Genetic Determinants of Autoimmune Disease and Coronary Vasculitis in the MRL-\textit{lpr/lpr} Mouse Model of Systemic Lupus Erythematosus

Lingjie Gu, Ari Weinreb, Xu-Ping Wang, Debra J. Zack, Jian-Hua Qiao, Richard Weisbart and Aldons J. Lusis

\textit{J Immunol} 1998; 161:6999-7006; http://www.jimmunol.org/content/161/12/6999

---

**References**
This article cites 49 articles, 16 of which you can access for free at: http://www.jimmunol.org/content/161/12/6999.full#ref-list-1

**Subscription**
Information about subscribing to \textit{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
Genetic Determinants of Autoimmune Disease and Coronary Vasculitis in the MRL-\textit{\textit{lpr}/lpr} Mouse Model of Systemic Lupus Erythematosus\textsuperscript{1}

Lingjie Gu,*† Ari Weinreb,* Xu-Ping Wang,* Debra J. Zack,‡ Jian-Hua Qiao,* Richard Weisbart,‡ and Aldons J. Lusis*\textsuperscript{2}

MRL-\textit{\textit{lpr}/lpr} (MRL/lpr) mice are a model of human autoimmune disease. They exhibit a number of characteristics of systemic lupus erythematosus, including anti-DNA Abs, anti-cardiolipin Abs, immune complex-mediated vasculitis, lymphadenopathy, and severe glomerulonephritis. Although the autoimmune disorder is mediated primarily by mutation of the \textit{Fas} gene (\textit{lpr}), which interferes with lymphocyte apoptosis, MRL/lpr mice also have other predisposing genetic factors. In an effort to identify these additional factors, we have applied quantitative trait locus (QTL) mapping using an intercross between MRL/lpr mice and the nonautoimmune inbred strain BALB/c\textit{J}. A complete linkage map spanning the entire genome was constructed for 189 intercross progeny, and genetic loci contributing to features of the autoimmunity were identified using statistical analytic procedures. As expected, the primary genetic determinant of autoimmune disease in this cross was the \textit{Fas} gene on mouse chromosome 19, exhibiting a lod score of 60. In addition, two novel loci, one on chromosome 2 (lod score, 4.3) and one on chromosome 11 (lod score, 3.1), were found to contribute to levels of anti-DNA Abs. Interestingly, the chromosome 19 and chromosome 11 QTLs, but not the chromosome 2 QTL, also exhibited associations with anti-cardiolipin Abs (lod scores, 38.4 and 2.6). We further examined the effects of these QTLs on the development of coronary vasculitis in the F2 mice. Our results indicate that the QTLs on chromosomes 11 and 19 also control the development of vasculitis, demonstrating common genetic determinants of autoantibody levels and vasculitis. \textit{The Journal of Immunology}, 1998, 161: 6999–7006.

\textit{S}ystemic lupus erythematosus (SLE)\textsuperscript{3} is an autoimmune disease characterized by the massive production of autoantibodies, the presence of circulating immune complexes, glomerulonephritis, and vasculitis (1, 2). Anti-dsDNA Abs correlate with lupus glomerulonephritis, and autoantibodies to cardiolipin, a membrane phospholipid, have been associated with thrombocytopenia, fetal loss, and vascular thrombosis (3). The heritable nature of human SLE has been well supported by the high concordance rate of 24% in monozygotic twins vs 2% in dizygotic twins (4), although the disease is clearly multifactorial, with a significant environmental contribution. As yet, the genetic factors involved are largely unknown. With the advantages of unlimited pedigree size, controlled environment, and capability of genetic manipulations, animal models are being used as tools to dissect the complex genetic contributions in several autoimmune disease models (5–7).

The MRL/lpr mouse, which carries the \textit{lpr} mutation, has been shown to have many features of generalized autoimmune disease, including extensive production of anti-DNA Abs, glomerulonephritis, vasculitis, and massive lymphadenopathy, closely resembling the immunopathologic features of human SLE. The \textit{lpr} mutation was identified as the defective \textit{Fas} apoptotic gene (8). This mutation leads to a breakdown of the central and/or peripheral tolerance, which results in the failure of proper clearance of CD4/CD8-negative T cells. The MRL/+ strain differs genetically by <1% from the MRL/lpr strain and develops a late-onset form of autoimmune disease. Furthermore, when the \textit{lpr} mutation was transferred to other mouse backgrounds, relatively mild autoimmune manifestations were observed (9). For example, relatively little autoimmune disease occurs when the \textit{lpr} mutation is transferred onto a strain C3H background. Taken together, these results point to the requirement of other background genes contributing to the autoimmune manifestations in MRL/lpr mice. Previously, in a backcross between strains MRL/lpr and CAST/Ei, genes other than the \textit{Fas} locus regulating glomerulonephritis and autoantibody production were identified on chromosomes (Chr) 7 and 12 (10), although the QTLs obtained for these loci were of marginal significance, with Chr\textit{lod} scores of 3.0 and 2.9, respectively. In an effort to understand the multigenic predisposition underlying MRL/lpr autoimmune manifestations, we established an intercross mouse model generated from the MRL/lpr and BALB/c\textit{J} parental strains. Here we report the identification of two gene-containing intervals on Chr 2 and 11 in addition to the \textit{Fas} locus that contribute to autoantibody production in this intercross mouse model. We also examined the effects of these QTLs on the development of vasculitis. The results indicate that vasculitis is determined by the \textit{Fas} gene mutation and by the Chr 11 QTL, indicating shared genetic determinants with autoantibody levels.
Materials and Methods

