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Genetics

Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice

(recombinant inbred strains/hyperalphalipoproteinemia/apolipoprotein A-II/chromosome 1)

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ABSTRACT **High density lipoprotein (HDL) is the major plasma lipoprotein found in mice fed normal laboratory chow containing 4% fat. When female mice from some inbred strains, such as C57BL/6, are fed a high fat diet (1.25% cholesterol, 15% fat, and 0.5% cholic acid), the levels of HDL-cholesterol decrease by about 50%, and lipid staining lesions form in the aorta within 14 weeks. In other strains of mice, such as C3H and BALB/c, HDL-lipid levels decrease only slightly, and few or no aortic lesions are observed at 14 weeks. The genetic basis of these phenotypic differences was analyzed by using recombinant inbred strains derived from C57BL/6 and BALB/c and also from C57BL/6 and C3H/He. The two phenotypes segregated as simple Mendelian traits, and no recombination was observed between them. Thus, HDL-cholesterol levels and susceptibility to atherosclerosis appear to be determined by the same gene (or by two closely linked genetic factors that are a maximum of 1.7 centimorgans apart). This gene was named *Ath-1*, for atherosclerosis susceptibility, with alleles *r* for resistance and *s* for susceptibility. *Ath-1* maps on chromosome 1 near *Alp-2*, a gene that determines the structure of apolipoprotein A-II, one of the two major proteins found in HDL. *Ath-1* is clearly separable from *Alp-2*, and the distance between these genes is 6.0 centimorgans with a standard error of 4.2 centimorgans. In humans, levels of HDL are inherited and are inversely correlated with atherosclerosis; familial hyperalphalipoproteinemia is associated with high levels of HDL-cholesterol and decreased risk of heart disease. The human trait phenotypically resembles *Ath-1* in the mouse.**

Epidemiological studies have shown that increased levels of high density lipoprotein (HDL) cholesterol are associated with a reduced risk of heart disease (1–3). Additionally, the genetic trait known as familial hyperalphalipoproteinemia is characterized by elevated levels of HDL-cholesterol, increased longevity, and reduced risk of coronary heart disease (4, 5). Taken together, these findings suggest a positive linkage between increased HDL levels and protection against coronary heart disease. The mechanism by which HDL protects against coronary heart disease is not yet understood, but several lines of evidence suggest that HDL may be involved in reverse cholesterol transport, a process by which excess cholesterol is removed from cells and carried to the liver for excretion in the bile (6, 7).

Considerable variation in susceptibility to atherosclerosis has been demonstrated among inbred strains of mice (8, 9). A susceptible strain, C57BL/6, and two resistant strains, C3H and BALB/c, were characterized for cholesterol and lipoprotein levels before and after being fed an atherogenic diet containing 1.25% cholesterol, 15% fat, and 0.5% cholic acid (10). HDL-cholesterol levels in the atherosclerosis-susceptible strain fell to half the normal value, while the HDL levels in both resistant strains decreased only slightly (10). All three strains showed similar increases in low density lipoproteins (LDL) and very low density lipoproteins (VLDL).

We now report on the genetic basis for the differences in atherosclerotic lesion formation and HDL-cholesterol levels among strains C57BL/6, C3H, and BALB/c. The genetic analysis used recombinant inbred (RI) strains made between the atherosclerosis-susceptible strain C57BL/6 and one of the two atherosclerosis-resistant strains. The use of RI strains provides a means of analyzing whether a single phenotypic difference is caused by one or multiple genes and whether two different phenotypes are determined by the same or closely linked genes; it also provides a means of determining the map location of genes (11, 12). Among these three strains, the phenotypes of atherosclerosis susceptibility and HDL-cholesterol levels are both inherited as single Mendelian traits. They appear to be determined by the same or closely linked genetic factors located on mouse chromosome 1 near a cluster of genes determining the structure and regulation of apolipoprotein molecules. Despite this proximity, it is clear that the new gene, designated *Ath-1* (for atherosclerosis susceptibility), is distinct from previously known genes in this region.

