Quantitative Trait Locus Analysis of Plasma Lipoprotein Levels in an Autoimmune Mouse Model
Interactions Between Lipoprotein Metabolism, Autoimmune Disease, and Atherogenesis

Lingjie Gu, Michael W. Johnson, Aldons J. Lusis

Abstract—The autoimmune MRL/lpr mouse strain, a model for systemic lupus erythematosus, exhibited an unusual plasma lipoprotein profile, suggesting a possible interaction of autoimmune disease and lipoprotein metabolism. In an effort to examine the genetic basis of such interactions, and to study their relationship to atherogenesis, we performed a quantitative trait locus analysis using a total of 272 (MRL/lpr×BALB/cJ) second generation (F2) intercross mice. These mice were examined for levels of total plasma cholesterol, HDL cholesterol, VLDL and LDL cholesterol, unesterified cholesterol, autoantibodies, and aortic fatty streak lesions. Using a genome scan approach, we identified 4 quantitative trait loci controlling plasma lipoprotein levels on chromosomes (Chrs) 5, 8, 15, and 19. The locus on Chr 15 exhibited lod scores of 11.1 for total cholesterol and 6.7 for VLDL and LDL cholesterol in mice fed an atherogenic diet, and it contains a candidate gene, the sterol regulatory element binding protein-2. The locus on Chr 5 exhibited lod scores of 3.8 for total cholesterol and 4.1 for unesterified cholesterol in mice fed an atherogenic diet, and this locus has been observed in 2 previous studies. The locus on Chr 8 exhibited a lod score of 3.1 for unesterified cholesterol in mice fed a chow diet. This locus contains the lecithin-cholesterol acyltransferase gene, and decreased activity of the enzyme in the MRL strain suggests that this gene underlies the quantitative-trait locus. The locus on Chr 19 exhibited a lod score of 8.4 for HDL cholesterol and includes the Fas gene, which is mutated in MRL/lpr mice and is primarily responsible for the autoimmune phenotype in this cross. That the Fas gene is responsible for the HDL quantitative-trait loci is supported by the finding that autoantibody levels were strongly correlated with HDL cholesterol levels (r=0.37, P<0.0001) among the F2 mice. HDL cholesterol levels were in turn significantly associated with aortic fatty streak lesions among the F2 mice (r=-0.17, P=0.006). Further, there was a threshold effect of autoantibody levels on the development of fatty streak lesions (r=0.45, P=0.004 for 42 F2 mice with anti-dsDNA Ab over 0.5 OD). Our results support the concept that the high prevalence of coronary artery disease in systemic lupus erythematosus is due in part to a reduction of HDL cholesterol levels resulting from the autoimmune disease. (Arterioscler Thromb Vasc Biol. 1999;19:442-453.)

Key Words: linkage analysis ■ antibodies, antinuclear ■ genes ■ lupus erythematosus, systemic ■ HDL

Plasma lipoprotein levels are an important determinant of atherosclerosis, the major cause of coronary artery disease (CAD) and stroke. High levels of LDL and VLDL are strongly associated with increased incidence of CAD, whereas HDL has a protective effect.1–4 Although a number of mendelian disorders contributing to lipoprotein metabolism, such as those of the LDL receptor in familial hypercholesterolemia and cholesterol ester transfer protein deficiency in hyperalphalipoproteinemia, have been elucidated,1,5,6 they explain only a small fraction of the population variance of plasma lipoprotein levels. As yet, relatively little is known of the genetic factors contributing to common, complex genetic variations. Studies of such variations in humans are complicated by dietary influences and genetic heterogeneity. Animal models have significant advantages for the analysis of complex traits;7–11; in particular, planned breeding can be performed and the environment can be controlled. The mouse, the most useful mammal for genetic studies, has been developed as a model for the analysis of lipoprotein metabolism and other traits relevant to atherosclerosis. In previous studies in our laboratory, we have identified several quantitative-trait loci (QTL) for lipoprotein metabolism in mice, and some of the underlying genes were elucidated using a positional candidate gene strategy.10,11

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We have screened a number of inbred strains of mice for variations in plasma lipoprotein metabolism and noted that certain autoimmune strains, such as MRL/lpr mice and NZB/BINJ mice, exhibited significantly altered lipoprotein levels compared with other inbred strains. The observation that MRL/n mice, lacking the lpr mutation, exhibited very different lipoprotein profiles compared with MRL/lpr mice suggested that the unusual lipoprotein profiles were related to autoimmunity. Previous studies have also shown that MRL/lpr mice, a model for systemic lupus erythematosus (SLE), develop severe coronary lipid lesions and increased myocardial infarction when fed an atherogenic, high fat diet. This suggests that the autoimmune background may contribute to the abnormal lipoprotein metabolism and CAD seen in the MRL mice. In an attempt to dissect these interactions, we examined the genetic determinants of lipoprotein metabolism and atherogenesis in the MRL strain.

