point of reference. All data were related to values acquired for the corresponding O20 reference mouse and “log-transformed to obtain approximately normal distributions. Statistical eval-

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RESULTS

Establishing a Panel of Inflammation-Related Assays to Phenotype Macrophage Characteristics. BMMF were obtained by differentiation of bone marrow cells in vitro in the presence of colony-stimulating factor-1. This procedure yielded sufficient primary macrophages from individual mice to perform a series of inflammation-related assays. BMMF from strain O20 were used to select the optimal conditions to establish a panel of inflammation-related assays, because this strain was used as a common point of reference throughout these studies. Harvesting supernatants at 6 and 24 h after stimulation with 100 ng/ml LPS appeared to be most informative to determine secretion of TNF-α, IL-12p40, and NO. At 6 h, TNF-α levels were near maximal, IL-12p40 levels were about half-maximal, and NO levels were not detectable yet; whereas at 24 h, TNF-α levels had dropped, IL-12p40 levels were near maximal, and NO levels were high (data not shown). Stimulation of O20 BMMF with 1 ng/ml LPS resulted in 2-fold higher levels of TNF-α and 2-fold lower levels of NO than stimulation with 100 ng/ml LPS (Fig. 1), indicating that BMMF were differentially activated by low and high concentrations of LPS. Expression of iNOS and COX-2, the enzymes responsible for the increased production of NO and prostaglandins during inflammation, respectively (29, 30), was visualized by immunofluorescent staining. Hardly any staining was observed at time points of interest, reaching maximal levels at 24 h (Fig. 2). Their relative expression levels were estimated by computer-aided analysis of digital images.

The following experiments were selected to compose the panel of inflammation-related assays to be applied to linkage analysis studies: treatment of BMMF with 1 ng/ml and 100 ng/ml LPS followed by detection in supernatants of TNF-α at 6 h, IL-12p40 at 6 h and 24 h, and NO at 24 h after stimulation; and treatment of BMMF with 100 ng/ml LPS followed by determination of expression levels of iNOS and COX-2 at 24 h after stimulation. A pilot experiment was performed in which the characteristics for this panel of assays of O20 BMMF were compared with B10.O20 BMMF, the parental strains of the O20-congenic B10.O20 (OcB) recombinant congenic strains that were previously used to map Sluc lung cancer susceptibility loci (31). About 1.5–2-fold differences were observed for all assays except for secretion of NO (data not shown), demonstrating that these assays were influenced by genetic variation between O20 and B10.O20. Although the magnitude of the differences appeared to be modest, it should be emphasized that large differences are not required to allow mapping of numerous susceptibility genes with relatively large effects, as demonstrated by the mapping studies of Sluc loci (7–9). Hence, this panel of inflammation-related assays was used for linkage analysis to phenotype macrophage characteristics.

Results of Linkage Analysis. Mapping genes involved in multigenic traits is greatly facilitated by reducing genetic complexity. Previously, we demonstrated that O20 and OcB-9 differ from each other in 11 Sluc loci, whereas genetically OcB-9 carries only 12.5% B10.O20-derived genome onto an O20 genetic background (7–9). Here, genetic complexity was even further reduced by making use of the OcB-9-derived strains NTX-10 and NTX-20, two strains that together differ from the common background strain O20 in only 10% of their genome that harbors seven Sluc loci. The B10.O20-derived genomic segments that were contained within NTX-10 and NTX-20 are listed in Table 1. First, BMMF were cultured from 30 (NTX-10 × O20)F2 mice and exposed to the panel of inflammation-related assays. Genotypes of F2 mice were determined using simple sequence length polymorphism markers located within each of the segregating genomic segments (Table 1; see “Materials and Methods”). One marker per segregating segment and sex of the mice were incorporated together in a statistical model to analyze their effects on each of the assays. Table 2 summarizes the results and presents both suggestive linkage data (nominal P values < 0.05) and genome-wide significant linkage data (P values < 0.05 after correction according to the...
rules of Lander and Kruglyak (28). Significant linkage was obtained for sex of the mice with expression of iNOS; for D4Mit12 with secretion of TNF-α, with expression of iNOS, and with differential effects of low and high concentrations of LPS on secretion of IL-12; and for D8Mit3 with secretion of TNF-α. In addition, D19Mit60 was suggestively linked to expression of iNOS and to effects of sex of the mice with various inflammation-related assays. Statistical analysis revealed genome-wide significant linkage for D8Mit125 with secretion of TNF-α and suggestive linkage for D4Mit54 and sex of the mice with various assays (Table 2), confirming the presence of loci on chromosomes 4, 8, and 19 that largely overlapped the B10.O20-derived genomic regions in NTX-10 (Table 1). Although the marker D4Mit12 was not B10.O20-derived in NTX-20, several other nearby markers were, allowing improvement of the mapping position of the locus on chromosome 4. In addition, NTX-20 could be used to confirm a locus on chromosome 8 and to provide additional evidence for a locus on chromosome 19. Therefore, BMMF from 35 (NTX-20 × O20)F2 mice were genotyped and phenotyped for the panel of inflammation-related assays. Statistical analysis revealed genome-wide significant linkage for D8Mit125 with secretion of TNF-α and suggestive linkage for D4Mit54 and sex of the mice with various assays (Table 2), confirming the presence of loci on chromosomes 4