MATERIALS AND METHODS

Materials. Oil red O was obtained from Aldrich; hemotoxylin light green and O.C.T. embedding medium were from Fisher; electrophoretic grade agarose was from Bio-Rad; GelBond plastic support medium for gels was from FMC, Rockland, ME; and other chemicals were from Sigma. Normal chow was Purina chow containing 4% fat. Preparation of an atherogenic diet, which contains by weight 15% fat, 1.25% cholesterol, and 0.5% cholic acid, has been described (8).

Animals. Mice were obtained from The Jackson Laboratory except for the set of RI lines between C57BL/6J and A/J (A × B and B × A), which were a gift from M. Nesbitt (University of California, San Diego, CA). The strains used, unless indicated otherwise, were C57BL/6J, C3H/HeJ, BALB/cJ, and recombinant inbreds. Earlier data showed that C57BL/6ByJ and BALB/cByJ were comparable to C57BL/6J and BALB/cJ in HDL-lipid levels and atherosclerotic lesion formation (8). This is important because the

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Abbreviations: HDL, high density lipoprotein; RI, recombinant inbred strain; apoA-II, apolipoprotein A-II; BXH, a recombinant inbred strain derived from C57BL/6J and C3H/HeJ; CXB, a recombinant inbred strain derived from BALB/cBy and C57BL/6By; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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recombinant inbred set CXB used C57BL/6By and BALB/cBy as parents. All animals were housed in a temperature-controlled facility with a 12-hr light/dark cycle.

Experimental Design. Female mice, 2–4 months of age, were used. Blood samples were obtained from animals just before they were placed on the atherogenic diet and after 4 weeks of atherogenic diet consumption. Changes in HDL-lipid levels were stable from 3 to 14 weeks on the atherogenic diet (10), and 4 weeks was selected as the standard protocol for determining the HDL phenotype. Mice were maintained on the atherogenic diet for 14 weeks and then were sacrificed after an overnight fast; the hearts and the beginning portion of the aorta were removed, fixed, and evaluated for lesions.

Histology. The formalin-fixed hearts were embedded in gelatin and sectioned on a cryostat as described (10, 13). The number of oil red O staining lesions was counted in a standardized region of the aorta as described (13). Hearts were coded so that the scoring was done blindly as to identity of the heart.

Analytical Methods. Blood was collected into tubes containing EDTA (1 mM final concentration). Plasma was prepared by sedimenting cells at 1000 × *g* for 15 min at 5°C. Plasma was stored at 4°C up to 1 week before being analyzed. Cholesterol was determined according to the procedure of Rudel and Morris (14). HDL-cholesterol was determined after the removal of LDL and VLDL by selective precipitation with PEG (15) or phosphotungstate (16). Agarose gel electrophoresis of mouse plasma was performed by a modification (10) of the method of Noble *et al.* (17), which separates β lipoproteins (LDL and VLDL) from lipoproteins of higher mobility (HDL). The determination of apolipoprotein A-II (apoA-II) isoforms by isoelectric focusing was carried out as described by Lusis *et al.* (18).

Phenotypic Scoring. For lesion formation in the aorta, the phenotype of resistance to atherosclerosis was defined as 0–0.4 lesion per mouse after 14 weeks of the atherogenic diet; the phenotype of susceptibility to atherosclerosis was defined as more than 0.8 lesion per mouse. For HDL-cholesterol or HDL-lipid levels, the phenotype of resistance to atherosclerosis was defined as an HDL-cholesterol level of 60–90 mg/dl after 4 weeks on the atherogenic diet, a value that is comparable to the HDL-cholesterol levels in mice fed chow. The phenotype of atherosclerosis susceptibility was defined as an HDL-cholesterol level of 30–45 mg/dl, a value that represents a 40–50% decrease in HDL-cholesterol levels as compared to the values of mice fed chow. RI strains did not exhibit intermediate phenotypes, and there was no ambiguity in scoring the phenotype of each strain. After precipitation of LDL and VLDL, HDL was scored as HDL-cholesterol levels determined by chemical assay or by HDL-lipid levels determined after agarose gel electrophoresis and staining with sudan black. The chemical assay measures both free cholesterol and cholesterol ester, whereas staining by sudan black measures neutral lipids such as triglyceride and cholesterol ester. Since murine HDL contains almost no triglyceride (19, 20) and since cholesterol ester accounts for 87–93% of the total cholesterol (19, 20), the two methods are nearly equivalent. HDL-lipid, as measured by sudan black staining of agarose gels, was the method used preferentially for scoring the HDL phenotype because agarose gels require only a small quantity of plasma. The same mouse can be used for HDL-lipid levels before and after being fed an atherogenic diet and for lesions at 14 weeks. HDL-lipid levels, estimated visually or by densitometric scanning, were compared to Precilip, a human plasma standard, or to standards made from pooled plasma of C57BL/6 mice fed chow or the atherogenic diet.

