Identification of the *Mhc* Region as an Asthma Susceptibility Locus in Recombinant Congenic Mice

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Mouse models of allergic asthma are characterized by airway hyper-reactivity (AHR), Th2-driven eosinophilic airway inflammation, high allergen-specific IgE (anti-OVA IgE) levels in serum, and airway remodeling. Because asthma susceptibility has a strong genetic component, we aimed to identify new asthma susceptibility genes in the mouse by analyzing the asthma phenotypes of the *Leishmania major* resistant (*lmr*) recombinant congenic (RC) strains. The *lmr* RC strains are derived from C57BL/6 and BALB/c intercrosses and carry congenic loci on chromosome 17 (*lmr1*) and 9 (*lmr2*) in both backgrounds. Whereas the *lmr2* locus on chromosome 9 contributes to a small background-specific effect on anti-OVA IgE and AHR, the *lmr1* locus on chromosome 17 mediates a strong effect on Th2-driven eosinophilic airway inflammation and background-specific effects on anti-OVA IgE and AHR. The *lmr1* locus contains almost 600 polymorphic genes. To narrow down this number of candidate genes, we performed genome-wide transcriptional profiling on lung tissue from C.*lmr1* RC mice and BALB/c control mice. We identified a small number of differentially expressed genes located within the congenic fragment, including a number of *Mhc* genes, polymorphic between BALB/c and C57BL/6. The analysis of asthma phenotypes in the C.B10-H2b RC strain, carrying the C57BL/6 haplotype of the *Mhc* locus in a BALB/c genetic background, reveals a strikingly similar asthma phenotype compared with C.*lmr1*, indicating that the differentially expressed genes located within the C.B10-H2b congenic fragment are the most likely candidate genes to contribute to the reduced asthma phenotypes associated with the C57BL/6 allele of *lmr1*.

Keywords: allergic asthma; quantitative trait locus; recombinant congenic mice; chromosome 17; mouse model

Allergic asthma is a disease that is mainly characterized by high allergen-specific IgE levels in serum, airway hyperreactivity to nonspecific bronchospasmogenic stimuli, Th2-driven pulmonary inflammation, and airway remodeling. The genetic susceptibility to develop asthma has been shown to be complex and is

CLINICAL RELEVANCE

We identify the mouse *Mhc* region as a quantitative trait locus for airway eosinophilia and hyperresponsiveness in a mouse model for experimental asthma. These findings will allow further identification of asthma genes and the dissection of their contribution to the disease process.

influenced by many genes. At the end of 2007, polymorphisms in 173 genes had been associated with asthma and related phenotypes (1). However, only 33 of these associations were replicated in five or more populations (2). This discrepancy can partially be explained by differences in phenotype and phenotyping method. In addition, gene-gene and gene-environment interactions have been shown to play major roles in the expression of a genetic susceptibility to develop asthma (3). For example, Battle and coworkers recently showed an ethnicity-specific epistatic interaction between IL-4R α and IL-13 among African Americans (4). Based on functional data, which are available for 63 genes out of the 173, asthma susceptibility genes can be subdivided into four functional categories: (1) innate immunity and immune modulation, (2) Th2 cell differentiation, (3) epithelial integrity, and (4) lung function and airway remodeling (2).

Inbred mouse strains also differ in their susceptibility to experimentally induced asthma (5). This genetic variation between inbred strains can be exploited to identify asthma susceptibility genes. One approach to this end is the characterization of recombinant congenic strains, which consist of a series of inbred strains containing fractions of a donor genome on an otherwise homogeneous genetic background (6). Recombinant congenic (RC) strains have been used as a powerful tool for the mapping of genes involved in complex traits (7, 8). Using a model of experimental allergic asthma in the CcS/Dem series of RC strains (8), we have previously established the applicability of RC strains for the mapping of asthma-related quantitative trait loci (QTL) (9). Different intensities and types of responses were observed throughout the series of strains, indicating independent segregation of multiple asthma susceptibility loci, in theory allowing the identification of the genes involved in the different asthma manifestations (7, 9).

To identify novel asthma susceptibility loci in the mouse, we used the *lmr* series of RC strains (10). In parallel to the CcS/Dem strains, the *lmr* strains were developed to map genes involved in *Leishmania major* infection. Two *L. major* resistance (*lmr*) loci have been studied into detail, the *lmr1* locus on chromosome 17 and the *lmr2* locus on chromosome 9 (10). Both loci were previously identified as *L. major* susceptibility loci in

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a classical F2 cross with C57Bl/6 and BALB/c (10, 11). An impaired wound healing process was shown to play a major role in the increased susceptibility of BALB/c compared with C57Bl/6, resulting in significantly smaller lesions, less inflammation, and faster collagen deposition in C.lmr1/2, the *lmr1/2* RC strain in the BALB/c genetic background (12, 13). In addition, differences in activities of Th-cell subsets have been shown to contribute to *Leishmania* susceptibility (14). Because an impaired wound-healing response at the level of the airway epithelium and a strong allergen-specific Th2 response are thought to contribute to the pathogenesis of allergic asthma (15), we hypothesized that the *lmr* loci might also contribute to asthma susceptibility. Here, we evaluate the asthma manifestations induced by an ovalbumin (OVA)-driven allergic asthma model in *lmr1/2* RC strains on BALB/c and C57Bl/6 backgrounds and identify *lmr1* as an asthma susceptibility locus in the mouse. We used transcriptional profiling on lung tissue to narrow down the *lmr1* locus to polymorphic genes that are differentially expressed between the two genetic backgrounds. We identified a small number of candidate genes, including a number of *Mhc* genes. Finally, we used the C.B10-*H2b* RC strain to directly test the effect of the haplotype of the *Mhc* locus on the asthma phenotype in the BALB/c genetic background.