**Autoantibody levels** for this period of time.

levels in either parental strain compared with those in age- and sex-
of autoimmune disease and atherogenesis in this cross. As shown in Table
the last 2 mo because we were also interested in examining the interaction
tain relevant tissues were stored frozen. The atherogenic diet was used for
overnight, bled, and sacrificed. Plasma samples were again taken, and cer-

Premier Laboratory Diets, Madison, WI). Parental mice were separated
and 0.5% cholic acid with a total fat content of 15% (Teklad 9022, Teklad
another 2 mo. The HF diet contains 7.5% cocoa butter, 1.25% cholesterol,
fast. All F2 mice were then fed a high fat, high cholesterol diet (HF) for
they were 3.5 mo of age. Plasma samples were collected after an overnight
mouse chow diet (Ralston Purina, St. Louis, MO) containing 4.5% fat until

When the animals were sacrificed, the heart and proximal aorta were dis-
sected and washed to remove blood. The basal portion of the heart and root
of the aorta were embedded in OCT compound (Miles, Elkhart, IN) and
frozen quickly on dry ice as previously described (12). For a semiquanti-
tative assessment of vasculitis in the coronary arteries, every fifth 10-μm
heart section was collected from the lower portion (the site of the first cut)
to the aortic root, stained with oil red O and hematoxylin, and counter-
stained with fast green FCF (12). Vasculitis was defined by clear vessel
wall destruction, cellular infiltration, and abnormal lipid accumulation as
previously described (12). A total of about 70 sections were evaluated for
each animal. The semiquantitation of vasculitis was determined by the
number of positive sections that contained vasculitis as previously
described (12).

**Genomic analysis**

Genomic DNA was isolated from mouse tails. Genotyping was performed
by PCR amplification of simple sequence repeat (microsatellite) markers (13, 14) using PCR primer pairs (MapPairs) purchased from Research Genet-
ics (Huntsville, AL). Primer pairs were first screened for polymorphic
bands between MRL/"lpr" and BALB/c parental strains (data not shown). A total of >100 markers were used to construct a complete linkage map in
189 (MRL/"lpr" × BALB/c)F2 mice. A list of these markers as well as the
linkage data are available from A. J. Lusis.

All genomic regions scored were examined for the possibility of seg-
regation distortion. Four regions, linked to markers D2Mit227, D7Mit71,
D1Mit47, and D19Mit93, exhibited evidence of a non-Mendelian distribu-
tion of alleles (data not shown), raising the possibility of effects on prenatal
or postnatal viability. However, none of these overlapped with the three
autoimmune QTLs described above.

**Statistical analysis**

Phenotypic values are presented as the mean ± SD. Analysis of variance,
regression analysis, and correlation analysis were performed on Macintosh
computers using the StatView (Abacus Concept, Berkeley, CA) applica-
tion. Linkage analysis among the microsatellite markers used was per-
formed using the MAPMAKER (15) and Map Manager (16) programs. The
MAPMAKER/QTL and QT Manager subprograms were used for quanti-
tative trait linkage analysis as described for F2 intercrosses (17–19). Both
analyses yielded similar results. Phenotypes were normalized using either
the log(trait) or sqrt(trait) functions when necessary. A lod score >4.3 indi-
cates significant linkage, while a lod score of 2.8–4.3 indicates sugges-
tive linkage (also see Discussion), as determined by Lander and Kruglyak
for the intercross free model analyses (18).

**Results**

Autoantibody levels among parental MRL/"lpr" and BALB/cJ mice, F1 mice, and F2 intercross mice

Parental MRL/"lpr" and BALB/cJ mice as well as (MRL/"lpr" × BALB/cJ)F1 and F2 mice were examined for quantitative traits