RESULTS

Atherosclerosis Susceptibility in Parental Strains and Hybrids. When female mice of strains C57BL/6, BALB/c, and C3H were on a low fat diet of normal chow, HDL transported most of the neutral lipid (Fig. 1). From composition studies, about 90% of this neutral HDL-lipid is cholesterol ester (19, 20). HDL also transports phospholipid (19, 20), but sudan black does not stain phospholipid. When animals were fed a high fat atherogenic diet, the quantity of cholesterol transported by LDL and VLDL increased dramatically and to approximately the same extent in all three mouse strains (Table 1). In the two resistant strains, BALB/c and C3H, HDL-cholesterol levels in mice on the atherogenic diet remained in the 60–90 mg/dl range typical of chow-fed animals, but in the susceptible strain C57BL/6, the HDL-cholesterol levels decreased by about 50% into the range of 30–45 mg/dl (Fig. 1 and Table 1). Thus, it appears that the critical difference among the strains is in the level of HDL and not in the level of VLDL and LDL.

Because HDL-lipid determinations using agarose gel electrophoresis can be carried out using the small volumes of plasma obtained from a live mouse, whereas chemical determinations of HDL-cholesterol require sacrificing animals to obtain sufficient plasma, we used the electrophoretic procedure in subsequent experiments. The HDL-lipid levels of mice fed the atherogenic diet for 4 weeks are depicted in Fig. 2 for strains C57BL/6, BALB/c, and C3H. Strain BALB/c had HDL levels that were intermediate compared to C57BL/6 and C3H. The range of HDL-lipid levels did not overlap for strains C57BL/6 and C3H; the range for BALB/c and C57BL/6 met but also did not overlap.

The expression of atherosclerosis susceptibility and HDL-lipid levels were tested in parental strains and in F_1 progeny from crosses between C57BL/6 and the resistant strains, C3H/He and BALB/c (Table 2). After 14 weeks on the atherogenic diet, the average number of aortic lesions per mouse was 1.2 ± 0.2 (mean ± SEM) for the atherosclerosis-susceptible strain C57BL/6 and 0 for both resistant strains, C3H and BALB/c (Table 2). Lesion formation in F_1 progeny between C57BL/6 and the resistant strains appeared to be intermediate. However, these values differed from C57BL/6 with only borderline significance ($P = 0.05$ and 0.06, respectively), so the question of whether or not lesion formation is truly intermediate in heterozygotes requires additional testing. On the atherogenic diet, HDL-lipid levels in the (C57BL/6 × BALB/c)F_1 mice resembled the BALB/c parent while those in the (C57BL/6 × C3H)F_1 mice appeared to be intermediate. Although C3H mice have higher HDL-lipid levels than BALB/c, the levels in the two F_1 heterozygotes were rather similar.

We also tested an F_1 between the two resistant strains, BALB/c and C3H/HeJ. If resistance to atherosclerosis was due to two different recessive genes, the F_1 hybrid between the resistant parents might have had lesions, indicating that the genes determining resistance in BALB/c and C3H were different. However, the (C3H × BALB/c)F_1 hybrid was resistant to lesion formation (Table 2).