An intercross between autoimmune MRL/lpr and non-autoimmune BALB/cJ strain mice, which also differ significantly for lipoprotein levels and fatty streak lesion susceptibility, was constructed. Four major chromosomal intervals controlling lipoprotein levels, on chromosomes (Chrs) 5, 8, 15, and 19, were identified. Three of these appear to result from nonautoimmune related genetic variations. On the other hand, the Chr 19 QTL for HDL cholesterol levels appears to result from the mutant Fas gene carried in the MRL/lpr mice. The Fas gene controls apoptosis of lymphocytes, and the lpr mutation of the Fas gene, the primary cause of autoimmune disease in MRL mice, leads to the accumulation of autoantibodies. We observed a significant negative correlation of autoantibody levels with HDL cholesterol levels (\( r = -0.37, P < 0.0001 \)) in the F2 mice, and HDL levels were inversely associated with atherosclerotic lesions in the aorta (\( r = -0.17, P = 0.006 \)). Moreover, there appeared to be a threshold effect of autoantibody levels on lesion development, as only mice with high autoantibody levels exhibited a strong association between autoantibody levels and the size of aortic lesions. These findings indicate that autoimmunity may promote atherogenesis in part by decreasing HDL cholesterol levels, providing a possible explanation for the high incidence of CAD in SLE patients.

**Methods**

**Animals and Diets**

Inbred MRL/lpr and BALB/cJ mice were purchased from the Jackson Laboratory (Bar Harbor, ME). MRL/lpr and BALB/cJ mice were crossed to produce the first generation (F1), and brother-sister mating of F1 mice produced a total of 272 second generation (F2) animals. After weaning for 21 days after birth, mice were housed 2 to 3 per cage with free access to food and water and a 12-hour light/dark cycle. All animals, parental as well as F2 mice, were maintained on a Purina Mouse Chow diet containing 4.5% fat until 3.5 months of age, when plasma samples were collected after an overnight fast. The mice were then fed a high fat, high cholesterol diet (HF) for another 2 months. The HF diet contained 7.5% cocoa butter, 1.25% cholesterol, and 0.5% cholic acid with a total fat content of 15% (Teklad 9022, Teklad Premier Laboratory Diets). Parental mice were separated into 2 groups. One group was kept on the chow diet, while the other group was put on the HF diet for another 2 months. The animals were then fasted overnight, bled, and euthanized by cervical dislocation following isoflurane anesthesia. Plasma samples were again collected and certain relevant tissues were stored frozen. The HF diet results in hyperlipidemia and aortic fatty streak lesion formation in certain strains of mice.

**Lipoprotein Assays**

Mice were bled retro-orbitally under isoflurane anesthesia (Forane, Anaquest) at 3.5 and 5.5 months of age after overnight fasting. Blood was collected directly through heparinized capillary tubes (Becton Dickinson) and plasma was separated by centrifugation. Cholesterol assays were performed in 96-well microtiter plates (Costar No. 3598) using a Biomek 1000 Automated Laboratory Workstation (Beckman). Plasma lipids were determined as described, including total cholesterol (TC), HDL cholesterol, and VLDL and LDL (V/LDL) cholesterol. HDL cholesterol was measured after the addition of heparin and manganese to precipitate apolipoprotein B containing V/LDL, followed by centrifugation at 15 000 g for 15 minutes. The supernatant solution was then used for determination of HDL cholesterol.

**Autoantibody Quantitation**

IgG antibodies to double-stranded (ds) DNA were quantitated by ELISA using calf thymus DNA (Sigma Chemical Co) coated on 96-well polystyrene microtiter plates (Dynatech), as described. Briefly, sera were added in 1:100 dilution in 0.5% hen egg albumin/phosphate buffered saline tween. One hundred \( \mu L \) of the dilution was added to each well in duplicate. Plates were incubated at 4°C overnight and washed 3 times with phosphate buffered saline solution. One hundred \( \mu L \) of goat anti-mouse IgG alkaline phosphatase (So. Biotech lot No. J194-Y894), diluted 1:3000 in 0.5% hen egg albumin/phosphate buffered saline tween, was added to each well and incubated at room temperature for 1 hour. The wells were then washed 3 times in phosphate buffered saline solution and developed by adding phosphatase substrate in diethanalamine buffer (Sigma 104). The absorbencies at 450 nm were determined using a plate reader.