### Table I. Differences of autoantibody levels among parental MRL/"lpr" and BALB/cJ mice, (MRL/"lpr" × BALB/cJ)F1

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Sex</th>
<th>Sample Size</th>
<th>dsDNA Ab</th>
<th>ssDNA Ab</th>
<th>Cardiolipin Ab</th>
<th>Vasculitis Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRL/&quot;lpr&quot;</td>
<td>Male Chow</td>
<td>4</td>
<td>0.73 ± 0.41</td>
<td>1.57 ± 0.39</td>
<td>0.27 ± 0.13</td>
<td>2 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Female Chow</td>
<td>5</td>
<td>0.80 ± 0.19</td>
<td>2.51 ± 0.23</td>
<td>0.39 ± 0.26</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>BALB/c</td>
<td>Male Chow</td>
<td>5</td>
<td>0.71 ± 0.41</td>
<td>1.34 ± 0.66</td>
<td>0.31 ± 0.03</td>
<td>6.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Female Chow</td>
<td>4</td>
<td>0.94 ± 0.34</td>
<td>1.64 ± 0.35</td>
<td>0.28 ± 0.09</td>
<td>9 ± 0.5</td>
</tr>
<tr>
<td>F1</td>
<td>Female Chow</td>
<td>6</td>
<td>0.03 ± 0.01</td>
<td>0.00 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>2 ± 0.05</td>
</tr>
<tr>
<td>F2</td>
<td>Male HF</td>
<td>5</td>
<td>0.32 ± 0.11</td>
<td>0.70 ± 0.15</td>
<td>0.36 ± 0.05</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Female HF</td>
<td>134</td>
<td>0.33 ± 0.31</td>
<td>0.80 ± 0.48</td>
<td>0.29 ± 0.28</td>
<td>6.7 ± 1.4</td>
</tr>
</tbody>
</table>

* Autoantibody levels are expressed as mean ± SD in OD units. Parental mice and F1 mice were measured at the same time and levels can be accurately compared. F2 mice were measured at a different time, and results were adjusted according to the same positive control used.

* For 2 mo prior to bleeding and sacrifice, the mice were maintained on either a chow or a HF diet. As discussed in the text, the diet did not significantly influence plasma autoantibody levels for this period of time.

* The differences of autoantibody levels between MRL/"lpr" and BALB/c parental mice are significant for all comparisons (using ANOVA method): A1: p = 0.006; A2: p < 0.0001; A3: p = 0.008; B1: p < 0.0001; B2: p < 0.0001; B3: p = 0.02; C1: p = 0.03; C2: p = 0.01; C3: p = 0.02; and D1: p = 0.007; D2: p < 0.0001; D3: p = 0.04. The differences of autoantibody levels between mice on HF diet and chow diet are not significant. There is also no sex difference.

* Eight female MRL/"lpr" mice and eight female BALB/cJ mice on atherogenic diet were examined for vasculitis development at 5.5 mo of age. Vasculitis scores represent the number of cryosections of heart exhibiting coronary vessel vasculitis described under Materials and Methods.
associated with autoimmune disease at 24 wk of age. MRL/lpr and BALB/cJ differed significantly ($p < 0.05$ for all comparisons) in the levels of anti-dsDNA Ab, anti-ssDNA Ab, and anti-cardiolipin Ab for both sexes and both diets (Table I). F1 female mice had intermediate anti-dsDNA and anti-ssDNA Ab levels compared with the parental strains and similar anti-cardiolipin Ab levels as the MRL/lpr strain. They differed significantly from age- and sex-matched parental BALB/cJ mice for all Ab levels ($p < 0.005$ for all comparisons) and from MRL/lpr mice for anti-ssDNA Ab levels ($p = 0.005$). These autoantibody levels of F1 mice indicated the involvement of genes from MRL/lpr as well as BALB/cJ mice. Anti-DNA mAbs, mAbs 3E10, 5C5, and 5C6, bind ssDNA and dsDNA, but showed no reactivity to cardiolipin, indicating that the anticardiolipin ELISA used in our study was not cross-reactive with DNA epitopes (data not shown). As expected for a genetically determined trait, the variance in autoantibody levels among F2 mice was greater than that among F1 mice. The sex differences among both parental strains and F2 mice were not significant. In addition to the autoantibody levels, the two parental strains differed in their susceptibility to the development of coronary vasculitis (Table I).

An F2 intercross consisting of 272 mice was constructed by intercrossing (MRL/lpr × BALB/cJ)F1 mice. At 24 wk of age, the F2 mice were examined for the above autoantibody traits associated with autoimmune disease. As a quantitative measure of autoimmune disease, we determined the levels of autoantibodies and spleen weight. As discussed below, we also performed semiquantitative analyses of coronary vasculitis to examine the relationship of vasculitis with generalized autoimmune disease.

As shown in Table II, we observed significant correlations among autoantibody levels and spleen weight in both male and female mice. Among them, the Spearman rank correlation coefficients for anti-dsDNA Ab levels and anti-ssDNA Ab levels were 0.78 ($p < 0.0001$) in male mice and 0.78 ($p < 0.0001$) in female mice. The Spearman rank correlation coefficients for anti-dsDNA Ab levels and anti-cardiolipin Ab levels were 0.80 ($p < 0.0001$) in male mice and 0.78 ($p < 0.0001$) in female mice. Spleen weight correlated best with ssDNA Ab levels, with Spearman rank correlation coefficients of 0.55 ($p < 0.0001$) in male mice and 0.46 ($p < 0.0001$) in female mice. Interestingly, anti-cardiolipin Ab levels have higher correlation coefficients with anti-dsDNA Ab levels (0.80 for males and 0.79 for females) than with anti-ssDNA Ab levels (0.60 for males and 0.58 for females).

