Genetic Control of Severe Egg-Induced Immunopathology and IL-17 Production in Murine Schistosomiasis

Patrick M. Smith, Mara G. Shainheit, Lindsey E. Bazzone, Laura I. Rutitzky, Alexander Poltorak and Miguel J. Stadecker

*J Immunol* 2009; 183:3317-3323; Prepublished online 12 August 2009;
doi: 10.4049/jimmunol.0901504
http://www.jimmunol.org/content/183/5/3317

---

**References**
This article cites 60 articles, 22 of which you can access for free at:
http://www.jimmunol.org/content/183/5/3317.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

---

*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2009 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Genetic Control of Severe Egg-Induced Immunopathology and IL-17 Production in Murine Schistosomiasis

Patrick M. Smith,† Mara G. Shainheit,† Lindsey E. Bazzone,* Laura I. Rutitzky,* Alexander Poltorak,*† and Miguel J. Stadecker2*†

Infection with the trematode parasite Schistosoma mansoni results in a distinct heterogeneity of disease severity, both in humans and in an experimental mouse model. Severe disease is characterized by pronounced hepatic egg-induced granulomatous inflammation in a proinflammatory cytokine environment, whereas mild disease corresponds with reduced hepatic inflammation in a Th2 skewed cytokine environment. This marked heterogeneity indicates that genetic differences play a significant role in disease development, yet little is known about the genetic basis of dissimilar immunopathology. To investigate the role of genetic susceptibility in murine schistosomiasis, quantitative trait loci analysis was performed on F2 progeny derived from SJL/J and C57BL/6 mice, which develop severe and mild pathology, respectively. In this study, we show that severe liver pathology in F2 mice 7 wk after infection significantly correlated with an increase in the production of the proinflammatory cytokines IL-17, IFN-γ, and TNF-α by schistosome egg Ag-stimulated mesenteric lymph node cells. Quantitative trait loci analysis identified several genetic intervals controlling immunopathology as well as IL-17 and IFN-γ production. Egg granuloma size exhibited significant linkage to two loci, D4Mit203 and D17Mit82, both of which were inherited in a BL/6 dominant manner. Furthermore, a significant reduction of hepatic granulomatous inflammation and IL-17 production in interval-specific congenic mice demonstrated that the two identified genetic loci have a decisive effect on the development of immunopathology in murine schistosomiasis. The Journal of Immunology, 2009, 183: 3317–3323.

In nature, complex traits that affect disease are the rule rather than the exception. Most common human diseases are complex traits whose phenotypes vary widely in a population and are influenced by multiple genes as well as by gene–gene and gene–environment interactions. Much of the genetic variation that leads to disease susceptibility is governed by loci that have quantitative effects on the disease phenotype. Identification and analysis of these loci is an important measure in understanding the molecular basis of these complex traits, and quantitative trait loci (QTL) analysis can be used to localize chromosomal regions harboring genetic variants that control susceptibility or resistance. However, it has been difficult to identify QTL for complex traits in humans because most have low penetrance, requiring large numbers of subjects to identify, and phenotypes can be affected by environmental factors. Many human and mouse diseases share similar phenotypes and, because there is synergy between their genomes (1), it is possible to use rodent models to identify chromosomal regions linked to disease susceptibility in humans (2). This has made mouse models of disease particularly important for biomedical research and many QTL found in mice have corresponded with or led to the identification of QTL in homologous regions of the human genome (3–6). QTL are critically important to understanding the molecular mechanisms that underlie disease and are responsible for most of the genetic variability in disease susceptibility.