**Fatty Streak Lesions**

At the time when animals were euthanized, the heart and proximal aorta were dissected and washed. The basal portion of the heart and the root of the aorta were embedded in OCT compound and frozen on dry ice. The region beginning at the aortic root and continuing toward the aortic arch was sectioned for a distance of approximately 400 \( \mu m \). Every 10 \( \mu m \) section was collected, stained with oil red O and hematoxylin, and counter-stained with fast green. Each of these sections was reviewed by light microscopy and then evaluated quantitatively. The cross-sectional area of lipid-containing lesions was determined using a microscope eyepiece grid (20×20-grid disk No. 478, AO Scientific Instruments). The areas of all lesions in each section were averaged to give a total lesion area per section.

**Genotypic Analysis**

Genomic DNA was isolated from mouse tails. Genotyping was done by polymerase chain reaction (PCR) amplification of microsatellite markers using PCR primer pairs (MapPairs) purchased from Research Genetics. Primer pairs were first screened for polymorphic bands between MRL/lpr and BALB/cJ parental strains (data not shown), using the standard PCR conditions suggested by Research Genetics.

**Statistical Analysis**

Phenotypic values are presented as the mean±SEM. ANOVA, regression analysis, and correlation analyses were performed on Macintosh computers using Statview (Abacus Concepts Inc) application. Linkage analysis of the microsatellite markers used was performed using the MAPMAKER and Map Manager programs. The MAPMAKER/QTL and QT Manager subprograms were used for quantitative trait linkage analysis as described for F2 intercrosses, and both analyses yielded similar results. Phenotypes were sometimes normalized using either the log (trait) or square (trait) functions. Calculations by Lander and Kruglyak suggest that,
for intercross free model analysis, a lod score over 4.3 indicates significant linkage, while a lod score of 2.8 to 4.3 indicates suggestive linkage (also see Discussion). Due to the nature of the algorithms used for maximal likelihood estimation in QTL analysis, lod score based on the mathematical model may not necessarily reflect the true strength of the association of the locus with the trait. Factors such as map distance, species, and trait distribution also influence the thresholds for significance. Therefore, we performed permutation analysis according to the method of Churchill and Doerge, using the QT Manager program. We determined the maximal lod score peaks of 1000 randomly permuted trait data to test in our data set how often a QTL could actually occur due to chance. By taking a 95% cut off from the distribution obtained with the permuted 1000 maximal lod scores, a realistic significance

TABLE 1. Lipoprotein Levels in MRL/lpr, BALB/cJ, and (MRL/lpr×BALB/cJ)F1 Mice*

<table>
<thead>
<tr>
<th></th>
<th>MRL/lpr</th>
<th></th>
<th>BALB/cJ</th>
<th></th>
<th>F1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF (n=5)</td>
<td>188±5</td>
<td>142±3†</td>
<td>252±29‡</td>
<td>147±31†</td>
<td></td>
</tr>
<tr>
<td>Chow (n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>126±6†</td>
<td>129±2†</td>
<td>85±10‡</td>
<td>93±20</td>
<td></td>
</tr>
<tr>
<td>V/IDL cholesterol, mg/dl</td>
<td>63±4</td>
<td>14±2†</td>
<td>167±30</td>
<td>55±14†</td>
<td></td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>3.8±1.2</td>
<td>22±1†</td>
<td>5.6±0.5‡</td>
<td>40±3</td>
<td></td>
</tr>
<tr>
<td>UC, mg/dl</td>
<td>37±2</td>
<td>20±1†</td>
<td>52±10‡</td>
<td>22±3‡</td>
<td></td>
</tr>
</tbody>
</table>

*All the mice were 5.5 months of age, including 3.5 months fed chow diet and 2 months fed either chow diet (Chow) or atherogenic (HF) diet. All the values are expressed as mean±SEM.
†Indicates significant differences between MRL/lpr and BALB/cJ mice for the same sex and same diets, P<0.05, by Student’s t test.
‡Indicates significant differences between female MRL/lpr, BALB/cJ, and F1 mice on atherogenic diet, P<0.05 by ANOVA.