### Table II. Strength of association among autoantibody levels and spleen weight in (MRL/lpr × BALB/cJ)F2 mice

<table>
<thead>
<tr>
<th></th>
<th>dsDNA Ab</th>
<th>ssDNA Ab</th>
<th>Cardiolipin Ab</th>
<th>Spleen Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dsDNA Ab</td>
<td>0.78</td>
<td></td>
<td>0.80 (n = 117)</td>
<td>0.46 (n = 118)</td>
</tr>
<tr>
<td></td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
</tr>
<tr>
<td>ssDNA Ab</td>
<td>0.71 (n = 134)</td>
<td>0.60 (n = 117)</td>
<td>0.55 (n = 122)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
<td></td>
</tr>
<tr>
<td>Cardiolipin Ab</td>
<td>0.79 (n = 134)</td>
<td>0.58 (n = 134)</td>
<td>0.37 (n = 118)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
<td></td>
</tr>
<tr>
<td>Spleen weight</td>
<td>0.35 (n = 132)</td>
<td>0.46 (n = 134)</td>
<td>0.34 (n = 132)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
<td>($p &lt; 0.0001$)</td>
</tr>
</tbody>
</table>

* Strength of association expressed as Spearman’s rho and $p$ values. Values to the top right of the diagonal are measured in male mice, and values to the bottom left are measured in female mice.

### Identification of chromosomal loci controlling autoantibody levels

In an effort to identify loci contributing to autoantibody levels, we performed QTL analysis on 189 (MRL/lpr × BALB/cJ)F2 intercross animals. A total of 109 polymorphic microsatellite markers were used to construct a linkage map designed to cover the whole mouse genome at intervals of 20 centimorgan (cM) or less. Due to a failure in some cases to identify informative markers, gaps of $>20$ cM were present in three chromosomal regions (These three gaps are located at Chr 5 between D5Mit105 and D5Mit10 with a distance of 20.5 cM, at Chr 8 between D8Mit555 and D8Mit530 with a distance of 23.6 cM, and at Chr 16 between D16Mit555 and D16 Mit173 with a distance of 31.5 cM). Each F2 mouse was genotyped for the above 109 markers and phenotyped for the levels of anti-dsDNA, anti-ssDNA, and anti-cardiolipin Abs. The distributions of these values are presented in Fig. 1. The broad range of Ab levels reflects the large differences in the parental strains. The majority of F2 animals showed values between the parental extremes. The individuals with values outside the parental strain ranges presumably resulted from the recombining of genetic components in the F2 mouse; that is, certain F2 mice exhibited higher levels of autoantibodies than did the parental strain MRL/lpr mice due to the inheritance of unique combinations of MRL/lpr and BALB/cJ alleles. We then performed statistical analysis combining both genotypes and phenotypes. Analysis using the MAPMAKER-QTL statistical program and the QT Manager program yielded similar results (only QTL results are shown).

We observed that the Fas locus on Chr 19 was the major gene controlling the autoantibody levels (maximum lod, 53 for anti-dsDNA Abs; Table III and Fig. 2). This locus explained about 83% of the variance in autoantibody levels observed among the F2 mice. As expected, mice homozygous for the MRL/lpr alleles of the Fas gene exhibited much higher autoantibody levels than those heterozygous or homozygous for the BALB/cJ alleles, indicating recessive inheritance with the MRL allele condoning high expression of autoantibodies. The peak QTLs for all three autoantibodies exhibited coincident locations (Fig. 2). Mice homozygous for the MRL/lpr allele at the Fas gene locus exhibited somewhat elevated autoantibody levels compared with BALB/cJ mice (Fig. 3; $p < 0.0001$ for the difference of dsDNA Ab levels between CC and MC groups), suggesting that the Fas mutation is not completely recessive. This was supported by QTL analysis of F2 mice when mice homozygous for the mutation were eliminated (see below).
In addition, a locus on Chr 11 closely linked to marker D11Mit70 showed suggestive linkage with all autoantibody levels (lod = 3.1 for anti-dsDNA Abs, lod = 2.1 for anti-ssDNA Abs, lod = 2.6 for anti-cardiolipin Abs). The peak QTLs for all three autoantibodies coincided (Fig. 2). This locus has not previously been reported in studies of autoimmune-related traits in mice. It is noteworthy that among the F2 mice, those heterozygous for the D11Mit70 marker tended to exhibit higher autoantibody levels than those homozygous at the locus for either the MRL/lpr or BALB/cJ alleles. This suggests an interaction between BALB/cJ and MRL/lpr alleles in the development of autoimmune disease, consistent with the observation that a subset of F2 mice exhibited higher autoantibody levels than the MRL/lpr parental strain.

In addition to the three QTLs reported here, we have carefully examined in our cross any loci for the trends of association with autoantibody levels. The only genomic region in which we observed a trend of association was at marker D5Mit32 for ssDNA Ab (lod score = 2.1; Table III), overlapping with a region reported previously to be linked to mortality in New Zealand mice (20).