MATERIALS AND METHODS

A full description of the methods can be found in the online supplement.

Mice

Animal housing and experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (DEC) at the University of Groningen. BALB/cAnBradley, C57Bl/6J, B6.lmr1/2, B6.lmr1, B6.lmr2, and C.lmr1/2 mice were obtained from the Walter and Eliza Hall Institute in Melbourne, Australia. BALB/cAnBradley, C.lmr1, and C.lmr2 mice were bred and phenotyped at the animal facilities of the University of Groningen, The Netherlands. C.B10-*H2b*/LilMcDj (BALB.B) and BALB/cJ control mice were obtained from The Jackson Laboratory (Bar Harbor, Maine).

Experimental Asthma Protocol

Mice were phenotyped at age 5 to 9 weeks, with six to nine mice per group, as described previously (16). In brief, mice were intraperitoneally sensitized with 10 μ g OVA in 2.25 mg alum adjuvant (Pierce, Rockford, IL). On Days 21, 24, and 27, the animals were challenged for 20 minutes with 10 mg OVA/ml in saline. Serum was isolated, and airway responsiveness to methacholine were measured at Days 18 and 28 in conscious, unrestrained mice as described previously (17).

Analysis of Airway Inflammation and OVA-Specific Immunoglobulin Levels in Serum

Lungs were lavaged through a tracheal cannula with five 1-ml aliquots of saline. Differential BAL cell counts were obtained from cytospin preparations stained with Diff-Quick (Merz and Dade A.G., Duding, Switzerland). Levels of OVA-specific IgE, IgG1, and IgG2a in serum were measured as described previously (16).

Isolation of RNA, Amplification, Hybridization, and Analysis of Microarray Data

Individual lung lobes were isolated, quickly frozen in liquid nitrogen, and stored at -70°C . Total RNA was isolated using TriReagent (MRC, Cincinnati, OH). The 3'IVT-Express labeling kit (Affymetrix, Santa Clara, CA) was used to synthesize biotin-labeled cRNA using 100 ng of RNA as input. Hybridization of cRNA to Affymetrix HT Mouse Genome 430 PM Plate Arrays was performed using 4.5 μ g of cRNA as input material.

Average signal intensities for each probe set within arrays were calculated by and exported from Affymetrix's Expression Console

(version 1.1) software using the RMA method. Pairwise comparisons were used to statistically resolve gene expression differences between groups using the R/MAANOVA analysis package (18). Differentially expressed genes were detected by using F_s (18, 19). Statistical significance levels of the pairwise comparisons were calculated by permutation analysis (1,000 permutations) and adjusted for multiple testing using the false discovery rate, q-value, method (20). Differentially expressed genes were declared at a false discovery rate q-value threshold of 0.05.

Mouse Single Nucleotide Polymorphism and other Polymorphism Data

Polymorphic genes were identified by the presence of single nucleotide polymorphisms (SNPs) or indels in exon sequences between BALB/cJ and C57Bl/6J (reference strain) mice as obtained from the Sanger website (<http://www.sanger.ac.uk/cgi-bin/modelorgs/mousegenomes/snps.pl>).

Polymorphisms between C57Bl/6J, C57Bl/10J, and BALB/c mice in the Mhc locus were derived from the mouse phenome database at The Jackson Laboratory (<http://phenome.jax.org/SNP/>) using the CGD imputed strain set.

Statistical Analysis

Comparison of immunoglobulin and cytokine ELISA data obtained before and after OVA challenge were performed using signed rank tests. The different strains were compared with their background strains using Mann Whitney U-test. Statistical analyses were performed using SAS for Windows version 9.0 (SAS-Institute, Cary, NC).

RESULTS

Asthma Phenotypes of *lmr1/2* Double Congenic Mice

We tested the hypothesis that the previously identified *lmr1* and *lmr2* loci contain genes that contribute to the genetic predisposition to develop asthma. The *lmr1/2* RC mice carry both loci: the BALB/c alleles on C57Bl/6 background in case of B6.lmr1/2 or the C57Bl/6 alleles on BALB/c background in the C.lmr1/2 strain. To test our hypothesis, we assessed the asthma phenotypes of the RC strains C.lmr1/2 and B6.lmr1/2 in comparison to that of the respective parental strains, BALB/c and C57Bl/6. First, we characterized OVA-specific immunoglobulin responses in the RC strains and the parental strains. In all four strains, we found that OVA-specific IgE (anti-OVA IgE) serum levels were significantly increased after OVA inhalation challenge (Figure 1A). B6.lmr1/2 mice displayed a strongly elevated anti-OVA IgE level in comparison to C57Bl/6 after sensitization and after the OVA challenges. In contrast, the C.lmr1/2 strain and the parental BALB/c strain had similar anti-OVA IgE levels after sensitization. Nevertheless, the C.lmr1/2 strain displayed a reduced anti-OVA IgE response after OVA challenges as compared with BALB/c mice (Figure 1A).