Schistosomes are parasitic trematode helminths that infect and cause widespread disease in vertebrates and are responsible for over 200 million human infections that cause ~200,000 deaths per year (7). Infection with one such species, Schistosoma mansoni, results in a granulomatous inflammatory and fibrosing reaction in the liver and intestine against tissue-trapped parasite eggs. Granuloma formation is the result of a host adaptive immune response mediated by CD4 T cells specific for schistosome egg Ags (SEA) (8–10). Following infection with S. mansoni, most humans develop mild intestinal schistosomiasis, however, S–10% develop the severe hepatosplenic form of the disease, which can lead to portal hypertension, splenomegaly, and death (7). Despite similar environmental conditions and parasite loads, this marked variability also exists among mouse strains. For example, CBA/J and SJL/J mice develop pronounced hepatic granulomatous inflammation, whereas C57BL/6 (BL/6) mice develop significantly smaller lesions with less inflammation (11,12). Severe immunopathology corresponds with an increase of the proinflammatory cytokines IFN-γ and IL-17, which are the products of the Th1 and Th17 subsets of CD4 T cells, respectively. Of these, the Th17 subset is a more likely marker of severe disease (13–16). Conversely, following infection, BL/6 mice produce low levels of IFN-γ and IL-17 and high amounts of IL-4 and IL-10, which results in a milder form of pathology (12,14).

The outcome of schistosome infection depends on complex innate and adaptive immune responses, including regulatory mechanisms such as those afforded by alternatively activated macrophages (17) and regulatory T cells (18). Based on the polygenic nature of the immune response and the striking heterogeneity...
in egg-induced immunopathology, it has long been thought that disease severity is, at least in part, under genetic control, prompting the search for host genetic factors that account for these differences. Human studies performed in areas where schistosomiasis is endemic have suggested that several HLA alleles are associated either negatively or positively with disease intensity (19–22) and identified two genetic loci, designated Sm1 and Sm2, that control susceptibility to infection and severe hepatic fibrosis, respectively (23, 24). An earlier study from our laboratory identified genetic susceptibility to infection and severe hepatic fibrosis, respectively in a population from Sri Lanka with schistosomiasis. In this study, a comprehensive QTL analysis of an F2 progeny of the BALB/c × DBA/2 cross was performed, using congenic animals to identify loci that influence disease phenotypes. The results of this study suggest that several genetic loci, including one on chromosome 17, influence disease severity and that these loci may be involved in the pathogenesis of schistosomiasis.

**Materials and Methods**

**Mice and infection**

C57BL/6J (BL/6), SJL/J, and B6SJLJ1/J (F1) mice, 5–6 wk old, were purchased from The Jackson Laboratory. One hundred fifty male and female BL/6 × SJL/J F1 mice were bred in-house by F1 brother × sister mating. The interval-specific congenic mice, as described below, were also bred in our colony at Tufts University School of Medicine (Boston, MA). All mice were maintained at the Animal Facility at Tufts University School of Medicine in accordance with the American Association for the Assessment and Accreditation of Laboratory Animal Care guidelines. All F1, F2, and congenic mice, together with age-matched SJL/J and BL/6 parental controls, were infected between 6 and 8 wk of age by i.p. injection with 85 cercariae of *S. mansoni* (Puerto Rico strain). Cercariae were obtained from infected *Biomphalaria glabrata* snails, provided by Dr. Fred Lewis (Biomedical Research Institute, Rockville, MD) through National Institutes of Health/National Institute of Allergy and Infectious Diseases Contract N01-AI-55270. All mice were studied after 7 wk of infection.

**Histopathology assessment by morphometric analysis**

Liver samples from all mice were fixed in 10% buffered formalin and processed for routine histopathological analysis: 5-μm sections were stained with H&E. The extent of hepatic granulomatous inflammation around schistosome eggs was measured by computer-assisted morphometric analysis using Image-Pro Plus software (Media Cybernetics). The lesions were assessed blindly by an observer unaware of the experimental parameters. To accurately reflect the true magnitude of the granulomatous inflammation, only those granulomas with a single visible central egg were counted. A minimum of 20 granulomas were counted per section with more than one section counted per liver. Mean granuloma size was measured in square micrometers ± SEM.