Since the lpr mutation has an overwhelming effect on the autoimmune manifestations of the MRL/lpr mouse, and a major interest was to identify other background genes, we performed QTL analysis with lpr/+ and +/+ subpopulations. Specifically, all the mice that were homozygous for the MRL/lpr genotype at the Fas locus were removed, and a total of 144 mice were left in this subpopulation. The results from MAPMAKER-QTL analysis are presented in Table III. The Fas locus showed a reduced, but still significant, contribution to autoantibody levels, indicating an incomplete recessive phenotype of the lpr mutation as has been previously reported (21). In addition, a locus on Chr 2 closely linked to marker D2Mit12 showed a lod score of 4.3 for anti-ssDNA Ab levels and a lod score of 2.6 for anti-dsDNA Ab levels. The failure to identify this locus in the entire sample presumably resulted from the overwhelming contribution of lpr homozygous animals to the autoantibody distributions in F2 mice. It is also noteworthy that the Chr 11 locus was not significantly associated with autoantibody levels in this subpopulation, suggesting that the Chr 11 locus interacts with the Fas gene. The fact that QTL peaks for anti-dsDNA and anti-ssDNA Abs on Chr 2 coincided (Fig. 2) indicates that they are determined by the same gene. When separated by genotype at the D2Mit12 marker, individuals with the MM genotype at the Chr 2 locus had increased anti-ssDNA autoantibody levels compared with mice with CC and MC genotypes, which had similar levels of anti-ssDNA autoantibodies. Thus, the Chr 2 locus represents one of the genetic factors contributed by the MRL genetic background predisposing to autoimmune disease. It acts in a recessive fashion to control anti-DNA autoantibody levels (Fig. 3).

It is noteworthy the levels of anti-cardiolipin Abs in the F1 mice slightly exceeded those in MRL mice, which were, in turn, higher than those in BALB/c mice (Table I). Thus, there is a difference in the genetic control of the specificity of anti-cardiolipin Abs compared with anti-DNA Abs. Nevertheless, at the two QTLs that exhibited linkage with anti-cardiolipin Ab levels (Chr 11 and Chr 19), the inheritance pattern for anti-cardiolipin Ab levels resembled those for anti-DNA Ab levels. Thus, at the Fas gene locus, mice homozygous for the MRL allele exhibited elevated anti-cardiolipin levels compared with heterozygous mice and mice homozygous for the BALB/c allele. Similarly, at the Chr 11 locus, heterozygous mice exhibited the highest levels of anti-cardiolipin Ab, suggesting an interaction between the MRL and BALB/c alleles (data not shown).

Table III. QTL Analysis of autoantibody levels and vasculitis in (MRL/lpr × BALB/cJ)F2 intercross mice

<table>
<thead>
<tr>
<th>Phenotypic Trait</th>
<th>Locus</th>
<th>Maximum lod</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTL analysis in lpr/lpr, lpr/+ population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dsDNA Ab</td>
<td>D11Mit70</td>
<td>3.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ssDNA Ab</td>
<td>D11Mit70</td>
<td>2.1</td>
</tr>
<tr>
<td>Cardiolipin Ab</td>
<td>D11Mit70</td>
<td>2.1</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>D11Mit70</td>
<td>2.2</td>
</tr>
<tr>
<td>QTL analysis in lpr/+ and +/+ subpopulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dsDNA Ab</td>
<td>D2Mit12</td>
<td>2.6</td>
</tr>
<tr>
<td>ssDNA Ab</td>
<td>D2Mit12</td>
<td>4.3</td>
</tr>
<tr>
<td>Cardiolipin Ab</td>
<td>Fas</td>
<td>5.1</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>Fas</td>
<td>2.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Autoantibody and vasculitis values were measured in F2 mice of 5.5 mo of age, including 3.3 mo on chow diet and 2 mo on HF diet.

<sup>b</sup> The most closely linked marker typed in the cross.

<sup>c</sup> Analysis was done using log transformation of the phenotype.
We also performed QTL analysis with spleen weight in the F2 mice. The only QTL observed with this trait is on Chr 19, coincident with the QTL for autoantibodies, with a peak lod score of 59.0. This locus for spleen weight exhibited the same pattern of inheritance as it did with the dsDNA Ab levels (data not shown). The failure to detect the Chr 2 and 11 loci using spleen weight presumably reflects the fact that spleen weight is a less precise measurement of autoimmune disease than autoantibody levels.

Common genetic control of autoantibody levels and vasculitis

MRL/lpr mice also develop severe vasculitis; some evidence suggests that the vasculitis is mediated in part by the autoantibodies (12, 22). In a previous study, we examined in detail vasculitic lesion formation in the arteries of MRL/lpr mice. We showed that when mice are fed a HF diet, concentric and transmural lesions with a large amount of lipid accumulation occur in coronary arteries of MRL/lpr mice, but not in a variety of other strains examined. These lesions are prone to infiltration of inflammatory cells and exhibit extensive deposition of Ig (9). In an attempt to examine the relationship of vasculitis with autoantibodies, we scored the vasculitic lesion formation in the coronary arteries of the F2 mice. The severity of vasculitis was scored by determining the number of sections positive for vasculitis as described in Materials and Methods. As shown in Table IV, we observed significant associations of vasculitis with the autoantibody levels in 252 (MRL/lpr × BALB/cJ)F2 mice. The correlation coefficients were 0.35 ($p < 0.0001$) for both dsDNA Ab and cardiolipin Ab, and 0.33 ($p < 0.0001$) for ssDNA Ab. We noticed that vasculitis correlated equally well with dsDNA Ab and cardiolipin Ab levels, but less well with ssDNA Ab levels.