For OVA-specific IgG1 (anti-OVA IgG1), B.lmr1/2 showed a significant increase in anti-OVA IgG1 serum levels after OVA inhalation challenges, in contrast to the parental C57Bl/6 strain. The opposite was observed in the BALB/c background, where C.lmr1/2 showed significantly lower anti-OVA IgG1 levels after challenge compared with the parental strain BALB/c (Figure 1B). In case of OVA-specific IgG2a (anti-OVA IgG2a), the C57Bl/6 parental strain showed higher levels after sensitization than the RC B6.lmr1/2 strain, whereas the BALB/c parental strain had lower anti-OVA IgG2a levels in serum compared with C.lmr1/2. After challenge, the opposite was observed, with higher anti-OVA IgG2a serum levels in B6.lmr1/2 and lower in C.lmr1/2 compared with their respective background strains (Figure 1C). Taken together, these data indicate that the *lmr1/2* loci affect the OVA-specific serum responses of IgE, IgG1, and

Figure 1. Asthma phenotypes of *lmr1/2* recombinant congenic (RC) strains in both backgrounds. Serum levels of ovalbumin (OVA)-specific IgE (A), IgG1 (B), and IgG2a (C) before (solid bars) and after (open bars) OVA inhalation challenges. (D) Airway hyper-responsiveness to increasing doses of methacholine. Total numbers of eosinophils in bronchoalveolar lavage (BAL) (E) and levels of Th2 cytokines in BAL (F). #Significantly different compared with before challenge with $\alpha \leq 0.05$. ##Significantly different compared with before challenge with $\alpha \leq 0.01$. *Significantly different from background strain with $\alpha \leq 0.05$. **Significantly different from background strain with $\alpha \leq 0.01$.

IgG2a in a consistent fashion, with the C57BL/6 alleles being associated with an increased anti-OVA IgG2a response and the BALB/c alleles being associated with increased anti-OVA IgE and anti-OVA IgG1 responses.

The mouse model of allergic asthma is also characterized by the induction of AHR to methacholine inhalation. Before challenge, all four strains showed comparable airway reactivity to methacholine (data not shown). Only the BALB/c parental strain displayed an increased AHR after OVA challenges, whereas no AHR was observed in the parental C57BL/6 and the RC B6.*lmr1/2* strains or in the RC C.*lmr1/2* strain (Figure 1D).

We also tested the levels of eosinophils and Th2 cytokines in BAL fluid after OVA challenges. B6.*lmr1/2* showed significantly higher levels of IL-4 and IL-5 and higher eosinophil counts in the BAL compared with C57BL/6, whereas the C.*lmr1/2* showed significantly lower levels of IL-4, IL-5, and IL-13 and lower eosinophil counts in the BAL than the parental BALB/c strain (Figures 1E and 1F). No IFN- γ was detectable in BAL of challenged mice. Taken together, these data indicate that the *lmr1/2* loci present in these RC mice contribute to susceptibility for allergic asthma phenotypes in the OVA-driven mouse model.

Asthma Phenotypes of *lmr1* and *lmr2* Single Congenic Mice

To identify which congenic locus, *lmr2* on chromosome 9 or *lmr1* on chromosome 17, is responsible for the altered asthma phenotypes, we measured the critical asthma parameters in the single congenic strains carrying the *lmr1* or the *lmr2* congenic region. Here, the *lmr1* mice carry the chromosome 17 locus, either the BALB/c allele on C57BL/6 background in case of B6.*lmr1* or the C57BL/6 allele on BALB/c background in the C.*lmr1*. The B6.*lmr2* and C.*lmr2* mice carry the congenic chromosome 9 locus in the C57BL/6 and BALB/c background, respectively.

Both RC strains and background strains showed similar levels of anti-OVA IgE before challenge. B6.*lmr1* showed significantly more anti-OVA IgE after challenge compared with C57BL/6, whereas no differences were observed between C57BL/6 and B6.*lmr2* (Figure 2A). In contrast, the C.*lmr2* mice showed significantly lower anti-OVA IgE compared with BALB/c

mice, whereas no significant differences were found between BALB/c and C.*lmr1* (Figure 2B).

Eosinophil counts and IL-4 and IL-5 levels in BAL were significantly increased in B6.*lmr1* mice compared with the background strain C57BL/6, whereas no difference could be observed between C57BL/6 and B6.*lmr2* (Figures 2C and 2E). The BALB/c background displayed consistent results: the C.*lmr1* strain shows significantly lower eosinophil counts as well as IL-4 and IL-5 levels than the BALB/c background strains, whereas the C.*lmr2* strain displayed comparable eosinophil counts and Th2 cytokine levels in BAL (Figures 2D and 2F). None of the tested strains showed measurable amounts of IFN- γ in BAL (data not shown).

We observed no induction of AHR after OVA challenges in the RC mice on a C57BL/6 background (Figure 3A). On the BALB/c background, C.*lmr1* and C.*lmr2* showed a significantly reduced PenH compared with BALB/c at methacholine dosages of 12.5 mg/ml and higher (Figure 3B). These data indicate that the C57BL/6 alleles of the *lmr1* and *lmr2* loci seem to contribute to the AHR resistant phenotype in the BALB/c background observed in C.*lmr1/2*. Because we have phenotyped the airway responses in the RC strains using whole-body plethysmography, which is an indirect and disputed measure of airway responsiveness (21), we aimed to confirm our data by using an invasive method to directly assess airway resistance in the C.*lmr1* RC mice and their respective background strain, the BALB/c. We performed invasive measurement of airway resistance the day after the last OVA challenge. The airway resistance of the C.*lmr1* RC strain was strongly suppressed as compared with the parental BALB/c strain, underscoring the suppressive effect of the C57BL/6 allele on the AHR in the BALB/c background (Figure 4).