**Cell preparations and cytokine determinations**

Mesenteric lymph nodes (MLN) were removed aseptically from mice 7 wk after infection. Single-cell suspensions were prepared from each individual mouse by teasing the lymph nodes in RPMI 1640 medium supplemented with 10% FCS (Atlanta Biologicals), 4 mM L-glutamine, 80 U/ml penicillin, and 100 μg/ml streptomycin, 1 mM sodium pyruvate, 10 mM HEPES and 1× nonessential amino acids (all obtained from BioWhittaker), as well as 0.1% 2-ME. Erythrocytes were lysed by treatment with Tris-ammonium chloride buffer (pH 7.2; Sigma-Aldrich) for 15 min on ice. Cells were washed and live cells that excluded trypan blue were counted and re-suspended at 5 × 10⁸ cells/ml in complete RPMI 1640 medium. Bulk MLN cells were cultured with or without 15 μg/ml of SEA, prepared as previously described (25). After 48 h, the culture supernatants were removed, filtered, and stored at −36°C until analyzed by ELISA. For measurement of IL-17, IFN-γ, and TNF-α standard cytokines, Abs and protocols were obtained from R&D Systems, whereas for IL-4, IL-5, and IL-10 standard cytokines, Abs and protocols were obtained from BD Pharmingen.

**Genetic mapping**

DNA was extracted for genotyping from tail biopsies using DNeasy tissue kit (Qiagen) or DirectPCR (tail) (Viagen Biotech) according to manufacturers’ instructions. Single-strand conformation polymorphic loci were selected from available polymorphic microsatellite markers (mouse genome informatics or www.cird.jhmi.edu) and primers were obtained from Integrated DNA Technologies. Microsatellite marker positions were obtained from The Jackson Laboratory Mouse Genome Database (www.informatics.jax.org). A panel of 100 primer sets that readily distinguish BL/6 and SJL alleles was used for genotyping the F2 mice. Together, the markers spanned all 19 autosomes and the X chromosome with an average intermarker distance of <20 Mb (10–40 cM). Standard PCR was performed at an annealing temperature of 55°C for each primer pair. Amplified products were electrophoresed in 3–4% agarose gels and visualized by ethidium bromide staining with UV transillumination. Data were analyzed by Mapmaker QTL and R/QTL using the J/QTL interface (The Jackson Laboratory). Mapmaker is a QTL mapping program that tests whether markers show evidence of linkage to the tested phenotypes. J/QTL is a graphical user interface for R/QTL, which is a powerful statistical software program used for mapping QTL in experimental crosses. Mapmaker and R/QTL programs were also used to determine epistatic interactions between loci. Both programs returned similar results. The linkage was considered highly significant if the logarithm of the odds (LOD) favoring linkage score exceeded 3.3 according to the system of Lander and Kruglyak (26) in the context of a genome search using an intercross study.

**Congenic strain development**

B6SJLF1/J mice were backcrossed to SJL/J mice to produce N2 animals. Selection of breeders at this stage and subsequent backcrosses was based on genotyping at three markers within and flanking the QTL on chromosome 4 (between D4Mit308 at 123.8 Mb and D4Mit256 at 154 Mb, including peak marker D4Mit203) and chromosome 17 (between D17Mit133 at 25 Mb and D17Mit180 at 57 Mb, including peak marker D17Mit82). Mice were backcrossed for eight additional generations with selection for these markers followed by intercrossing of N2 animals to isolate donor homozygotes. The resultant mice constituted the finished congenic strains, SJL.B6-D4Mit203, SJL.B6-D17Mit82, and double congenic SJLB6-D4Mit203/D17Mit82, representing mice that were homozygous for a donor (BL/6) segment from the QTL identified by peak marker D4Mit203, D17Mit82, or both, respectively.

**Statistical analysis**

One-way ANOVA was used to determine statistically significant differences between groups of mice. A p value <0.05 was considered significant and statistical analysis was performed with GraphPad Prism (GraphPad Software).