Figure 1. Distribution of lipoprotein levels of mice fed the chow diet (A) and the HF diet (B) among (MRL/lpr×BALB/cJ) F2 mice. Parental MRL/lpr and BALB/cJ values are the average of 4 to 5 female mice fed the same diet as the F2 mice. F1 values are the average of 7 female mice at 5.5 months of age fed the HF diet. M indicates MRL/lpr; C, BALB/cJ; and F1, (MRL/lpr×BALB/cJ), first generation.
Results

Inheritance of Plasma Lipoprotein Levels in an F2 Intercross Between Strains MRL/lpr and BALB/cJ

Parental MRL/lpr and BALB/cJ mice as well as (MRL/lpr×BALB/cJ) F1 and F2 mice were examined for lipoprotein levels. Both parental strains and F1 mice were separated into 2 groups. One group was fed a normal chow diet until 5.5 months of age, while the other group was fed a chow diet until 3.5 months of age and then an atherogenic diet for another 2 months (see Methods). MRL/lpr until 3.5 months of age and then an atherogenic diet for

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threshold for a given experimental data set was determined. The results were similar to the threshold criteria proposed by Lander and Kruglyak.

Results

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MRL/lpr and BALB/cJ mice of both sexes were used to produce F1 mice. Brother-sister mating of F1 mice produced 272 F2 mice with 128 males and 144 females. All the mice were fed a chow diet for 3.5 months, bled, and then given an atherogenic HF diet rich in fat and cholesterol (see Methods) for another 2 months. They were again bled and euthanized. Lipoprotein levels in F2 mice were measured, and the distributions of these values are presented in Figure 1. The broad range of lipoprotein levels presumably reflects the oligogenic nature of the control of lipoprotein metabolism.

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 factors in the F2 mice; that is, certain F2 mice exhibited higher or lower levels of lipoproteins than either parental strain due to the inheritance of unique combinations of MRL/lpr and BALB/cJ alleles. Table 2 shows the relationship of various lipoprotein parameters on the chow and HF diets.

Mapping Chromosomal Loci Controlling Lipoprotein Levels

To identify loci contributing to differences in lipoprotein levels, we performed QTL analysis on 189 (MRL/lpr×BALB/cJ) F2 intercross mice. A total of 105 polymorphic microsatellite markers were used to construct a linkage map (Figure 2) designed to cover the entire mouse genome at intervals of ≈20 centimorgans (cM). Due to a failure in some cases to identify informative markers, gaps of more than 20cM were present in 3 chromosomal regions. We then performed statistical analysis of the relationships between genotypes and phenotypes using the Mapmaker-QTL and the QT Manager programs. Both programs yielded similar results (data not shown). A summary of all the QTLs exhibiting lod scores >2.0 are presented in Table 3. Most of these QTLs are located on 4 Chr, with several lipoprotein phenotypes coinciding at the same positions.

UC, TC, and V/LDL cholesterol levels fed the atherogenic diet exhibited QTLs at the position D5Mit10 (Figure 3A), with lod scores of 4.1, 3.8, and 2.8, respectively (Table 3). Mice homozygous for the MRL allele of D5Mit10 exhibited lower UC levels, TC levels, and V/LDL cholesterol levels at 5.5 months of age than those heterozygous or homozygous

### Table 2: Strength of Association* of Lipoprotein Levels in (MRL/lpr×BALB/cJ) F2 Mice

<table>
<thead>
<tr>
<th>Lipoprotein Parameter</th>
<th>TC Cholesterol</th>
<th>V/LDL Cholesterol</th>
<th>UC Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>260</td>
<td>260</td>
<td>260</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.28</td>
<td>−0.32</td>
<td>0.69</td>
</tr>
<tr>
<td>n</td>
<td>261</td>
<td>260</td>
<td>260</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V/LDL cholesterol</td>
<td>0.93</td>
<td>−0.09</td>
<td>−0.07</td>
</tr>
<tr>
<td>n</td>
<td>261</td>
<td>261</td>
<td>260</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>UC</td>
<td>0.90</td>
<td>0.32</td>
<td>0.14</td>
</tr>
<tr>
<td>n</td>
<td>261</td>
<td>261</td>
<td>262</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>&lt;0.0001</td>
<td>0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.09</td>
<td>0.33</td>
<td>−0.04</td>
</tr>
<tr>
<td>n</td>
<td>261</td>
<td>261</td>
<td>262</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>&lt;0.0001</td>
<td>0.51</td>
<td>0.28</td>
</tr>
</tbody>
</table>