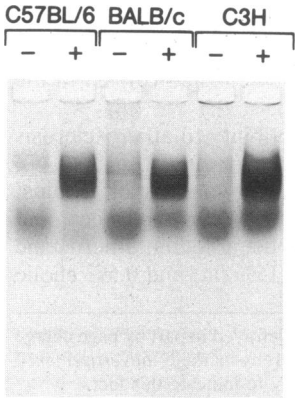

FIG. 1. Agarose gel electrophoresis of plasma from strains of mice fed a low or high fat diet. Mice were fed a chow (−) or an atherogenic (+) diet containing 4% or 15% fat, respectively, for 4 weeks. Each lane represents 15 μl of plasma pooled from three female mice of each strain. The upper band is LDL and VLDL; the lower band is HDL. Sudan black stain indicates neutral lipids (cholesterol ester and triglycerides).

Table 1. Cholesterol levels in female mice of strains C57BL/6, BALB/c, and C3H

Cholesterol	Diet	Exp.	Cholesterol, mg/dl C57BL/6	BALB/c	C3H
Total	LF	1	69 ± 4	73 ± 5	113 ± 12
		2	66 ± 14	93 ± 10	84 ± 3
Total	Ath	1	146 ± 57	111 ± 32	119 ± 9
		2	192 ± 26	181 ± 10	207 ± 32
HDL	LF	1	65 ± 7	65 ± 6	87 ± 10
		2	62 ± 9	69 ± 14	69 ± 13
HDL	Ath	1	31 ± 6	72 ± 11	62 ± 8
		2	39 ± 7	64 ± 8	69 ± 9
LDL, VLDL	LF	1	5	8	25
		2	4	15	24
LDL, VLDL	Ath	1	115	49	46
		2	153	117	138

Results are means ± SD. Experiments 1 and 2 were done in separate laboratories with slightly different protocols. Exp. 1, done by R.C.L., used 4–6 mice per group, sampled mice after 3 weeks on the atherogenic diet, and precipitated the LDL and VLDL with phosphotungstate. Exp. 2, done by B.P., used 3 mice per group, sampled mice after 4 weeks on the atherogenic diet, and precipitated LDL and VLDL with PEG. The LDL and VLDL values are calculated by subtracting mean HDL-cholesterol values from total cholesterol values, so there are no standard deviations. Exp. 2 was reported earlier (10) and is included here for comparison. LF, low fat; Ath, atherogenic.

Survey of RI Strains. RI strains are a relatively recent tool in mouse genetics that is replacing the more laborious backcross analysis. RI strains are constructed by crossing two progenitor strains and establishing a series of new homozygous inbred strains from their progeny. Each new RI strain consists of a unique mixture of genes in a homozygous state derived from the two parental strains. The set of RI strains permits rapid linkage analysis because alleles of linked genes tend to be linked in the same combination as in the parents, whereas unlinked genes are randomized. Data for segregation of markers is cumulative. Each RI strain is typed for a given marker once, and a new genetic variant can be mapped by comparing its strain distribution pattern of alleles to the strain distribution patterns of previously typed markers. Concordant distributions indicate linkage. Sets of RI strains have been constructed between C57BL/6J and C3H/HeJ (BXH) and between BALB/cBy and C57BL/6By (CXB).

Plasma from female mice of each recombinant inbred strain, which had been fed the atherogenic diet for 4 weeks, was scored as having either the S phenotype (reduced levels of HDL-lipid like the susceptible C57BL/6 parent) or the R phenotype (having higher levels of HDL-lipid like the resistant parent). The aortas were monitored for lesion formation at 14 weeks. RI strains with mean number of lesions per mouse of 0.8 or more were scored as susceptible to atherosclerosis (S phenotype); strains with 0–0.4 lesion per animal were scored as resistant to atherosclerosis (R phenotype).