**Results**

**SJL mice develop significantly larger liver granulomas than BL/6 mice and in an F2 progeny, enhanced pathology correlates with an increase in proinflammatory cytokines**

Seven weeks after infection with 85 cercariae, SJL mice developed large liver granulomas (mean size, 221 ± 19.8 μm² × 10³), while in BL/6 mice they were significantly smaller (mean size, 137 ± 13.5 μm² × 10³) (Fig. 1A). In this study, SJL mice

![FIGURE 1.](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
were used because they develop the most severe liver pathology of any strain previously examined (11). F1 mice developed small granulomas, close to those in BL/6 mice, indicating that low pathology was dominant. To further elucidate the genetic basis of granuloma formation, F2 mice were studied in a similar manner. The F2 progeny displayed a wide range in granuloma size with some reaching that displayed by either the SJL or BL/6 parental strains (Fig. 1A).

Because proinflammatory cytokines, particularly IL-17, have been shown to be of pathogenic significance in schistosomiasis (14, 15), we analyzed SEA-induced cytokine production by MLN cells from SJL, BL/6, F1 and F2 mice. SJL mice produced significantly higher levels of IL-17 than BL/6 mice, whereas F1 mice displayed an intermediate phenotype, although levels were closer to those of BL/6 (Fig. 1B). Cytokine analysis in F2 mice again revealed a wide variation in IL-17 production and a significant correlation of the proinflammatory cytokines IL-17, IFN-γ, and TNF-α with granuloma size (Fig. 2, A–C). In contrast, the Th2 cytokines IL-4 and IL-5, as well as the anti-inflammatory cytokine IL-10, exhibited no significant correlation to granuloma size (Fig. 2, D–F). Because granuloma size and cytokine values did not follow a normal distribution, data were reanalyzed after log transformation and yielded similar results (data not shown).

QTL analysis of immunopathology identified significant linkage to granuloma formation and IL-17 production

Linkage analysis of granuloma size identified a statistically significant linkage to two loci, D4Mit203 on chromosome 4 (peak position 129.2 Mb, LOD 3.4, p < 0.001, 95% confidence interval CI 115–142 Mb) and D17Mit82 on chromosome 17 (peak position 33.9 Mb, LOD 6.0, p < 0.0001, 95% CI 21–40 Mb). An additional locus was also identified on chromosome 9 with a suggestive linkage to granuloma size (peak position 95.2 Mb, LOD 2.5, p < 0.05, 95% CI 63–105 Mb) (Fig. 3). F2 mice that were homozygous for the SJL (SS) and BL/6 (BB) alleles at D4Mit203 or D17Mit82 developed larger and smaller granulomas, respectively, while heterozygous F2 mice (SB) developed small granulomas equal to those homozygous for BL/6, indicating that both loci were inherited in a BL/6 dominant manner (Fig. 4). We further analyzed the extent of control these loci exert on immunopathology by their effect on cytokine production and found that IL-17 and IFN-γ responses were also inherited in a BL/6 dominant manner. There was no significant difference in TNF-α, IL-4, IL-5, and IL-10 production between homozygous and heterozygous mice at either locus, suggesting that they had no effect on the production of these cytokines (Fig. 4).

Further analysis of F2 mice disclosed that those that were homozygous for the SJL allele at both D4Mit203 and D17Mit82 had significantly increased pathology compared with those that were homozygous for BL/6 at each locus. Interestingly, while F2 mice that expressed the BL/6 allele only at D4Mit203 developed small granulomas close to mice that expressed BL/6 alleles at both D4Mit203 and D17Mit82, mice that expressed the BL/6 allele only at D17Mit82 still developed larger granulomas similar to mice that expressed SJL alleles at both loci, suggesting that D4Mit203 plays a more significant role in pathology (Fig. 5).