*Strength of association are expressed as Fisher’s r to z correlation coefficients and P values using Statview 4.0 (Abacus Concepts Inc) application. Values to the top right of the diagonal are measured in F2 samples collected at 5.5 months of age including 2 months fed the atherogenic diet. Values to the bottom left are measured in F2 samples collected at 3.5 months of age including 2 months fed the chow diet, and values to the bottom right of the diagonal are measured in F2 samples collected at 3.5 months of age from mice fed the chow diet, and values to the bottom right of the diagonal are measured in F2 samples collected at 5.5 months of age including 2 months fed the atherogenic diet.
for the BALB/cJ allele (Figure 3B), whereas the mice homozygous for the BALB/cJ allele were not different from heterozygous mice. This indicates a recessive pattern of inheritance. The peak QTLs for all 3 lipoprotein levels exhibited coincident locations, suggesting that they all result from the same gene (Figure 3B).

UC levels on a chow diet exhibited a suggestive QTL at the marker D8Mit242 (Figure 4A), with a lod score of 3.1 (Table 3). Coincident with this QTL was a QTL for TC levels on a chow diet, exhibiting a peak lod score of 2.1. Mice homozygous for the MRL allele of D8Mit242 exhibited higher TC levels than those homozygous for the BALB/cJ allele. Heterozygotes exhibited intermediate levels. In addition, mice homozygous for the MRL allele of D8Mit242 exhibited higher V/LDL and UC levels than those homozygous for the BALB/cJ allele (Figure 4B). A promising candidate gene, lecithin-cholesterol acyltransferase (LCAT), is located precisely under the peak lod score. The LCAT activity was previously reported to be significantly decreased (by 41%) in MRL/lpr mice compared with strain ICR mice, suggesting...
that the increased UC levels in mice of the MRL genotype are due to deficient LCAT function.

TC, V/LDL cholesterol, and UC levels on the atherogenic diet exhibited significant QTLs at the position D15Mit17 (Figure 5A), with lod scores of 11.1, 6.7, and 4.6, respectively (Table 3). Mice homozygous for the MRL allele of D15Mit17 exhibited higher TC and V/LDL cholesterol on the atherogenic diet than those homozygous for the BALB/cJ allele. Heterozygotes exhibited levels intermediate between the parental strains (Figure 5B). The peak QTLs for these 3 lipoprotein levels exhibited coincident locations.

Chr 19 contained QTLs controlling levels of HDL cholesterol (lod score 8.4) and TC (lod score 3.8) fed the atherogenic diet (Figure 6A, Table 3). The Fas gene is located about 1 cM from the marker D19Mit12 and is a very attractive candidate gene underlying the Chr 19 QTL (see below). Mice homozygous for the MRL allele of D19Mit12 exhibited lower HDL cholesterol levels than those homozygous for the BALB/cJ allele (Figure 6B). The peak QTLs for the above 3 lipoprotein levels exhibited coincident locations (Figure 6A).

Interactions Between Autoimmune Disease, Lipoprotein Metabolism, and Atherosclerosis

The MRL/lpr mouse has many features of generalized autoimmune disease. As a quantitative measure of autoimmune disease, we determined the levels of anti-dsDNA antibodies in both parental strains as well as F1 and F2 mice. MRL/lpr mice exhibited significantly higher levels of autoantibodies than BALB/cJ mice (with OD numbers after ELISA assays of 0.94 ± 0.34 versus 0.05 ± 0.01). F1 mice had levels of anti-dsDNA antibodies intermediate between the parental strains. The anti-dsDNA antibody levels of the F2 mice exhibited a nonnormal distribution (Figure 7). Previous studies have shown that the Fas gene mutation is the primary determinant of autoimmune disease, including autoantibody levels, in...
MRL/lpr mice. We confirmed this finding in the present cross. In particular, the D19Mit12 marker yielded a highly significant QTL for anti-dsDNA antibody levels in the F2 mice (lod score 60). The other 3 lipoprotein QTLs identified in this cross, on Chrs 5, 8, and 15, showed no evidence of linkage to autoantibody levels in either the entire F2 population or the subpopulation that was homozygous for lpr or the subpopulation without the lpr mutation (data not shown).

