domains (*7*), there may be another cDNA that encodes a secreted form of ADA. If the ADA on the cell surface is encoded by the same cDNA as the internal ADA, then (i) ADA may be expressed on the surface after binding to CD26 inside the cells; (ii) membrane ADA may be expressed by a yet-to-be defined mechanism not requiring hydrophobic domains, as is predicted from the secretions of the interleukin-1s and the fibroblast growth factors; or (iii) the ADA expressed on the cell surface may be derived from dead cells.

15. T. Tanaka *et al.*, unpublished data. The soluble form of CD26 was purified by concanavalin-A and anti-CD26 (1F7) affinity chromatography from culture supernatants of CHO cells transfected with mutant CD26 lacking the codons for amino acids 3 to 9. SDS-PAGE followed by a staining with Coomassie brilliant blue revealed one single band of 110 kD.
16. The purity of the calf spleen ADA was greater than 90% by SDS-PAGE. Calf spleen ADA was used because human ADA was not available, but we presume that the positive results with calf ADA were highly suggestive of the similar interaction between human CD26 and human ADA. The use of calf ADA was also suggested on the basis of evidence that characteristics of the binding between ADA and the ADA binding protein are well conserved among various species (*20*).
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# Atherosclerosis in Transgenic Mice Overexpressing Apolipoprotein A-II

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**Concentrations of plasma high density lipoprotein (HDL) are inversely correlated with atherosclerotic coronary artery disease. The two most abundant protein constituents of HDL are apolipoproteins A-I and A-II (apoA-I and apoA-II). ApoA-I is required for assembly of HDL and, when overexpressed in transgenic mice, confers resistance to early atherosclerosis. The present studies reveal that transgenic mice that overexpress mouse apoA-II had elevated HDL-cholesterol concentrations but, nevertheless, exhibited increased atherosclerotic lesion development as compared to normal mice. The HDL in the transgenic mice was larger and had an increased ratio of apoA-II to apoA-I. Thus, both the composition and amount of HDL appear to be important determinants of atherosclerosis.**

High density lipoproteins (HDL) are particles found in the blood plasma that contain approximately equal parts lipid and protein. The most abundant proteins of HDL are apolipoproteins A-I and A-II (apoA-I and apoA-II). Low concentrations of HDL in the plasma are associated with increased risk of atherosclerotic coronary artery disease (CAD), but the mechanisms responsible have remained elusive (*1–5*). HDL may protect against CAD by removing excess cholesterol from peripheral tissues and transporting it to the liver, a process termed "reverse cholesterol transport". Alternatively, the association of atherosclerosis with low HDL levels may reflect the metabolism of triglyceride-rich and other atherogenic lipoproteins, as suggested by the observation that rare genetic syndromes resulting in less HDL are not always associated with increased CAD risk (*2, 5, 6*). Fractionation of HDL by electrophoresis and immunochemical procedures has revealed several subpopulations differing in size and apolipoprotein composition (*7–9*). Several, but not all, studies have shown that HDL containing both apoA-I and apoA-II are less effective at promoting cholesterol efflux from tissue culture cells than are HDL containing apoA-I alone (*10–14*). We report here that overexpression of mouse apoA-II in transgenic mice results in an increase in the number and size of fatty streak atherosclerotic lesions, even though total plasma HDL-cholesterol levels are significantly elevated.

Transgenic mice overexpressing human apoA-I show elevated plasma HDL-cholesterol (*15, 16*) in association with reduced aortic fatty streak development when maintained on atherogenic diets high in fat and cholesterol (*17*). Whether this is a result of alterations in HDL subpopulations or increased HDL levels is unclear. This observation suggests that HDL is directly involved in the pathogenesis of atherosclerosis. Studies of naturally occurring variations of apoA-II gene expression in mice suggest that apoA-II can influence both HDL amounts and apolipoprotein compositions (*18, 19*). To examine the contribution of HDL subpopulations to the early stages of atherosclerosis, we have constructed transgenic mice overexpressing mouse apoA-II.