QTL analysis of cytokine production from in vitro SEA-stimulated MLN cells identified one major and several minor loci linked to increased IL-17 production. D4Mit203 (peak position 24 Mb, LOD 3.4, 95% CI 10–33 Mb) was significantly linked to increased IL-17 production. D4Mit203 and D17Mit82 were significantly linked to granuloma size in S. mansoni-infected F2 mice. The markers D4Mit203 (LOD of 3.4) and D17Mit82 (LOD of 6.0) were significantly linked to granuloma size. LOD scores are represented on the y-axis and chromosomal positions on the x-axis. Vertical dashed lines represent chromosomal breaks. Results are based on analysis performed by J/QTL (5000 permutations).
linked to IL-17 production \((p < 0.01)\) and analysis of F2 mice showed that this locus was inherited in a BL/6 dominant manner (Table I). Suggestive linkage to IL-17 production was also detected at the previously described loci \(D4Mit203\) (LOD 1.8) and \(D17Mit82\) (LOD 2.2), as well as at \(D3Mit191\) (peak position 101.5 Mb, LOD 2.3, 95% CI 75–120 Mb) and \(D9Mit269\) (peak position 87.8 Mb, LOD 2.1, 95% CI 61–104 Mb) (Table I). Statistical analysis using J/QTL and mapmaker programs revealed an epistatic interaction between \(D4Mit211\) and two markers, \(D3Mit191\) \((p < 0.01)\) and \(D9Mit269\) \((p < 0.001)\), but not between \(D9Mit269\) and \(D3Mit191\). Increased IFN-\(\gamma\) production showed minor linkage to two loci, \(D5Mit233\) (peak position 53 Mb, LOD 2.2, 95% CI 35–71 Mb) and \(D10Mit148\) (peak position 44.7 Mb, LOD 2.5, 95% CI 21–59 Mb). The locus containing \(D5Mit233\) was inherited in a BL/6 dominant manner, whereas the locus containing \(D10Mit148\) was inherited in an intermediate fashion (Table I). No linkage was detected for TNF-\(\alpha\), IL-4, IL-5, or IL-10 production (not shown).

\(SJL.B6-D4Mit203\) and \(SJL.B6-D17Mit82\) congenic mice exhibit significantly reduced granuloma size and IL-17 production in comparison with parental high pathology SJL mice

We were most interested in the loci identified for granuloma size, \(D4Mit203\) and \(D17Mit82\), because they had an effect on overall disease development as opposed to production of a specific cytokine. To assess the effect of these loci directly on immunopathology, we produced interval-specific congenic mice that were homozygous for the BL/6 allele at these loci on an otherwise SJL background. For this purpose, F1 mice were backcrossed to the SJL strain and mice were selected at each generation for heterozygosity at \(D4Mit203\) and \(D17Mit82\). After nine generations, these mice were intercrossed and selected for homozygosity at each locus, resulting in the production of the interval-specific congenic mice \(SJL.B6-D4Mit203\) and \(SJL.B6-D17Mit82\).

Following schistosome infection, both \(SJL.B6-D4Mit203\) and \(SJL.B6-D17Mit82\) mice exhibited a significant reduction in granuloma size when compared with SJL mice and littermate controls (Fig. 6A), with an even greater reduction observed in mice that were congenic for both loci, suggesting that there may be an additive effect. Both congenic mouse strains also had significantly lower production of IL-17 by SEA-stimulated MLN cells (Fig. 6B), again with a greater effect seen in the double congenic mice; however, neither locus significantly affected IFN-\(\gamma\) production (Fig. 6C). There was no effect on IL-4, IL-5, or IL-10 production in either congenic mouse (not shown).

Discussion

The availability of a well-established mouse model of human infectious disease with strain-specific differences in immunopathology provided a unique opportunity to investigate the genetic basis of a complex disease phenotype. It is clear that the course of infection with \(S. mansoni\) is profoundly affected by the host genome and identification of genes responsible for the variability in immunopathology among infected individuals will provide a greater understanding of its mechanisms of pathogenesis. The results of our analysis of a progeny from a cross between high (SJL) and low (BL/6) pathology mouse strains showed that F1 mice developed low pathology, indicating that the BL/6 background is dominant. Further analysis of an F2 progeny revealed a significant correlation between granuloma formation and SEA-induced levels of proinflammatory, but not anti-inflammatory cytokines. Our current observations confirm that severe immunopathology occurs in a proinflammatory cytokine environment dominated by both Th17 and Th1 cell subsets, suggesting that IL-17 and IFN-\(\gamma\) are an important
part of a larger and more complex series of biological events that result in severe disease. Predicted early death of mice with high pathology at ~8 wk postinfection precluded examination of liver fibrosis, which is not fully developed at this time.