Fine-Mapping the *lmr1* Locus by Analysis of Differentially Expressed Genes

Our data identify the *lmr1* locus as a QTL for AHR, Th2 responses, and eosinophilic airway inflammation in the C.*lmr1* RC strain. Next, we aimed to identify the susceptibility genes contained within the *lmr1* locus. We first genotyped a large panel of polymorphic SNPs and microsatellite markers in the C.*lmr1* RC strain. This analysis revealed that the D17Mit185,

Figure 2. Airway inflammation in *lmr1* and *lmr2* RC strains in both backgrounds. OVA-specific IgE in single congenic mice on C57BL/6 background (A) and BALB/c background (B) before (solid bars) and after (open bars) OVA inhalation challenges. Total numbers of eosinophils in BAL of single congenic mice on C57BL/6 background (C) and BALB/c background (D). Th2 cytokine levels of BAL fluid from single congenic mice on the C57BL/6 background (E) and BALB/c background (F). #Significantly different compared with before challenge with $\alpha \leq 0.05$. ##Significantly different compared with before challenge with $\alpha \leq 0.01$. *Significantly different from background strain with $\alpha \leq 0.05$. **Significantly different from background strain with $\alpha \leq 0.01$.

located at 68.8 Mbp, was the first marker with a BALB/c genotype on chromosome 17 in C.lmr1 (Figure 5). Hence, *lmr1* spans the larger part of chromosome 17 and contains close to 600 polymorphic genes, precluding the efficient identification of the relevant susceptibility gene(s). Several approaches can be taken to further fine-map this locus, such as F2 crosses to generate recombinant haplotypes of the *lmr1* locus that can then be phenotyped in the asthma protocol, an approach successfully used to identify the TIM family of AHR and Th2 inflammation susceptibility genes (22). However, this approach requires the breeding and genotyping of very large numbers of mice, and we preferred to take a more straightforward approach to susceptibility gene identification. To this end, we performed whole-genome transcriptional profiling on lung material derived

from C.lmr1 RC mice and BALB/c control mice to identify genes located within the *lmr1* locus that were differentially expressed between the two strains. We hypothesized that combining the gene expression data with the known polymorphisms between C57BL/6 and BALB/c on the *lmr1* locus would lead toward identification of the relevant susceptibility genes present in this rather large QTL. A similar approach has previously been applied by Karp and colleagues to identify complement factor 5 as an asthma susceptibility gene located within a known QTL on mouse chromosome 2 (23).

We performed OVA inhalation challenges as well as a PBS inhalation control treatment in C.lmr1 and BALB/c control mice. Individual lobes of the lungs of these mice were used for RNA isolation or histological evaluation to confirm the asthma phe-

Figure 3. Airway hyperresponsiveness in *lmr1* and *lmr2* RC strains in both backgrounds. AHR to increasing doses of methacholine of single congenic mice on the C57BL/6 background (A) and BALB/c background (B). *Significantly different from background strain with $\alpha \leq 0.05$.

Figure 4. Airway resistance of C.lmr1 and BALB/c mice in response to methacholine. The peak airway resistance (cm H₂O/s/ml) of C.lmr1 RC mice and the BALB/c parental strain in response to increasing doses of intravenously administered methacholine are shown.

notypes in the mice. In BALB/c and C.lmr1 backgrounds, OVA challenges readily induced a pronounced eosinophilic airway inflammation that was absent from PBS-challenged mice, confirming the induction of an asthma phenotype (Figure 6).

To identify genes differentially expressed between C.lmr1 and BALB/c, we compared the transcriptional profiles of the PBS-challenged mice from both genotypes and those of OVA-challenged mice from both genotypes. This analysis revealed the presence of a total of 101 genes that were differentially expressed at a genome-wide level of significance between C.lmr1 and BALB/c in PBS- or OVA-challenged mice. As expected, the majority of these genes ($n = 60$; Table 1) were located within the congenic region, although we also identified a number ($n = 41$) of additional differentially expressed genes located outside of the *lmr1* locus. More than half of the differentially expressed genes located within the *lmr1* locus were identified in both experimental conditions, whereas nearly all of the genes located outside of the *lmr1* locus were found to be differentially expressed only in PBS- or in OVA-challenged mice.

The identity of the differentially expressed genes located within the *lmr1* locus revealed that a large proportion of these (20 out of 60) belonged to the *Mhc* genes. The *lmr1* locus does encompass the entire *Mhc* region, and the two background strains are polymorphic for the *Mhc* genes. We identified differential expression of class I (11), class II (7), and class III (2) *Mhc* genes (Table 1). The observation of differential *Mhc* gene usage between C.lmr1 and BALB/c by our approach is a confirmation of the power of this analysis to identify differentially expressed genes in the locus. Moreover, because several *Mhc* genes have been identified as candidate genes in human asthma (24, 25), it is of interest to further dissect the contribu-

tion of these polymorphisms to the asthma phenotype in our screen.