We further performed QTL analysis with vasculitis in our F2 mice. Two QTLs on Chr 19 and 11, located at the same positions as the QTLs for autoantibodies, were observed with lod scores of 7.6 and 2.2, respectively (Table III and Fig. 2). It is interesting that the Chr 2 QTL for anti-DNA Abs did not contribute to either vasculitis or anti-cardiolipin Ab levels. As shown in Fig. 4, both Chr 19 and 11 QTLs had significant impact on...
vasculitis formation. Specifically, mice homozygous for the MRL/\(lpr\) alleles of the \(Fas\) gene exhibited much higher vasculitis scores than those heterozygous or homozygous for the BALB/cJ alleles \((p < 0.0001\), by analysis of variance), indicating recessive inheritance. For the Chr 11 QTL, mice heterozygous for the \(D11Mit70\) marker exhibited higher vasculitis scores than those homozygous at the locus for either the MRL/\(lpr\) or BALB/cJ alleles \((p = 0.05\), by analysis of variance), suggesting an interaction between BALB/cJ and MRL/\(lpr\) alleles in the development of vasculitis. Again, the Chr 2 QTL did not exhibit a significant relationship with coronary vasculitis in either the whole F2 population or the \(lpr/\) and \(+/+\) subpopulations using analysis of variance analysis. This raises the possibility that the Chr 2 locus contributes to autoimmune disease by a mechanism that has less impact on vasculitis. Alternatively, the semiquantitative nature of the scoring of coronary vasculitis may have observed a relationship, although it is noteworthy that the Chr 11 locus, which exhibited a lower lod score than the Chr 2 locus, was significantly associated with vasculitis.

**Discussion**

In our (MRL/\(lpr\) × BALB/cJ)F2 intercross, we identified two QTLs, on Chr 2 and Chr 11, in addition to the \(lpr\) mutation on Chr 19, for autoantibody levels. These two loci have not been previously observed in genetic studies of mouse models, including a previous study with MRL mice (10). The loci contain a number of interesting candidate genes, as discussed below. The loci on Chr 2 and 19 exhibited recessive inheritance, and the MRL alleles conferred high expression of autoantibodies. The Chr 11 locus, however, appears to involve an interaction between MRL and BALB/c alleles, as heterozygous mice exhibited the highest levels of autoantibodies. Our results also showed that the Chr 11 and 19 QTLs contribute to the development of anti-cardiolipin antibodies and coronary vasculitis.

The three mouse models that have been most commonly used to study the human autoimmune disease SLE are the MRL/\(lpr\) mouse model, the (NZB × NZW)F1 mouse model, and the B × SB mouse model, which carry the disease-accelerating Yaa gene on the Y Chr (5, 6, 7, 23). Most genetic studies of SLE have been conducted in NZB/W-related strains using either backcross or intercross approaches (20, 24–30). An elegant study by Morel et al. (24) using a backcross between NZM/Aeg2410 and C57BL/6 mice resulted in the identification of three chromosomal loci containing recessive glomerulonephritis susceptibility genes on Chr 1, 4, and 7. Each locus contained several potentially interesting candidate genes. Other studies confirmed these loci and revealed additional loci contributing to the autoimmune phenotype. Summaries of all chromosomal regions contributing to SLE-related phenotypes were derived from studies of NZB-related strains and MRL mice were published recently (5–7). Overall, there are 12 loci from NZB/W-related strains in addition to the MHC gene, and two loci from the MRL strains in addition to the \(lpr\) (\(Fas\)) and \(gld\) (\(Fas\) ligand) genes, that have been shown to be associated with or to have a trend of association with lupus-related traits (6). These loci reside on 14 separate Chr, including Chr 1, 4–7, 9, 11–14, and 16–19. In our study of an (MRL/\(lpr\) × BALB/cJ)F2 cross, we identified two QTLs, on Chr 2 and 11, in addition to the \(Fas\) gene on Chr 19. The Chr 11 QTL centered around D11Mit70 (52 cM from the centromere) (31), which lies about 16 cM from D11Mit114/Lbw8 (36 cM from the centromere) (31). Lbw8 was previously linked to anti-chromatin Abs production (20). The same area was shown later in other studies to overlap with regions associated with nephritis (28, 30). The Chr 2 QTL, however, did not overlap with any of the SLE loci identified before, although the same place was shown to contain a link to type 1 diabetes, IDD13 (32).