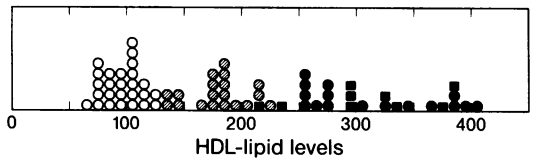

FIG. 2. HDL-lipid levels in mice fed atherogenic diet. HDL-lipid levels were determined by densitometry of agarose gels using a pooled plasma sample of C57BL/6 females fed chow as a standard. The standard was set to equal 200, which is equal to 65 mg of cholesterol per dl. The open circles are female C57BL/6 (n = 26); the hatched circles are female BALB/c (n = 19); the closed circles are female C3H (n = 16); and the closed squares are male C3H (n = 9).

Table 2. Aortic lesions and agarose gel HDL-lipid levels in female mice of strains C57BL/6, BALB/c, C3H, and F_1 progeny fed an atherogenic diet

Strain	Lesion(s) per mouse	HDL-lipid levels Low fat	High fat
C57BL/6	1.2 ± 0.2 (6)	202 ± 7 (12)	101 ± 2 (16)
C3H	0 (5)	310 ± 14 (8)	261 ± 15 (11)
BALB/c	0 (5)	228 ± 11 (10)	174 ± 7 (12)
(C57BL/6 × C3H)F_1	0.7 ± 0.2 (6)	172 ± 5 (10)	197 ± 13 (7)
(C57BL/6 × BALB/c)F_1	0.6 ± 0.3 (6)	212 ± 5 (12)	168 ± 7 (12)
(C3H × BALB/c)F_1	0 (5)	ND	ND

HDL-lipid values are based on densitometric scans of agarose gels and are expressed as a percentage of a human plasma standard, Precilip. Number of animals studied is indicated in parentheses. ND, not determined. Results are means ± SEM.

Strains having an intermediate number of lesions per mouse were not found.

If the phenotypes of atherosclerosis susceptibility and HDL levels for mice fed the atherogenic diet are determined by the same genetic factor, then these phenotypes should cosegregate. Even one discordant RI strain (e.g., one that was susceptible to atherosclerosis but had high levels of HDL) would suffice to show that the phenotypes were determined by separate genes. The data in Table 3 show that the two phenotypes cosegregated for all 17 RI strains examined, indicating that atherosclerosis susceptibility and HDL levels are determined by the same gene or by two very closely linked genes. In unpublished experiments (B.P., D.M., R.C.L., and M. Nesbitt), we have also examined 30 RI strains from a set made between C57BL/6J and the resistant strain A/J; the phenotypes of lesion formation and HDL levels are completely concordant in these RI strains also. If the two phenotypes are determined by different genes, these genes must be very closely linked; the absence of recombinants among 47 RI strains can be used to calculate the maximum distance between the two markers at a 95% confidence interval by the formula (12):

$$r_{max} = R_{max}/(4 - 6R_{max}),$$

where r = probability of recombination in a single meiosis; R = probability of fixation of a recombinant genotype during the process of developing a RI strain; R_{max} = the upper 95% confidence limit of R based on binomial distribution (i.e., $1 - e^{(\ln 0.05)/n}$); and n = number of RI strains tested. By using these formulas the maximum distance between the two genes (if two genes exist) at 95% confidence would be 1.7 centimorgans.

We have named the gene that causes the phenotypic differences in atherosclerosis susceptibility *Ath-1* (for atherosclerosis susceptibility) with alleles *s* for susceptibility and *r* for resistance.

Mapping *Ath-1*. The location of *Ath-1* in the murine genome was determined by comparing the strain distribution pattern of *Ath-1* in RI strains to the strain distribution pattern of other polymorphisms mapped for these RI sets. As shown in Tables 4 and 5, *Ath-1* is on chromosome 1, closely linked to *Alp-2*, a gene that determines the structure of apoA-II (18, 19). ApoA-II is a major protein component of HDL. The most likely map order is *Ltw-4, Ath-1, Alp-2, Hdl-1, Ly-9* as determined by the RI strains.