The Asthma Phenotype of the C.B10-H2b Strain

To test whether the observed differential expression of the polymorphic *Mhc* genes could indeed contribute to the phenotypic differences between C.lmr1 and BALB/c in the mouse asthma model, we used the existing C.B10-H2b (BALB.B) strain of mice, which carries the C57Bl/10 haplotype of the *Mhc* locus on a BALB/c genetic background. Because C57Bl/6 and C57Bl/10 share the *b* haplotype of the *Mhc* locus, the BALB.B strain allows the analysis of the effects of the presence of this *Mhc* haplotype in a BALB/c genetic background on the asthma phenotype. Indeed, BALB.B mice showed a strongly reduced induction of AHR to methacholine compared with BALB/c (Figure 7A). Moreover, the number of eosinophils and the level of Th2 cytokines in BAL fluid were significantly lower in BALB.B mice compared with BALB/c control mice (Figures 7B and 7C). In contrast, the level of anti-OVA IgE was similar between the two strains before and after the OVA inhalation challenges (Figure 7D). We also measured TNF- α levels in BAL because the LTA/TNF- α /TAP-1 cluster, a known asthma susceptibility locus in human asthma (26–29), is part of the *Mhc* region and is contained within the BALB.B congenic fragment. TNF- α levels in BAL did not differ between the two strains (Figure 7E). Taken together, these data indicate that the Th2-driven eosinophilic airway inflammation and AHR are strongly reduced in BALB.B mice compared with BALB/c mice. These data largely mirror the phenotype of the C.lmr1 strain in the OVA-induced asthma model.

DISCUSSION

Asthma susceptibility has a large genetic component, and only a small portion of the heritability of asthma can be explained by the currently known asthma susceptibility loci. In this study, we used a novel approach toward the identification of asthma susceptibility genes using the mouse model of allergic asthma to screen the phenotypes of the *lmr* RC mice derived from C57Bl/6 and BALB/c intercrosses. Because BALB/c and C57Bl/6 mice have been shown to display divergent phenotypes in the mouse asthma model (30–33) and because *lmr1* and *lmr2* are QTLs for *L. major* resistance, which is affected by Th2-biased T-cell responses as well as an impaired wound healing response (14, 15), we hypothesized that the *lmr* loci could contain genes that contribute to the susceptibility for asthma development. Our experiments clearly show an influence of the *lmr* loci on all major asthma phenotypes in this mouse model: anti-OVA IgE responses, Th2-driven eosinophilic airway inflammation, and AHR. In dissecting the contribution of the individual *lmr* loci, we demonstrate a major role for the *lmr1*

Figure 5. Overview of the *lmr1* locus on mouse chromosome 17. Schematic representation of mouse chromosome 17. Indicated are the relative positions of the *lmr1* locus, as well as the known mouse quantitative trait loci for airway reactivity and the congenic fragment present in the C.B10-H2b RC strain.

Figure 6. Histologic evaluation of eosinophilic airway inflammation. Eosinophilic inflammation in the lungs of OVA-challenged mice was determined by staining for cyanide-resistant endogenous peroxidase activity with diaminobenzidine as in Ref. 50. (A) Representative example. (B) Digital images were obtained, and positive staining was quantified by morphometric analysis and expressed as volume percentages for both C.lmr1 and BALB/c groups ($n = 4-6$ per group). OVA = OVA-sensitized and OVA-challenged mice; PBS = OVA-sensitized and PBS-challenged mice.

locus on the Th2-cell activity and eosinophilic airway inflammation on both backgrounds, with an additional effect on IgE in the C57Bl/6 background and on AHR in the BALB/c background. The *lmr2* locus only shows a small BALB/c background-specific effect on IgE and AHR. We observe a reduced airway eosinophilia in the C57Bl/6 genetic background, which is in contrast to reported data in literature (32, 33). However, the relative induction of eosinophilic airway inflammation in BALB/c and C57Bl/6 is variable in the literature and seems to be dependent on the challenge protocol and the time of analysis (31, 34).

The *lmr1* locus was selected for further candidate gene identification in the BALB/c genetic background because it exerted dominant effects on eosinophilic airway inflammation and AHR. The congenic locus in the C.lmr1 strain spans 68.8 MBp on mouse chromosome 17 from the centromeric region onward (Figure 5). When comparing the C57Bl/6 and BALB/c genomic sequences, we identified almost 600 polymorphic genes located within the congenic fragment. To further fine-map the *lmr1* locus toward the identification of (candidate) susceptibility genes, we used genome-wide transcriptional profiling on lung tissue of C.lmr1 RC mice and BALB/c control mice. Annotation of the genes that are differentially expressed between the two genetic backgrounds to their chromosomal location allows for the identification of the differentially expressed genes located within the *lmr1* locus, which we hypothesized to include the susceptibility genes relevant to the C.lmr1 phenotype. A similar approach has been applied to identify candidate susceptibility genes for various mouse models of human disorders, including asthma (23), pertussis (35), type I diabetes (36), adiposity (37), tuberculosis (38), and anxiety (39). We found a total of 101 genes that were differentially expressed between the two strains with genome-wide significance, 60 of which were located within the *lmr1* locus (Table 1). The majority of these were found to be differentially expressed independently of experimental condition (38 out of 60), indicating a potential *cis*-regulation of their expression by polymorphisms between C57Bl/6 and BALB/c.