Previously, a complete linkage study using (MRL/\(lpr\) × CAST/Ei)F1 × MRL/\(lpr\) backcross mice by Watson and colleagues (10) identified two QTLs contributing to the development of glomerulonephritis in addition to the \(lpr\) mutation. These two QTLs resided on Chr 7 (lod score = 3.0) and 12 (lod score = 2.9). Since the submission of this paper, two studies were published where the MRL strain was used as a model for the genetic analysis of autoimmunity (33, 34). In an (MRL/\(lpr\) × C57BL/6-\(lpr\))F2 cross, four loci (Lmb1–4) with significant linkage to lymphadenopathy and/or splenomegaly were identified on Chr 4, 5, 7, and 10 (33). We failed to confirm the QTLs obtained in their studies. However, we used a different parental strain (BALB/cJ) than the CAST/Ei or C57BL/6 strains they used. Previous studies have shown that the genetic backgrounds of the nonautoimmune strains in these crosses can markedly alter the genes showing linkage with disease (27, 28, 30). C57BL/6 and CAST/Ei strains are phylogenetically very distant related to BALB/cJ, and it is quite possible that the choice of partner strains contributed to the different findings of these studies. Further, our study also provides a better understanding of the relationships of anti-DNA Abs and anti-cardiolipin Abs with vasculitis.

The maximum lod score we obtained for the Chr 11 locus (3.1) is strongly suggestive and overlaps with previous identified loci, whereas the lod score for the Chr 2 locus appears to be significant, as judged by the proposed thresholds by Lander and Kruglyak (18). Due to the nature of the algorithms used for maximal likelihood estimation in QTL analysis, a high lod score may not necessarily reflect the true strength of the association of the locus with the trait, even though it is true with respect to the mathematical model. Factors such as map distance, species, and trait distribution also influence the significance threshold (19, 35). Therefore, we determined the maximal lod score peaks of 1000 randomly permuted trait data to test in our dataset how often a QTL could actually occur due to chance, permitting the definition of a realistic significance threshold for a given experimental dataset (19). Using the method of Churchill and Doerge (19), as implemented in the QT Manager program, each trait was permuted 500–1000 times.

### Table IV. Relationship of autoimmune phenotypes with vasculitis score in (MRL/\(lpr\) × BALB/cJ)F2 mice

<table>
<thead>
<tr>
<th></th>
<th>dDNA Ab</th>
<th>ssDNA Ab</th>
<th>Cardiolipin Ab</th>
<th>Spleen Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasculitis score</td>
<td>(r = 0.35^a)</td>
<td>(r = 0.33)</td>
<td>(r = 0.35)</td>
<td>(r = 0.23)</td>
</tr>
<tr>
<td>(p &lt; 0.0001)</td>
<td>(p &lt; 0.0001)</td>
<td>(p &lt; 0.0001)</td>
<td>(p = 0.0002)</td>
<td></td>
</tr>
</tbody>
</table>

*a* Strength of association expressed as Spearman’s \(\rho\) and \(p\) values. 252 F2 animals were used for the analysis.

### Figure 4

**Differences in vasculitis scores at Chr 19 and 11 QTLs.**

Vasculitis scores were grouped according to the genotypes at the typed microsatellite markers closest to the QTL peaks. Significant differences for each comparison were determined using analysis of variance and are expressed as \(p\) values.
The resulting thresholds for significance were similar to those proposed by Lander and Kruglyak (18). That is, for the intercross free model analysis, a lod score of about 4.3 indicated significant linkage (the likelihood of a false positive being <5%), while a lod score of 2.8–4.3 indicated suggestive linkage.

The 95% confidence intervals (going 1 lod down from the maximum lod score) approximate the 95% confidence interval for the QTL for the Chr 2 and Chr 11 QTLs contain some interesting candidate genes. The genes for IL-1α and IL-1β lie about 4 cM proximal to marker D2Mit46 on Chr 2. IL-1 was shown to be overexpressed in MRL/lpr mice and may contribute to high levels of IgG production in MRL/lpr mice (36, 37). Another candidate gene in the Chr 2 confidence interval is the immune response-2 locus, which is about 6 cM proximal to marker D2Mit46. This locus was first characterized by the difference of response to erythrocyte antigen-1 among different inbred strains of mice, with the BALB/cJ strain being nonresponsive (38). In the confidence interval of Chr 11, a family of small inducible cytokines (Scya), in the BALB/cJ strain being nonresponsive (38). In the confidence interval of Chr 11, a family of small inducible cytokines (Scya), including Scya1–6, are located about 5–7 cM proximal to marker D11Mit70. These genes include a family of cytokines that function as proinflammatory chemotactarrents for CD4+ T cells, monocytes, and eosinophils and as activators of basophils to release histamine. Members of the β-chemokine RANTES family have also been implicated in a number of chronic inflammatory and autoimmune processes (39).