We hypothesized that the Fas gene may influence HDL cholesterol levels and promote fatty streak lesion development through effects on autoantibody levels. To test this hypothesis, we compared autoantibody levels to HDL cholesterol levels in the 272 (MRL/lpr X BALB/cJ) F2 intercross mice. As seen in Table 4, anti-dsDNA antibody levels were inversely correlated with HDL cholesterol levels (r 0.37, P 0.0001). These results support the conclusion that the Fas gene mutation underlies the Chr 19 QTL for HDL cholesterol levels.

SLE patients exhibit about a tenfold increased rate of premature CAD compared with control populations. The above results suggested that this could be due, in part, to effects of autoimmune disease on lipoprotein metabolism. To test this hypothesis, we examined aortic fatty streak lesions in the parental strains and in their F2 progeny. Consistent with previous findings, BALB/cJ mice are relatively resistant to the development of fatty streak lesions in the aorta when challenged with an atherogenic diet, whereas strain MRL/lpr mice are moderately susceptible. The distribution of the fatty streak lesion scores in the F2 animals is shown in Figure 7. In the entire F2 population of 272 mice, the sizes of aortic lesions were not significantly associated with autoantibody levels (Table 4). However, there was a significant correlation between lesion size and autoantibody levels in the 44 mice homozygous for the lpr mutation (r 0.31, P 0.04). No correlation was observed in heterozygous mice or mice homozygous for the wild type Fas gene. This suggested a threshold effect on lesion development with respect to autoantibody levels. In support of this possibility, a more significant correlation was observed in animals with anti-dsDNA Ab levels over 0.5 OD (total 42 mice, r 0.45, P 0.004, Table 4). As expected from human epidemiological data and previous studies in mice, HDL cholesterol levels were significantly and inversely correlated with the size of aortic lesions (Table 4, r 0.17, P 0.006). The relatively low correlation coefficient is presumably due to the fact that the aortic lesion scores are determined by multiple genetic factors, such as LDL levels and factors acting at the level of the artery wall, in addition to HDL levels. Since autoantibody levels are strongly correlated with HDL cholesterol levels, one mecha-

Figure 4. Chr 8 QTL. A, Lod score plots for lipoprotein QTLs on Chr 8. TC values for mice fed the chow diet were transformed by taking the logarithm. UC values for mice fed the chow diet were transformed by taking the square root. B, Effects of Chr 8 QTL on lipoprotein levels. ANOVA was used to calculate the difference among all 3 genotypes.

Figure 5. Chr 15 QTL. A, Lod score plots for lipoprotein QTLs on Chr 15. TC and UC values for mice fed the HF diet were transformed by taking the logarithm. V/LDL cholesterol values for mice fed the HF diet were transformed by taking the square root. B, Effects of Chr 15 QTL on lipoprotein levels. ANOVA was used to calculate the difference among all 3 genotypes.
were observed when the mice homozygous for lpr showed no evidence of linkage to autoantibody levels and apparently unrelated to autoimmune disease because they atherogenesis. The other loci, on Chrs 5, 8, and 15, were link between autoimmunity, lipoprotein metabolism, and 60) and HDL cholesterol levels (lod score 8.4), providing a segregated strongly with both autoantibody levels (lod score 60) and HDL cholesterol levels (lod score 8.4), providing a link between autoimmunity, lipoprotein metabolism, and atherogenesis. The other loci, on Chrs 5, 8, and 15, were apparently unrelated to autoimmune disease because they showed no evidence of linkage to autoantibody levels and were observed when the mice homozygous for lpr were removed from the analysis. The possibility of an indirect effect of autoimmunity on these loci cannot, however, be ruled out because autoimmunity disease in MRL mice is under rules out because autoimmune disease in MRL mice is under strong candidate gene (Chr 8). The mechanisms by which these loci contribute to lipoprotein metabolism can now be further examined by analyzing the expression of candidate genes located within the QTLs and by isolation of the individual loci as congenic strains.