Our approach to fine-map the *lmr1* asthma susceptibility genes by genome-wide transcriptional profiling has two major drawbacks. First, polymorphisms between the two background strains could be present in the probes used to detect the levels of gene expression in the lung tissue RNA and might give false-positive outliers due to differences in cRNA annealing efficiency to the array. Such false-positive hits showing differential

expression would be expected to occur only within the *lmr1* locus and independently of experimental condition. In addition, expression arrays are potentially sensitive to false-negative results because the sensitivity of the array to detect differences in gene expression is dependent on the average expression level of the gene. As such, differences in relatively poorly expressed genes are much harder to identify as a significant difference between the two strains. Therefore, we might have missed some differentially expressed genes due to the limitations of our approach. Nevertheless, we expect these effects to be relatively minor given the strong supportive evidence of the asthma phenotype of the C.B10-*H2b* (BALB.B) RC strain, which carries the C57Bl/6 allele of the *Mhc* region in a BALB/c genetic background. A large number of these genes map to the *Mhc* locus, located on mouse chromosome 17 approximately between *col11a2* at 34.1 Mbp and *Zfp57* at 37.1 Mbp (40), which is entirely contained within the *lmr1* locus. Our analysis of the BALB.B asthma phenotype indicates that the presence of the C57Bl/6 haplotype of the *Mhc* locus in an otherwise homogeneous BALB/c background is sufficient to induce the reduced AHR and Th2 responses and airway eosinophilia in the mouse asthma model but does not affect the anti-OVA IgE response in serum (Figure 7). These results are strikingly similar to our results obtained with the C.lmr1 mice (see Figures 2 and 3). These data provide experimental proof for the notion that the C57Bl/6 allele of the *Mhc* locus, harboring the most strongly differentially expressed genes, renders the asthma manifestations to be at the level of the C.lmr1 RC strain. The BALB.B congenic region has been mapped to be located between coordinates 26.9 and 37.3 Mbp on mouse chromosome 17 (41). This includes not only the *Mhc* region (34.1–37.1 Mbp) but also some additional 7 Mbp located on the centromeric side of the *Mhc* locus. This latter region includes several polymorphic genes differentially expressed between the two strains, including *Glyoxalase1*, *Rab44*, and *Pim1* (Table 1). Consequently, although the BALB.B data allow us to narrow down the *lmr1* candidate susceptibility genes to a relatively small number, these still include *Mhc* genes and a small number of unrelated genes.

The *lmr1* locus and the BALB.B congenic locus specifically affect the Th2-driven eosinophilic airway inflammation and AHR in the BALB/c genetic background but have no effect on anti-OVA IgE. This discrepancy between the individual asthma phenotypes has been observed in genetic screens by us and by others (9, 42, 43). As a result, QTL or susceptibility genes also differ between the individual asthma phenotypes. Consequently, we designate the *lmr1* locus as a QTL for eosinophilic airway inflammation and allergen-induced AHR.

The *lmr1* locus overlaps with several previously identified QTLs for naive airway reactivity on mouse chromosome 17 (Figure 5). Two loci were identified in backcrosses between C57Bl/6 and A/J ([C57Bl/6 $\times$ A/J] $\times$ C57Bl/6) (44) and C3H and A/J ([C3H $\times$ A/J] $\times$ C3H) (45), both of which overlap with the BALB.B congenic region. In addition, a single study using RC strains derived from C57Bl/6 and A/J intercrosses reported the presence of two separate QTL for naive AHR on mouse chromosome 17, both overlapping with the *lmr1* locus but not with the BALB.B congenic region (46). Finally, a haplotype association mapping survey involving 36 inbred strains failed to find any association with chromosome 17 haplotypes to naive AHR (47). These data seem to indicate that there might be multiple QTL for naive AHR present within the *lmr1* locus and that the expression of these is dependent on the genetic background, which suggests the presence of genetic interactions.