Ath-1 is clearly a separate gene from *Alp-2* because three different RI strains are recombinant for the two genes. The separation of *Ath-1* and *Alp-2* was confirmed in the congenic strain B6.C-*H-25*^{c}, which was made by moving the closely linked histocompatibility marker *H-25* from BALB/c into

Table 3. Segregation of atherosclerosis resistance and HDL levels among RI strains between the atherosclerosis susceptible strain C57BL/6 and resistant strains C3H and BALB/c

Strain	*n*	Lesion(s) per mouse	HDL levels		*Ath-1* phenotype
			Low fat	Atherogenic	
C57BL/6	5	1.2 ± 0.2	4.0 ± 0.2	1.9 ± 0.2	S
C3H	5	0	4.8 ± 0.1	4.7 ± 0.1	R
BXH-7	5	1.2 ± 0.2	4.2 ± 0.2	2.1 ± 0.2	S
BXH-8	5	0.8 ± 0.2	3.9 ± 0.2	2.0 ± 0.1	S
BXH-10	5	1.5 ± 0.3	4.0 ± 0.1	2.1 ± 0.1	S
BXH-11	5	1.0 ± 0.4	4.2 ± 0.1	2.4 ± 0.2	S
BXH-3	5	0	4.3 ± 0.1	4.4 ± 0.1	R
BXH-4	5	0	4.5 ± 0.3	4.3 ± 0.1	R
BXH-6	8	0	4.5 ± 0.1	4.1 ± 0.1	R
BXH-9	5	0.2 ± 0.2	4.6 ± 0.2	4.0 ± 0.1	R
BXH-12	5	0	4.6 ± 0.1	4.5 ± 0.2	R
BXH-14	4	0.3 ± 0.3	4.6 ± 0.1	3.9 ± 0.1	R
C57BL/6	5	1.0 ± 0.4	4.0 ± 0.2	2.0 ± 0.2	S
BALB/c	5	0	4.2 ± 0.1	3.7 ± 0.1	R
CXB-E	7	1.9 ± 0.4	4.0 ± 0.2	2.1 ± 0.1	S
CXB-I	5	2.8 ± 0.2	4.1 ± 0.1	1.6 ± 0.2	S
CXB-D	5	0.4 ± 0.2	4.1 ± 0.1	3.8 ± 0.2	R
CXB-G	10	0	4.0 ± 0.1	3.5 ± 0.2	R
CXB-H	5	0.4 ± 0.2	3.8 ± 0.1	3.1 ± 0.2	R
CXB-J	10	0.3 ± 0.1	3.8 ± 0.1	3.7 ± 0.2	R
CXB-K	5	0	3.7 ± 0.1	3.5 ± 0.2	R
C57BL/6	5		215 ± 5	130 ± 19	S
C3H	6		283 ± 6	291 ± 3	R
BXH-3	6		220 ± 4	250 ± 4	R
BXH-4	6		203 ± 6	251 ± 6	R

Results are means ± SEM. HDL values <5 are based on visual estimates. Initially a densitometer was not available and the agarose gels were scored twice by two different technicians (i.e., four numbers were averaged per gel). The score was based on a comparison to standard pooled plasmas from C57BL/6 mice fed a low fat diet (assigned a score of 4) or C57BL/6 mice fed an atherogenic diet (assigned a score of 2). Scores of 1, 3, and 5 were assigned as being <, intermediate, or > the standard plasmas, respectively. When a densitometer was available, comparison of 50 visual estimates with densitometer readings gave a correlation coefficient of 0.85. HDL values >100 are based on densitometer readings compared to a standard pooled plasma of C57BL/6 fed a chow diet. The standard was arbitrarily given a value of 200. The strains in this third group were repeated because BXH-3 and BXH-4 appeared to be recombinant for *Ath-1* and *Alp-2*. R, phenotype of atherosclerosis resistance based on aortic lesions of 0–0.4 lesion per mouse and HDL-lipid levels in animals on the atherogenic diet in the range of 3.1–4.7 units (comparable to the HDL-lipid in resistant parental strains). S, phenotype of atherosclerosis susceptibility based on aortic lesions of >0.8 lesion per mouse and HDL-lipid levels in animals on atherogenic diet in the range of 1.6–2.4 units (comparable to C57BL/6 mice on atherogenic diet).