### Table 1 Maximal and minimal B10.02O-derived genomic segments inherited by NTX-10 and NTX-20

<table>
<thead>
<tr>
<th>Chr</th>
<th>Bordering markers and their position (cM)</th>
<th>Maximal (cM)</th>
<th>Minimal (cM)</th>
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<td>2</td>
<td>D2Mit5 (5)-D2Mit56 (41)</td>
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<td>4</td>
<td>D4Mit37 (57)-D4Mit42 (81)</td>
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<td>14</td>
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<td>6</td>
<td>D6Mit29 (37)-D6Mit10 (49)</td>
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<td>7</td>
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<td>7</td>
<td>D7Mit176 (27)-D7Mit98 (53)</td>
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<td>23</td>
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<td>8</td>
<td>D8Mit129 (32)-D8Mit125 (5)</td>
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<td>11</td>
<td>D11Mit24 (28)-D11Nds1 (44)</td>
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<tr>
<td>16</td>
<td>D16Mit101 (17)-D16Mit104 (5)</td>
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<td>19</td>
<td>D19Mit61 (9)-D19Mit63 (24)</td>
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<td>46</td>
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* The number of the chromosome (Chr) that contains a B10.O20-derived genomic segment.
* The SSLP markers that border the B10.O20-derived genomic segment and their position (in cM).

### Table 2 Linkage data of the (NTX-10 × O20)F2 and (O20 × NTX-20)F2 crosses

<table>
<thead>
<tr>
<th>Assay (LPS in ng/ml; duration in hours)</th>
<th>NTX-10 × O20 (or O20 × NTX-20)</th>
<th>P value (P) and corrected P (CP)</th>
<th>Sex</th>
<th>Marif1 D4Mit12 or D4Mit54</th>
<th>Marif2 D8Mit3 or D8Mit125</th>
<th>Marif3 D19Mit60</th>
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<td>TNF-α (1; 6h)</td>
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<td></td>
<td>CP</td>
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<tr>
<td></td>
<td>NTX-20</td>
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<td>0.012</td>
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<tr>
<td></td>
<td>NTX-10 and NTX-20</td>
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<td>TNF-α (100; 6 h)</td>
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<td>NTX-10 and NTX-20</td>
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* This column lists the type of inflammation-related assay. The amount of LPS used to stimulate BMMF (1 ng/ml or 100 ng/ml), and the duration of stimulation before the assay was performed (in hours). Assays: tumor necrosis factor (TNF-α), secretion of TNF-α, IL-12, secretion of IL-12p40; iNOS, expression of iNOS; IL-12 (1 versus 100). Differential effects of low and high concentrations of lipopolysaccharide (LPS) on secretion of IL-12p40.
* Suggestive linkage represented by nominal P values < 0.05 (P) and genome-wide significant linkage represented by corrected P values < 0.05 (CP), according to the rules of Lander and Kruglyak (28).
* Effects of sex of the mice on inflammation-related assays. Data represent P values.
* Effects of Marif1 on inflammation-related assays. P values listed for the NTX-10 × O20F2 cross were obtained using the marker D4Mit12, whereas P values listed for the O20 × NTX-20F2 cross and for the combination of both crosses were obtained using the marker D4Mit54.
* Effects of Marif2 on inflammation-related assays. P values listed for the NTX-10 × O20F2 cross were obtained using the marker D8Mit13, whereas P values listed for the O20 × NTX-20F2 cross and for the combination of both crosses were obtained using the marker D8Mit125.
* Effects of Marif3 on inflammation-related assays. All P values were obtained using the marker D19Mit60.