MHC genes influence the susceptibility to SLE both in humans (40, 41) and in NZB/NZW-related mouse strains (42). For example, replacing the H-2d haplotype of the NZB mouse with the H-2bw12 haplotype (a variant modifying the peptide binding groove of I-A), but not the H-2d haplotype, results in increased levels of anti-DNA Abs, whereas replacing the H-2d haplotype of the B × SB mouse with the H-2bw1 haplotype leads to decreased autoimmune symptoms (42). Most likely, other genetic factors, such as the differences in TCR repertoire, may act together with MHC genes in controlling disease susceptibility. However, in the present study, we did not observe linkage to the MHC locus on mouse Chr 17. Linkage to the MHC locus was also not revealed in previous (MRL/lpr × CAST/Ei)F1 × MRL/lpr backcross (10) and MRL-lpr × C57BL/6-lpr intercross studies (33). Thus, either the MHC locus (H-2d for MRL/lpr, H-2f for BALB/cJ) is irrelevant to the MRL/lpr model, or the effects were obscured by other strong genetic influences.

We also clearly observed that the lpr mutation functions to a significant extent in the heterozygous state (Table III). After removing the lpr/lpr homozygous individuals in our F2 population, we obtained lod scores of 13.7 for anti-dsDNA Abs and 10.9 for anti-ssDNA Abs, which still explains the majority of the variance for these two traits. Our result is consistent with a previous report (21). Since the influence of the lpr locus remains extremely large in the heterozygous mice, it is of interest whether the Chr 2 or 11 loci exert any effects in mice +/+ at the lpr locus. However, the number of animals in this subpopulation was insufficient for QTL analysis using the MAPMAKER-QTL program.

Anti-cardiolipin Abs shared the same Chr 19 and 11 QTLs with anti-DNA Abs, but not the Chr 2 QTL, indicating that the genetic controls of anti-cardiolipin Abs and anti-DNA Abs are not entirely the same. It is also noteworthy that anti-cardiolipin Ab levels of F1 mice were slightly higher than those of MRL/lpr mice, supporting a difference in the genetic control of this specificity compared with that of anti-DNA Abs. Nevertheless, the two loci influencing anti-cardiolipin Abs exhibited similar inheritance patterns for anti-DNA and anti-cardiolipin Ab levels. Abs detected by solid phase anti-cardiolipin immunoassays are heterogeneous. These Abs can bind to different epitopes of cardiolipin and can cross-react with other phospholipid and β2-glycoprotein I-cardiolipin complexes (43–45). Further, since the cardiolipin coated on the ELISA plates was stored at 4°C before use, it was likely to be oxidized. Abs against oxidized cardiolipin were also reported to recognize oxidized low density lipoproteins (46, 47). The ability of anti-cardiolipin to recognize these diverse epitopes could influence its clearance from the circulation and may explain in part why there is differing genetic control of anti-cardiolipin compared with anti-DNA Abs. For example, genetic factors are likely to influence the oxidation of phospholipids (discussed in Ref. 12), which could, in turn, affect the clearance of anti-cardiolipin Abs. However, the use of monoclonal anti-DNA Abs in these assays showed no evidence of cross-reactivity between DNA and cardiolipin.

Autoimmune vasculitis is characterized by the presence of autoantibodies in patient sera, such as anti-neutrophil cytoplasmatic Abs, anti-nuclear Abs (48–50), and anti-dsDNA Abs (51). Although the mechanism of this interaction has yet to be clearly established, autoantibodies have been shown to substantially up-regulate the expression of cell adhesion molecules, an early phase in the development of an inflammatory vascular lesions (48). Recently, anti-cardiolipin Abs were also shown to be significantly associated with vasculitis in autoimmune-prone (NZW × B × SB)F1 mice, suggesting that anti-cardiolipin Abs, and their proposed thrombogenic and vascular injury consequences, contribute to development of microvasculitis in lupus-prone mice (52). It is of interest to note that the QTLs associated with elevated anti-cardiolipin Abs (Chr 11 and 19), but not the QTL associated only with anti-DNA Abs (and not anti-cardiolipin Ab; Chr 2) were significantly associated with coronary vasculitis. We have, in our (MRL/lpr × BALB/cJ) intercross, demonstrated common genetic controls of vasculitis with autoantibodies. Our results provided a genetic link between these two most common features of lupus that will help to clarify the mechanisms underlying their interaction.

In conclusion, using an (MRL/lpr × BALB/cJ) intercross we identified two loci, on Chr 2 and 11, in addition to the lpr mutation on Chr 19, controlling anti-DNA Ab levels. The Chr 2 QTL was also shown to contribute to anti-cardiolipin Ab levels. In addition, there are undoubtedly many loci that cannot be detected with confidence without using a much larger number of animals than that used in this study. We also demonstrated common genetic controls of both vasculitis and autoantibodies by Chr 19 and 11 QTLs. The localization of disease-modifying genes from our mouse model may be useful in the prediction of loci contributing to human SLE genes due to the chromosomal conservation of linked genes between mice and humans. More importantly, genetic loci for complex disease can be isolated in mice using breeding strategies similar to those employed in the identification of histocompatibility loci, thereby providing more detailed molecular information about the underlying genes (53, 54).

Acknowledgments

We thank Drs. Boris Ivandic and Margareta Mehrabian for helpful discussions.

References