C57BL/6. This strain carries the C57BL/6 allele for susceptibility to atherosclerosis as shown by lesion formation and HDL levels (Table 6), and we confirmed the earlier report (18) that this congenic strain carries the BALB/c allele for *Alp-2*. It is therefore a recombinant for *Ath-1* and *Alp-2*.

The map distance between *Ath-1* and *Alp-2* can be calculated using the formula $r = R/(4 - 6R)$, where R in this instance is three recombinants in 17 RI strains, to give a map distance of 6.0 centimorgans with a standard error of 4.2 centimorgans (map distances are given as the distance ± the standard error in centimorgans).

DISCUSSION

These data indicate that the difference in atherosclerosis susceptibility between C57BL/6 and either BALB/c or C3H is due to a single major gene *Ath-1*, mapping on chromosome 1 near *Alp-2* and *Hdl-1*. That a single major gene is affecting the atherosclerosis phenotype is suggested by RI strains being either resistant or susceptible to lesion formation and having high or low HDL levels, respectively. There may be some question as to whether 0.2–0.4 lesion per mouse, as occurred in some RI strains classified as resistant, constitutes an intermediate phenotype suggesting the presence of some modifying genes with small effects. To answer the question of whether 0.2–0.4 lesion per mouse is significantly different from 0 lesion per mouse would require much larger numbers of animals than those used in these experiments. The fact that the susceptible strain CXB-I has lesions that are considerably higher than the parental strain C57BL/6 also suggests the presence of modifying genes. However, we can conclude that a single major gene is determining lesion formation.

Initially, we had some hesitation concerning the validity of this map location because 3 of 17 strains show a recombination between *Alp-2* and *Ath-1* and because the map order does require a double crossover event in strain BXH-4. However, subsequent crosses between C57BL/6 and BALB/c (D.M., B.P., and P. Holmes, unpublished data) and between C57BL/6 and C3H (27) confirmed the single gene difference and the map location. In these two crosses, seven recombinants for *Alp-2* and *Ath-1* were found in 144 tested chromosomes, indicating a map distance of 4.9 ± 1.8 centimorgans. This map distance agrees well with the distance of 6.0 ± 4.2 centimorgans calculated from the RI data.

It was somewhat surprising to find that HDL levels in F_1 mice were codominant in the cross between C57BL/6 and

Table 4. Strain distribution pattern of *Ath-1* and other loci on chromosome 1 in RI strains derived from C57BL/6 and C3H

Gene	BXH strain									
	3	4	6	7	8	9	10	11	12	14
Ltw-4	H	B	H	B	B	H	B	B	H	H
Ath-1	H	H	H	B	B	H	B	B	H	H
Alp-2	B	B	H	B	B	H	B	B	H	H
Hdl-1	B	B	H	B	B	H	B	B	H	H
Ly-9	H	B	H	B	B	H	B	H	H	H
H-25	H	B	B	B	B	H	B	H	H	H
Akp-1	H	H	B	B	B	H	B	B	H	H

B, genotype found in the C57BL/6 parent; H, genotype found in the C3H parent. *Ltw-4* controls a major protein variant of liver and kidney identified by 2-dimensional gel electrophoresis (21). *Alp-2* determines the structure of apoA-II and *Hdl-1* determines the size of HDL particles (18). *Ly-9* is a lymphocyte alloantigen locus (22), *H-25* is a histocompatibility locus (23), and *Akp-1* is alkaline phosphatase-1 (24). The approximate distance from *Ltw-4* to *Akp-1* is 15 centimorgans according to the 1986 mouse genetic map (28). Braces between genes indicate a crossover event.

Table 5. Strain distribution pattern of *Ath-1* and other loci on chromosome 1 in RI strains derived from C57BL/6By and BALB/cBy

Gene	CXB strain						
	D	E	G	H	I	J	K
Ltw-4	C	B	B	C	C	B	C
Ath-1	C	B	C	C	B	C	C
Alp-2	C	B	C	B	B	C	C
Hdl-1	C	B	C	B	B	C	C
Ly-9	C	B	C	B	B	C	C
H-25	C	B	C	B	B	B	C
Akp-1	C	B	C	B	B	B	C

B, genotype found in the C57BL/6 parent; C, genotype found in the BALB/c parent. Genetic markers are as described in Table 4. Braces between genes indicate a crossover event.