The magnitude of hepatic granulomatous inflammation was significantly linked to two loci, D4Mit203 and D17Mit82, which have also demonstrat ed linkage to other immune-mediated conditions. The locus on chromosome 4 contains overlapping QTL for the autoimmune diseases experimental autoimmune encephalomyelitis (EAE), insulin dependent diabetes, systemic lupus erythematosus (SLE), and psoriasis (27–30) and has several candidate genes implicated in immune signaling and regulation, including the signal- ing proteins LCK and map3k6, as well as the α-chain of the IL-22 receptor. This locus corresponds to a region on human chromosome 1, 45 Mb apart from the D15S252 marker that demonstrated linkage with infection levels of S. mansoni in humans (31). The locus identified on chromosome 17 has also been linked with EAE and SLE, and contains overlapping QTL for response to trypanosomiasis (Tir1), and resistance to malaria (Char3) and leishmaniasis (Lmr1) (32–34) (Table II). The most immunologically relevant candidate genes in this interval are TNF-α, which is prominent in acute inflammation, and Notch3, which has been shown to affect autoimmune disease (35). This locus corresponds to a region on human chromosome 5, 20 Mb from the DSS410 marker, which was used to identify the Sm1 locus that controls intensity of infection in humans (24). Another likely linkage is to MHC genes located near this region, as they have been suggested to play a role in human and murine schistosome infection (19–22, 36).

We produced congenic mice specific for the intervals containing D4Mit203 and D17Mit82. These intervals were chosen for closer analysis because they affect granuloma formation, which is a better measure of overall disease severity than individual cytokines that are a smaller part of a more complex response. Both congenic mice displayed significantly reduced granuloma size in comparison with SJL parental controls, with a greater reduction seen in SJL.B6-D4Mit203 congenic mice compared with SJL.B6-D17Mit82. These mice also produced significantly lower amounts of IL-17, but not IFN-γ, thus demonstrating that the BL/6 allele at each of these loci regulates pathology and IL-17 production. Interestingly, the locus containing D4Mit203 has a greater effect than D17Mit82 in the congenic mice. This is in agreement with the F2 progeny in that mice that contain the BL/6 allele at each of these loci regulates pathology and IL-17 production. Importantly, the smaller genetic differences in congenic mice reduces the intervals underlying these QTL, making it easier for future identification of candidate genes.

Genetic analysis of cytokine production identified one locus, D4Mit211, with a highly significant linkage to IL-17 production and 4 additional loci, D3Mit191, D4Mit203, D9Mit269, and D17Mit82, which were merely suggestive (26). Because D4Mit203 and D17Mit82 were also linked to granuloma size and it is known that IL-17 plays a significant role in the development of large granulomas, it is not surprising that there is suggestive linkage to these loci. Statistical analysis of the loci affecting cytokine production revealed epistatic interactions between D4Mit211 and D3Mit191 and between D4Mit211 and D9Mit269. D3Mit191 and D9Mit269 contain candidate genes encoding for the nuclear orphan receptor family members Rorc and Rora, respectively (Table II), which are critical for Th17 cell differentiation. The Rorc gene, which encodes for the transcription factor RORγ, was first identified as the transcription factor responsible for Th17 cell differentiation from naive cells (37), similar to Tbet for Th1 cells and Gata3 for Th2 cells (38). Since then it has been shown that in the absence of RORγ, a second transcription factor, RORα, was able to induce naive CD4 T cells to differentiate to Th17 cells (39). D4Mit211 was the only locus that was significantly linked to IL-17 production in our analysis and based on the candidate genes contained in the loci for D3Mit191 and D9Mit269, it is possible that this locus contains an unidentified transcription factor or other gene that is important for IL-17 production in vivo. One potential