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DISCUSSION

Dissecting the genetics of susceptibility to cancer is hampered by its multigenic nature and complex phenotype. Previously, we demonstrated that susceptibility to cancer is influenced by many TSGs that are frequently involved in genetic interactions, which illustrated the extensive complexity of genetic predisposition to cancer and emphasized the necessity to reduce this to identify new TSGs (7–9). Here, we applied a novel strategy in which reduction of genetic complexity was combined with reduction of phenotypic complexity. Genetic complexity was reduced by making use of highly related inbred strains of mice, strains that differed from each other in only 10% of their genome. Phenotypic complexity was reduced by selecting one of the biological processes known to contribute to tumorigenesis for analysis in vitro, instead of studying the complex multicellular and multistep development of cancer in vivo. The intimate relation between cancer and inflammation combined with the fact that macrophages are capable to mediate both tumorigenesis and inflammatory responses lead to the prediction that a subset of TSGs affect predisposition to cancer through their influence on macrophage characteristics. Using segregating crosses between strains that were known to differ in several lung tumor susceptibility loci, we report here the mapping of three macrophage-associated risk inflammatory factors Marif1, Marif2, and Marif3. These studies highlight several important features of complex multigenic traits. First, only 10% of the genome was analyzed for linkage (Table 1), yet three Marif loci were mapped, demonstrating once more that multigenic traits are truly influenced by many genes. Second, each Marif locus was linked to multiple inflammation-related assays (Table 2), supporting the notion that Marif genes function within molecular networks. As a consequence, each locus is expected to affect many assays, suggesting that even the restricted panel of inflammation-related assays that was applied here to determine macrophage characteristics might actually suffice to detect the majority of Marif loci. And third, the observation that allelic
variation of Marif loci can have opposite effects on one assay, for instance illustrated by the effects of Marif1 and Marif2 on secretion of TNF-α (Fig. 3, D and E), confirmed that phenotypic variation between parental strains will often represent an underestimation of the total amount of variation that can be detected by linkage analysis.

The Marif1 and Marif2 loci were each shown to modulate secretion of TNF-α, secretion of IL-12p40, and expression of iNOS (Table 2). However, the opposite direction of their effects on secretion of TNF-α (Fig. 3, D and E) was contrasted by the similar direction of their effects on expression of iNOS (Fig. 3F), whereas they required different concentrations of LPS to affect secretion of IL-12p40 (Fig. 3, G and H). Moreover, allelic variation in Marif3 resulted in opposite effects on secretion of IL-12p40 compared with Marif2 (Fig. 3H) and in opposite effects on the ratio of low versus high concentrations of LPS on secretion of IL-12p40 compared with Marif1 (Fig. 3I). Together, these data indicate that each locus influenced the molecular network of inflammation via different pathways, suggesting that their identification will help to unravel the mechanisms that determine macrophage versatility. Besides the inflammation-related assays that were linked to Marif loci, several assays from the panel revealed no linkage at all. For instance, no linkage was found with expression of COX-2 despite the observation that this assay was influenced by genetic variation between the parental strains O20 and B10.020. Most likely, genes that modify COX-2 expression are present within the 90% of the genome that was not covered by the crosses used in this study. Alternatively, loci whose main effects are masked by genetic interactions could not be detected by our analysis because the number of mice involved in the (O20 × NTX-10)F2 and (NTX-20 × O20)F2 crosses was not large enough to allow a systematic search for epistasis.