Table 6. Lesion formation in a C57BL/6By congenic strain carrying a segment of chromosome 1 from BALB/c

Strain	*n*	Lesions per mouse	*Ath-1*	*Alp-2*	*Hdl-1*
C57BL/6	6	1.1 ± 0.1	B	B	B
BALB/c	5	0	C	C	C
B6.C-*H-25*^{c}	6	1.8 ± 0.2	B	C	C

Results are means ± SEM. B, phenotype found in C57BL/6; C, phenotype found in BALB/c or C3H. B6.C-*H-25*^{c} is a congenic strain that has moved the chromosomal region surrounding the histocompatibility marker *H-25* from BALB/c into a C57BL/6By background by repeated backcrossing. Brace between genes indicates a crossover event.

C3H, but high HDL levels were dominant in the cross between C57BL/6 and BALB/c (Table 2). We presently have no explanation for this. Perhaps the *Ath-1*^{r} alleles in BALB/c and C3H are different or perhaps other strain differences are present. We have already noted (10) that HDL levels are affected by diet, sex, and testosterone treatment in BALB/c, but none of these factors affect the HDL levels in C3H mice. Furthermore, a comparison of relative HDL-lipid levels in all three strains fed an atherogenic diet show that C57BL/6 has about 100, BALB/c about 175, and C3H about 300 units. Thus BALB/c and C3H differ in several ways.

The mouse is being used increasingly as an experimental model for atherosclerosis research. Murine lipoproteins have been characterized, studies on the lipid transport have been initiated, and genetic variation in apolipoproteins have been examined (8–10, 13, 18–20). The present studies show that the sophisticated genetic system of the mouse can be used to add a genetic approach to the analysis of atherosclerosis. Moreover, because chromosomal organization has been preserved during evolution, the mapping of *Ath-1* in the mouse suggests the possibility of an equivalent gene at the homologous location in humans. So far, use of both human and murine data indicates clusters of apolipoprotein genes on homologous chromosome segments. The region of mouse chromosome 1 containing *Ath-1* also contains the genes for renin, peptidase, and apoA-II. The homologous region is present on human chromosome 1; it also contains the genes for renin, peptidase, and apoA-II (25). In addition to these structural genes, there is an interesting regulatory similarity between the mouse and human regions. The mouse locus *Hdl-1*, which is closely linked to *Alp-2*, determines the size of HDL particles by determining the molar ratio of apoA-I/apoA-II in the HDL particles (R.C.L., unpublished data). A similar locus occurs in the human (26), which also determines the apoA-I/apoA-II ratio. Alleles are recognized by a restriction site polymorphism, which maps on the 3′ side of the human apoA-II gene.

These similarities between the homologous regions of human and mouse chromosome 1 suggest that a human gene that is homologous to *Ath-1* may be located there. A candidate gene is the presently unmapped locus determining hyperalphalipoproteinemia. Affected individuals have levels of HDL-cholesterol in the 70–90 mg/dl range as compared to the average HDL-cholesterol value of 45 mg/dl (4). Families with hyperalphalipoproteinemia are characterized by increased longevity and reduced risk of heart disease (4). The trait was originally reported to segregate as an autosomal dominant trait, but recent evidence suggests that alleles at this locus may show additive inheritance. A family has now been described by Saito (5) in which both parents are hyperalphalipoproteinemic. Among their progeny, two individuals showed extremely high levels of HDL-cholesterol (>150 mg/dl), suggesting that the affected parents are heterozygous individuals and that the exceptional progeny are homozygous for hyperalphalipoproteinemia.

The present results illustrate the potential role that mouse genetic variants can play in analyzing the pathophysiology of common diseases and the genetic basis for susceptibility and also in providing experimental material for testing new therapeutic approaches.

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