This study was designed to address possible genetic interactions between autoimmune disease, lipoprotein metabolism, and atherogenesis. In an intercross between the common laboratory strain BALB/cJ and the autoimmune strain MRL/lpr, lipoprotein levels were determined by 4 major loci, on Chrs 5, 8, 15, and 19. The locus on Chr 19, contained the Fas gene that is mutated in MRL/lpr mice and is the primary cause of autoimmune disease in this strain. This locus segregated strongly with both autoantibody levels (lod score 60) and HDL cholesterol levels (lod score 8.4), providing a link between autoimmunity, lipoprotein metabolism, and atherogenesis. The other loci, on Chrs 5, 8, and 15, were apparently unrelated to autoimmune disease because they showed no evidence of linkage to autoantibody levels and were observed when the mice homozygous for lpr were removed from the analysis. The possibility of an indirect effect of autoimmunity on these loci cannot, however, be ruled out because autoimmunity disease in MRL mice is under complex genetic control.

These data add to our understanding of genetic factors contributing to lipoprotein metabolism in the mouse model. A summary of QTLs for lipoprotein metabolism identified in this cross and in previous genetic studies is presented in Figure 8. The locations of QTLs are indicated by boxes centered around the peak lod scores obtained by interval mapping, and candidate genes are indicated by crossbars and gene symbols (see Reference 33 for description of candidate genes). Two of the loci observed in this cross, on Chrs 15 and 19, exhibited highly significant lod scores (11.1 and 8.4, respectively), and 2 of the loci, on Chrs 5 and 8, exhibited suggestive lod scores (4.1 and 3.1, respectively). The latter 2 loci are supported by coincident QTLs observed in previous studies (Chr 5) and by a very strong candidate gene (Chr 8). The mechanisms by which these loci contribute to lipoprotein metabolism can now be further examined by analyzing the expression of candidate genes located within the QTLs and by isolation of the individual loci as congenic strains.

The Chr 5 QTL, centered around marker D5Mit10, occurs at about the same location as QTLs controlling TC and HDL cholesterol levels observed previously in 2 other genetic crosses, a (NZB/B1NJ×SM/J) F2 intercross and a (C57BL/6J×C3H/HeJ) F2 intercross. An interesting candidate gene located within this region is the class B scavenger receptor (Srb1), which has been reported recently to encode an HDL receptor. Recently, we mapped this gene to mouse Chr 5, adjacent to the microsatellite marker D5Mit10. The SRB-1 protein has been shown to bind various lipoproteins, including HDL, with high affinity. It is expressed primarily in liver and nonplacental steroidogenic tissues and mediates selective cholesterol uptake by a mechanism distinct from the classic LDL receptor pathway. A recent study in which SRB-1 was expressed using an adenoviral vector revealed a dramatic impact on HDL cholesterol levels. Another possible candidate gene located in the 95% confidence interval region of the Chr 5 QTL is the gene for mevalonate kinase, which converts mevalonate acid to 5-phospho-mevalonic acid in the cholesterol biosynthetic pathway.

It should be mentioned that while coincident loci mapped in various genetic crosses might represent the same underlying gene, the possibility of different genes cannot be ruled out.

The Chr 8 QTL, centered around marker D8Mit242, contains the LCAT gene, which is located within about 2 cM of marker D8Mit242. LCAT catalyzes the esterification of free cholesterol present in plasma lipoproteins, and deficiencies of LCAT result in decreases in HDL cholesterol levels and increases in UC levels. Recently, transgenic mice expressing the human LCAT gene were established and found to exhibit increased TC and HDL cholesterol levels and decreased V/LDL cholesterol levels. Interestingly, strain MRL/lpr mice exhibit about half the LCAT activity of strain ICR mice. The QTL on Chr 8 also leads to significantly increased V/LDL cholesterol and UC levels (Figure 4B), consistent with the hypothesis that LCAT deficiency in MRL/lpr mouse underlies the Chr 8 QTL in this cross. Northern analyses using age controlled female mice fed the chow diet showed no significant difference in LCAT mRNA levels between MRL/lpr and BALB/cJ mice (data not shown). Thus, it is
likely that any difference in LCAT expression occurs at the translational or posttranslational level.

The Chr 15 QTL is centered near the marker D15Mit17 and appears to be the major determinant of both V/LDL and HDL levels in this cross. QTLs controlling TC and V/LDL cholesterol levels occur at the same position in an (NZB/B1NJ × SM/J) F2 intercross and a (C57BL/6J × C3H/HeJ) F2 intercross. An interesting candidate gene, Srebfd2, lies within this region. This gene was recently mapped to Chr 15, adjacent to the microsatellite marker D15Mit31, within the 95% confidence region of the Chr 15 QTL. The SREBP-2 protein belongs to a family of basic-helix-loop-helix-leucine zipper transcription factors that recognize sterol regulatory element 1. Sterol regulatory element 1, a conditional enhancer in the promoters for the LDL receptor gene, HMG-CoA synthase gene, and a number of other sterol responsive genes, increases transcription at low concentrations of sterols and is inactivated when sterols accumulate.