TABLE 1. *LMR1* GENES DIFFERENTIALLY EXPRESSED BETWEEN C.LMR1 AND BALB/C MICE

Location	Gene Symbol	RFC PBS	Q-value PBS	RFC OVA	Q-value OVA	MHC class	C.B10-H2b
6124229-6124844	<i>Tulp4</i>	2.65	0.00	2.72	0.00		
6306455-6646628	<i>Tmem181</i>	-1.38	3.91×10^{-02}	-1.60	1.40×10^{-03}		
6808201-6813686	<i>Dynlt1</i>	-1.50	6.95×10^{-03}	ND	NS		
8138451-8172470	<i>Rshl2a / Rshl2b</i>	1.41	3.62×10^{-02}	1.47	1.20×10^{-02}		
8535581-8536945	<i>Prr18</i>	ND	NS	-1.66	4.02×10^{-02}		
15115571-15126399	<i>Gm3435</i>	-2.18	0.00	-2.26	0.00		
15842061-15908869	<i>Chd1</i>	ND	NS	1.55	4.88×10^{-02}		
15941318-15943205	<i>Rgmb</i>	1.77	3.33×10^{-02}	ND	NS		
21672505-21699826	<i>Zfp52</i>	1.92	4.04×10^{-03}	2.14	7.80×10^{-04}		
21705495-21710871	<i>BC049807</i>	ND	NS	1.56	2.51×10^{-02}		
21711279-21800327	<i>3110052M02Rik</i>	-1.86	1.39×10^{-02}	ND	NS		
22067092-22069398	<i>3110048L19Rik</i>	3.17	1.72×10^{-03}	3.59	4.35×10^{-04}		
22335458-22335831	<i>Gm4944</i>	2.87	2.05×10^{-04}	3.36	4.58×10^{-05}		
22474961-22498355	<i>6330416L07Rik</i>	1.65	3.58×10^{-02}	1.65	3.58×10^{-02}		
22498492-22514247	<i>Zfp758</i>	1.57	1.62×10^{-02}	1.71	2.88×10^{-03}		
22998692-23000920	<i>A630033E08Rik</i>	12.4	0.00	14.7	0.00		
22998692-23000920	<i>A630033E08Rik</i>	-2.06	6.96×10^{-03}	-1.76	2.98×10^{-02}		
23319882-23320523	<i>Zfp40</i>	2.10	3.43×10^{-02}	2.22	1.64×10^{-02}		
23956463-23961708	<i>Srrm2</i>	ND	NS	-1.41	3.58×10^{-02}		
24312377-24342505	<i>Tbc1d24</i>	2.51	2.93×10^{-03}	2.51	2.93×10^{-03}		
24489014-24547317	<i>Abca3†</i>	-1.55	1.41×10^{-02}	-1.46	2.70×10^{-02}		
24593511-24593945	<i>BC026600</i>	-2.21	2.16×10^{-02}	-2.68	2.93×10^{-03}		
24830134-24833090	<i>Gfer</i>	ND	NS	1.99	2.35×10^{-03}		
25025111-25025904	<i>Eme2</i>	3.56	0.00	3.04	0.00		
26218155-26227246	<i>Decr2</i>	-2.26	1.62×10^{-03}	-2.00	3.97×10^{-03}		
27700525-27702672	<i>AV039444</i>	ND	NS	1.57	3.91×10^{-03}		
29285239-29285925	<i>Rab44</i>	ND	NS	2.58	1.91×10^{-02}		*
29632605-29633056	<i>Pim1</i>	2.79	1.14×10^{-02}	3.44	1.61×10^{-03}		*
30729805-30749567	<i>Glo1‡</i>	-2.31	5.69×10^{-05}	-2.08	4.16×10^{-04}		*
31038821-31073465	<i>Glp1r</i>	4.54	1.63×10^{-03}	2.91	1.95×10^{-02}		*
31060152-31060536	<i>BM233846</i>	-13.95	0.00	-17.12	0.00		*
31075243-31077736	<i>2900001G08Rik</i>	4.42	7.92×10^{-04}	4.54	4.35×10^{-04}		*
31981201-31992692	<i>Sik1</i>	1.76	1.90×10^{-02}	2.15	1.25×10^{-03}		*
33823813-33824438	<i>March2</i>	1.94	1.02×10^{-03}	2.24	1.31×10^{-04}		*
34103775-34103873	<i>H2afb3</i>	12.0	0.00	10.6	0.00		*
34133107-34137144	<i>H2-D1†</i>	-189	0.00	-299	0.00	II	*
34272577-34276028	<i>H2-DMa</i>	ND	NS	2.08	1.87×10^{-02}	II	*
34375849-34390850	<i>H2-Ob</i>	2.33	2.00×10^{-02}	3.93	3.17×10^{-04}	II	*
34400205-34406290	<i>H2-Ab1</i>	ND	NS	2.08	1.13×10^{-02}	II	*
34419696-34419924	<i>H2-Aa†</i>	14.7	0.00	36.4	0.00	II	*
34476410-34478355	<i>H2-Eb2</i>	ND	NS	2.31	1.11×10^{-02}	II	*
34478947-34481590	<i>H2-Ea†</i>	-468	0.00	-622	0.00	II	*
35162926-35164861	<i>Ng23</i>	-1.98	3.62×10^{-03}	-2.03	1.81×10^{-03}	III	*
35175350-35181956	<i>Msh5</i>	-2.50	2.97×10^{-03}	-2.73	9.87×10^{-04}	III	*
35400049-35404127	<i>H2-D1‡</i>	ND	NS	1.62	2.70×10^{-02}	I	*
35400451-35404439	<i>H2-D1/L</i>	-112	0.00	-129	0.00	I	*
35457497-35483615	<i>BE136769</i>	7.52	0.00	8.78	0.00	I	*
35495945-36871453	<i>H2-K1</i>	-15.7	0.00	-20.57	0.00	I	*
35561795-35566998	<i>H2-Q1</i>	ND	NS	2.41	2.83×10^{-02}	I	*
35576133-35611504	<i>H2-Q7</i>	ND	NS	3.60	6.53×10^{-04}	I	*
35607046-35611498	<i>H2-Q10</i>	1.96	2.89×10^{-02}	2.52	1.64×10^{-03}	I	*
36093585-36106708	<i>Abcf1</i>	2.04	2.72×10^{-03}	2.13	1.25×10^{-03}	I	*
36136110-36136696	<i>H2-T24</i>	3.38	1.08×10^{-04}	2.42	2.88×10^{-03}	I	*
36136110-36136696	<i>H2-T24</i>	-1.98	1.41×10^{-02}	ND	NS	I	*
36167766-36221156	<i>H2-Q5</i>	-2.04	4.65×10^{-03}	-1.97	5.59×10^{-03}	I	*
36245850-36248615	<i>H2-T23</i>	ND	NS	1.63	2.98×10^{-02}	I	*
43594927-43596506	<i>Gpr116</i>	ND	NS	-2.08	3.84×10^{-02}		
44215820-44223061	<i>Enpp5</i>	2.17	1.65×10^{-02}	2.21	1.11×10^{-02}		
48559273-48560072	<i>Apobec2</i>	2.10	1.45×10^{-02}	2.03	1.49×10^{-02}		
51896624-51897581	<i>C230085N15Rik</i>	ND	NS	1.81	4.52×10^{-02}		
55836391-55851926	<i>Pot1b</i>	1.97	2.38×10^{-02}	2.29	3.91×10^{-03}		
57219488-57227053	<i>Tubb4</i>	2.04	3.76×10^{-03}	ND	NS		
65787125-65788038	<i>Mrps10</i>	2.65	0.00	ND	NS		

Gene ID and alignments on chromosome 17 of *Imr1* genes that are differentially expressed between C.Imr1 and BALB/c in PBS or OVA-challenged groups or both.