### Table I. Loci controlling cytokine production in S. mansoni-infected F2 mice

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Locus Peak Marker</th>
<th>Genotype</th>
<th>LOD Score</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17 production</td>
<td>D4Mit211</td>
<td>SS</td>
<td>3.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D3Mit191</td>
<td>0.632 ± 0.187 (n = 31)</td>
<td>SB</td>
<td>2.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D9Mit269</td>
<td>0.726 ± 0.331 (n = 34)</td>
<td>BB</td>
<td>2.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IFN-γ production</td>
<td>D5Mit233</td>
<td>SS</td>
<td>2.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D10Mit148</td>
<td>1.600 ± 0.899 (n = 39)</td>
<td>SB</td>
<td>2.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BB</td>
<td>2.5</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* LOD scores were calculated by the statistical program R/QTL using the graphical interface J/QTL.

* p values represent statistical significance for mode of inheritance between SS and SB groups.

\[ a \] Cytokine levels (ng/ml) are based on segregation according to genotype of F2 mice at the specified peak marker.

\[ b \] Cytokine levels (ng/ml) are based on segregation according to genotype of F2 mice at the specified peak marker.

\[ c \] Cytokine levels (ng/ml) are based on segregation according to genotype of F2 mice at the specified peak marker.
candidate is Runx11. Originally identified in humans as part of a fusion translocation protein associated with acute myeloid leukemia, the mouse homologue Runx11 (40) is a member of the runt-related family of transcription factors, one of which, Runx1, plays a significant role in Th17 differentiation by interacting with RORγt (41). These loci also contain overlapping QTL from the autoimmune diseases EAE, insulin dependent diabetes, SLE, and collagen-induced arthritis, as well as infectious diseases such as Salmonella typhimurium susceptibility, Borrelia burgdorferi-associated arthritis, and resistance to malaria (32, 42–50). All these QTL may be of interest because the schistosome infection shares mechanistic features with many autoimmune and infectious diseases.

Of the two loci linked with IFN-γ levels, the locus on chromosome 5 proved to be the most interesting. This locus has been associated with control of IFN-γ levels in a previous independent QTL analysis of S. mansoni infection (13), as well as in a model of Leishmania major infection (51). This interval has also been implicated in Lyme arthritis (49) and susceptibility to Listeria infection (52). The fact that this locus has been identified in multiple mouse strains and in different disease models highly supports the notion that it contains a candidate gene likely to control IFN-γ production during an immune response in general and not just specific to a particular disease model. One potential candidate gene is TLR6, which interacts with TLR2 as part of the innate immune response against a number of pathogens and in response to autoimmune diseases (53). The majority of loci controlling cytokine response in our model did not significantly affect granuloma formation. One possible reason for this is that immunopathology in schistosomiasis is a very complex trait and cannot be determined by genes regulating a single cytokine.

In humans, variation in the severity of schistosomiasis has been linked to a variety of HLA haplotypes, as well as two major loci outside the MHC, designated Sm1 and Sm2 (19–24). Sm1 and Sm2 are linked with a number of cytokines and cytokine receptor genes that influence the outcome of immune responses against pathogens, including IFN-γR1 (Sm2). CD4 T cell clones from individuals homozygous for the susceptibility allele of Sm1 tended to be of the Th1 type, whereas those homozygous for the resistance allele tended to be of the Th2 type (54). Furthermore, field studies demonstrated that severe clinical presentations of acute schistosomiasis correlated with a proinflammatory cytokine environment similar to the murine model (55–60). It is likely that humans, like mice, carry different subsets of susceptibility and resistance genes that predispose them to more severe disease. In humans, however, it is difficult to control for a number of variables, such as concomitant infections, intensity of infection, and other environmental factors, which hampers the identification of candidate genes. Many of these problems do not exist in mouse models, making it possible to map these genes in mice and then identify their human homologues, which can then be tested for their role in infection.

In sum, our genetic analysis of murine schistosomiasis has identified two genomic intervals that directly and significantly control granuloma formation and several others that control IL-17 and IFN-γ production. These loci have also been linked to a variety of other infectious and autoimmune disorders with similar pathogenic mechanisms, suggesting that they may contain genes that play important roles in the host immune response.

Disclosures
The authors have no financial conflict of interest.

References