The Chr 19 QTL is near the marker D19Mit12. Our results suggest that the Fas gene underlies this QTL. The lpr mutation carried by the MRL/lpr mice was defined as the defective Fas apoptotic gene. This mutation leads to a breakdown of the central and/or peripheral tolerance, which results in the failure to properly clear CD4/CD8 negative T cells. In a previous genetic study, as well as the present cross, the Fas gene was identified as the major defect underlying the autoimmune manifestations, especially autoantibody levels. Our results showed that HDL cholesterol levels were strongly correlated with the levels of autoantibodies in the F2 mice. For example, at 5.5 months of age, when MRL/lpr mice and most F2 mice homozygous for the lpr mutation exhibited severe autoimmune disease, levels of HDL cholesterol were correlated with levels of autoantibodies (r = 0.37, P < 0.0001). At 3.5 months of age, when the manifestations of autoimmune disease were less severe, weaker correlations between levels of HDL cholesterol and levels of autoantibodies (r = 0.21, P = 0.0001) were observed. Thus, our data provide strong evidence for a link between autoimmune disease and HDL metabolism.

The molecular mechanisms mediating the interaction between autoimmune disease and HDL metabolism are unknown, although there are some plausible explanations. First, inflammatory processes frequently influence lipoprotein levels, possibly through the actions of various cytokines and growth factors. For example, macrophage-colony stimulating factor dramatically influences the levels of circulating lipoproteins and the uptake of oxidized lipoproteins by macrophages. Thus, the effects of the Fas gene mutation on HDL metabolism could be secondary to the inflammation accompanying autoimmune disease. Since the lpr mutation leads to the accumulation of autoantibodies, another possibility is that the autoantibodies in MRL/lpr mice cross-react with oxidized lipoprotein particles, resulting in increased HDL turnover. Some evidence suggest that lipoproteins in MRL/lpr mice are prone to oxidation. Recently, monoclonal anticardiolipin autoantibodies established from (NZW × BXSB) F1 and apoE knockout mice were shown to cross-react with oxidized lipoproteins. Because HDL is the major

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<th>TABLE 4. Correlation of Fatty Streak Lesions With Autoantibody Levels and HDL Cholesterol Levels in (MRL/lpr × BALB/cJ) F2 Mice*</th>
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<tbody>
<tr>
<td>Anti-dsDNA Antibody</td>
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*aAll mice were 5.5 months of age, including 3.5 months being fed chow diet and 2 months being fed atherogenic diet. A total of 272 F2 mice were used to generate these data.
†Strength of association expressed as Spearman’s ρ and P values. Bold numbers indicate a correlation observed in 42 F2 mice with anti-dsDNA Ab levels over 0.5 OD.
carrier of lipid hydroperoxides in human plasma, the HDL in MRL/lpr mice may be recognized by autoantibodies, leading to increased HDL turnover. The presence of anticardiolipin antibodies in apoE knockout mice, which develop advanced atherosclerotic lesions, could also explain the low levels of HDL cholesterol observed in these mice.

Well over a dozen loci contributing to lipoprotein metabolism have now been reported in genetic studies of inbred strains of mice, including the concept that autoimmune disease negatively impacts HDL cholesterol levels, as F2 mice carrying the lpr mutation of the Fas gene, the primary determinant of autoimmune disease in this genetic cross, appears to have a major impact on HDL cholesterol levels, as F2 mice carrying the lpr mutation exhibited decreased levels of HDL cholesterol. HDL cholesterol levels were, in turn, significantly associated with the size of aortic atherosclerotic lesions. Thus, in this model, the autoimmune phenotype contributes to the development of atherosclerosis by depressing levels of HDL cholesterol. Some human studies are consistent with the concept that autoimmune disease negatively impacts plasma lipoprotein levels, although the magnitude of this effect would not explain the dramatic increase in CAD death in SLE patients.

Our data also suggest that autoimmune disease may impact more directly on atherosclerosis. Although we failed to observe a significant correlation between autoantibody levels and atherosclerosis in the entire F2 cross, a significant correlation was observed among mice expressing high levels of autoantibodies. This suggests a threshold effect.

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References


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