Definition of abbreviations: MHC class = annotation of genes contained within the *Mhc* region to MHC class I, II, or III genes (according to Ref. 40); ND = not determined; NS = not significant; Q-value OVA = Q-value (false discovery rate) in OVA comparison; Q-value PBS = Q-value (false discovery rate) in PBS comparison; RFC PBS = relative fold change C.Imr1 over BALB/c in PBS condition; RFC OVA = relative fold change C.Imr1 over BALB/c in OVA condition.

* C.B10-H2b genes annotated to the C.B10-H2b congenic fragment.

† Transcript identified by two independent probes.

‡ Transcript identified by three independent probes.

Figure 7. Asthma phenotype of the C.B10-H2b RC strain. (A) Airway hyperresponsiveness to increasing doses of methacholine of C.B10-H2b (BALB.B) and BALB/c mice. (B) Total numbers of eosinophils in BAL from BALB/c and BALB.B mice (as indicated). (C) Th2 cytokine levels in BAL fluid from BALB/c and BALB.B mice (as indicated). (D) OVA-specific IgE before (solid bars) and after (open bars) OVA inhalation challenges of BALB/c and BALB.B mice. (E) Levels of TNF- α in BAL fluid from BALB/c and BALB.B mice as indicated.

Only a single QTL for allergen-induced AHR on chromosome 17 has been identified (Figure 5) and overlaps with *lmr1* and the BALB.B congenic region (48). In contrast, a screen for allergen-induced AHR in a (C3H $\times$ A/J) $\times$ A/J backcross did not reveal any significant linkage to chromosome 17 (43).

The human *Mhc* genes are located in the syntenic region on chromosome 6. Genetic association with asthma (questionnaire-based asthma, AHR) have been found for HLA-G (24) and MICB (25), both nonclassical MHC class I genes (also known as MHC class Ib genes). Orthologous relationships of class I genes cannot be found between different mammalian orders and therefore are not found between primates and rodents (40). As a consequence, HLA-G and MICB lack a direct mouse homolog. Nevertheless, we identified a number of differentially expressed MHC class Ib genes in our genetic screen, including *H2-Q1*, *H2-Q7*, *H2-Q10*, *H2-T24*, and *H2-T23* (Table 1). These data underscore that MHC class Ib group genes might also contribute to susceptibility to AHR in the mouse model.

In addition to the MHC class Ib genes, the *LTA*, *TNF- α* , and *TAP-1* genes, which are part of the *MhcIII* region, have been shown to be associated with questionnaire-based asthma or AHR in different populations (27–29). We did not observe a differential expression of any of the homologous genes in the *LTA*/*TNF- α* /*TAP-1* cluster, nor did we observe differences in *TNF- α* levels in BAL obtained after OVA inhalation challenges of sensitized BALB.B and BALB/c mice (Figure 7E). Therefore, we think that the *LTA*/*TNF- α* /*TAP-1* cluster genes are not likely candidates for the *lmr1* QTL.

Although the identification of the *Mhc* genes as the most strongly differentially expressed genes within the *lmr1* locus is highly suggestive of a causal role for the MHC haplotype on the suppression of airway eosinophilia and AHR, we cannot exclude the possibility that other polymorphic genes present within the BALB.B region might be the relevant susceptibility genes.

For instance, the serine/threonine kinase PIM1 has been shown to be highly expressed in bronchial epithelial cells (49). Moreover, the expression level of *PIM1* was found to be inversely associated with lymph node metastases in non-small cell lung cancer, indicating that Pim1 might be regulating cellular processes, such as epithelial-to-mesenchymal transition, thereby affecting invasive growth and metastasis of the tumor cells. The *lmr* loci have been found to contribute to *Leishmania* resistance by affecting genes regulating the wound healing response (12, 13) rather than through an effect on immune function. Because wound healing and invasive growth of epithelial tumor cells depend on processes involving epithelial remodeling, *Pim1* might be a potential candidate gene for the suppressive effect of the *lmr1* locus on AHR. Further research is needed to identify the relevant susceptibility genes contained within the BALB.B congenic fragment present within the *lmr1* locus.

In conclusion, we have identified *lmr1* as a susceptibility locus for eosinophilic airway inflammation and AHR in the mouse using recombinant congenic strains derived from BALB/c and C57BL/6 mice. Transcriptional profiling of lung tissue from C.*lmr1* RC and BALB/c mice has identified a limited number of candidate genes located within the *lmr1* locus that are differentially expressed between the two strains, with a number of *Mhc* genes being strongly differentially expressed. Analysis of the asthma phenotype of the BALB.B RC strain indicates that the presence of the C57BL/6 haplotype at the *Mhc* locus in the BALB/c genetic background is sufficient for the reduced asthma phenotype observed in the C.*lmr1* strain, indicating that the small number of polymorphic, differentially expressed genes mapping to the BALB.B congenic fragment likely contain the relevant *lmr1* asthma susceptibility gene.

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