Absence of linkage with secretion of NO was an unexpected finding considering the fact that both Marif1 and Marif2 were linked to expression of iNOS, the enzyme responsible for the strong induction of NO production during an inflammatory response. However, also the O20 and B10.020 parental strains exhibited this discrepancy because they differed in their expression of iNOS while producing similar amounts of NO (data not shown), indicating that iNOS is not the only factor that regulates the amount of NO production and secretion.

Tumorigenesis is a process in which both the acquisition of genetic alterations within cancer cells as well as the interaction between cancer cells and their microenvironment play a prominent role. Therefore, one group of candidate genes for TSGs include oncogenes and tumor suppressor genes, exemplified by identification of Kras2 as a susceptibility gene for lung cancer and Ptpri as a susceptibility gene for colon cancer (32, 33). Another group of candidate genes for TSGs comprises genes that influence the (tumor) microenvironment, exemplified by identification of the group IIA secretory phospholipase A2 gene Pla2g2a as a susceptibility gene for ApcMin-induced intestinal neoplasia (34, 35). Our strategy specifically aimed to map TSGs that affect susceptibility to cancer through their influence on the (tumor) microenvironment and resulted in the mapping of Marif loci that influence the characteristics of macrophages, one of the nontransformed nonepithelial cell types that is frequently associated with tumors of epithelial origin. Although these data do not prove that Marif genes are TSGs, they each do affect parameters that have been implicated to affect tumorigenesis: mice deficient for TNF-α become more resistant to skin carcinogenesis (36), whereas mice that lack iNOS are more susceptible to intestinal cancer and less susceptible to lung cancer (37, 38). The adaptive immune response is strongly influenced by IL-12, and absence of IL-12p40 from antigen-presenting cells results in poor cytotoxic T lymphocyte responses against tumors (39). Because of the multitude of effects that macrophages have on the tumor microenvironment, Marif loci that affect tumor susceptibility through their function in macrophages are likely to influence both quantitative and qualitative features of various types of cancer (23). In addition, Marif loci are also expected to affect susceptibility to other diseases in whose etiology macrophages play an important role, like inflammatory diseases and autoimmune diseases. In compliance with these expectations, Marif1 and Marif2 colocalized with quantitative trait loci (QTLs) for susceptibility to lung cancer, Sluc6/Sluc21 and Sluc20, respectively (8, 9); with QTLs that determine lung tumor shape, Ltsd4 and Ltsd3, respectively (40); with susceptibility to intestinal cancer, Momi1/sluc1 and Scc8, respectively (11, 41, 42); with susceptibility to colitis, Cdc9 and Cdc94, respectively (43, 44); and with susceptibility to autoimmune diseases like diabetes and experimental allergic encephalomyelitis. Id11 and Eae14/Eae24, respectively (Refs. 45–47; Fig. 4). Moreover, Marif2 and Marif3 colocalized with QTLs for susceptibility to arthritis, Pgi4 and Pgia23, respectively (48, 49). This clustering of QTLs for susceptibility to various macrophage-associated diseases suggests that they could be influenced by one gene that affects macrophage characteristics, represented by the Marif locus. At present, the genomic segments to which the Marif loci have been mapped are relatively large, and each region contains several hundreds of genes, including many putative candidate genes that are involved in inflammation. It is interesting to note that Marif1 is tightly linked to the secretory phospholipase A2 gene Pla2g2a, which besides its effects on inflammation and predisposition to intestinal cancer was recently also shown to affect susceptibility to atherosclerosis when transgenically expressed by macrophages (50).

Additional investigations are required to identify the genes that affect macrophage characteristics in vitro followed by examination of their effects in vivo on quantitative and qualitative aspects of tumor development. These studies will extend our knowledge about the molecular mechanisms via which hematopoietic cells in general and macrophages in particular influence tumorigenesis, thereby increasing
our understanding of genetic predisposition to cancer and contributing to early diagnosis and prevention of cancer. Moreover, the possibility to manipulate non-tumorigenic hematopoietic cells ex vivo to modulate tumorigenesis in vivo may provide additional tools to improve cancer treatment further.

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Genetic Analysis of Macrophage Characteristics as a Tool to Identify Tumor Susceptibility Genes: Mapping of Three Macrophage-Associated Risk Inflammatory Factors, Marif1, Marif2, and Marif3

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