Transgenic mice were constructed using a genomic clone of the mouse apoA-II gene containing several kilobases of 5′ flanking and 3′ flanking DNA (*20*). Founder transgenic mice were backcrosses derived from a cross of C57BL/6J with C57BL/6J × DBA/2J heterozygotes. All of the mice used in this study were derived from a line developed from one of the founder mice by backcrossing for two generations to strain C57BL/6J mice, such that on average 93% of the genetic back-

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ground was derived from the C57BL/6J strain. Southern (DNA) blot analysis revealed that approximately 10 copies of the apoA-II gene were integrated into the genome in this line (*20*). C57BL/6J were used as the background strain because previous studies have shown that C57BL/6J mice are relatively susceptible to the development of early atherosclerotic lesions (fatty streaks) in the aorta when maintained on high fat, high cholesterol diets (*19, 21–23*).

On a low fat chow diet, the ratio of apoA-II to apoA-I in HDL of transgenic animals was increased about threefold (Table 1). Male transgenic mice exhibited higher apoA-II concentrations than female mice, whereas apoA-I concentrations were similar among all the groups (Table 1). Lipoproteins from transgenic mice and nontransgenic littermates were examined by density fractionation (Fig. 1A), agarose gel electrophoresis (Fig. 1B), and gel filtration chromatography (Fig. 1C). Lipoproteins were separated by density, utilizing ranges established for human lipoproteins: density less than 1.019 g/ml for very low and intermediate density lipoproteins (VLDL and IDL), density 1.019 to 1.063 g/ml for low density lipoproteins (LDL), and density 1.063 to 1.21 g/ml for HDL. HDL-cholesterol concentrations were elevated about twofold and the average HDL particle size was increased in the transgenic mice (Fig. 1C), consistent with previous studies of naturally occurring variations of apoA-II (*19*). The transgenic mice accumulated a particle rich in cholesteryl-ester. The particle was similar in density (d = 1.019 to 1.063 g/ml) and size (20.5- to 25-ml elution volume in Fig. 1C) to LDL. Upon agarose gel electrophoresis, the particle migrated slower than HDL and faster than LDL and VLDL (Fig. 1B), and it was present in only trace amounts in nontransgenic mice (Fig. 1B, d1.019 to d1.063). As judged by immunoblot analysis of lipoproteins separated

**Table 1.** Plasma lipoprotein concentrations and HDL apolipoprotein compositions in apoA-II transgenic mice and nontransgenic littermates. Lipid concentrations were determined as described (*19*). HDL-cholesterol concentrations were estimated after precipitation of apoB-containing particles (VLDL, IDL, and LDL) by heparin-manganese treatment (*35*). ApoA-II and apoA-I concentrations were determined as described (*18, 19*).

| | Chow diet | | Atherogenic diet | |
|---|---|---|---|---|
| | Transgenic | Nontransgenic | Transgenic | Nontransgenic |
| Males | | | | |
| Total cholesterol* | 233 ± 15 (16) | 82 ± 4 (11) | 431 ± 53 (7) | 274 ± 19 (8) |
| HDL-cholesterol* | 120 ± 7 (16) | 53 ± 3 (11) | 170 ± 25 (7) | 86 ± 12 (8) |
| ApoA-II* | 84 ± 5 (16) | 26 ± 2 (11) | 52 ± 11 (7) | 27 ± 4 (8) |
| ApoA-I* | 143 ± 12 (16) | 119 ± 10 (11) | 62 ± 19 (4) | 79 ± 25 (4) |
| Ratio apoA-II/apoA-I | 0.58 | 0.22 | 0.83 | 0.34 |
| Females | | | | |
| Total cholesterol* | 171 ± 14 (11) | 81 ± 7 (10) | 330 ± 27 (8) | 198 ± 23 (7) |
| HDL-cholesterol* | 93 ± 7 (11) | 46 ± 3 (10) | 98 ± 14 (8) | 47 ± 10 (7) |
| ApoA-II* | 60 ± 4 (11) | 23 ± 1 (10) | 48 ± 6 (8) | 31 ± 7 (7) |
| ApoA-I* | 127 ± 13 (11) | 119 ± 10 (10) | 87 ± 10 (4) | 76 ± 4 (4) |
| Ratio apoA-II/apoA-I | 0.47 | 0.19 | 0.55 | 0.41 |

*Values are expressed as mg/dl [mean ± SE (*n*)].

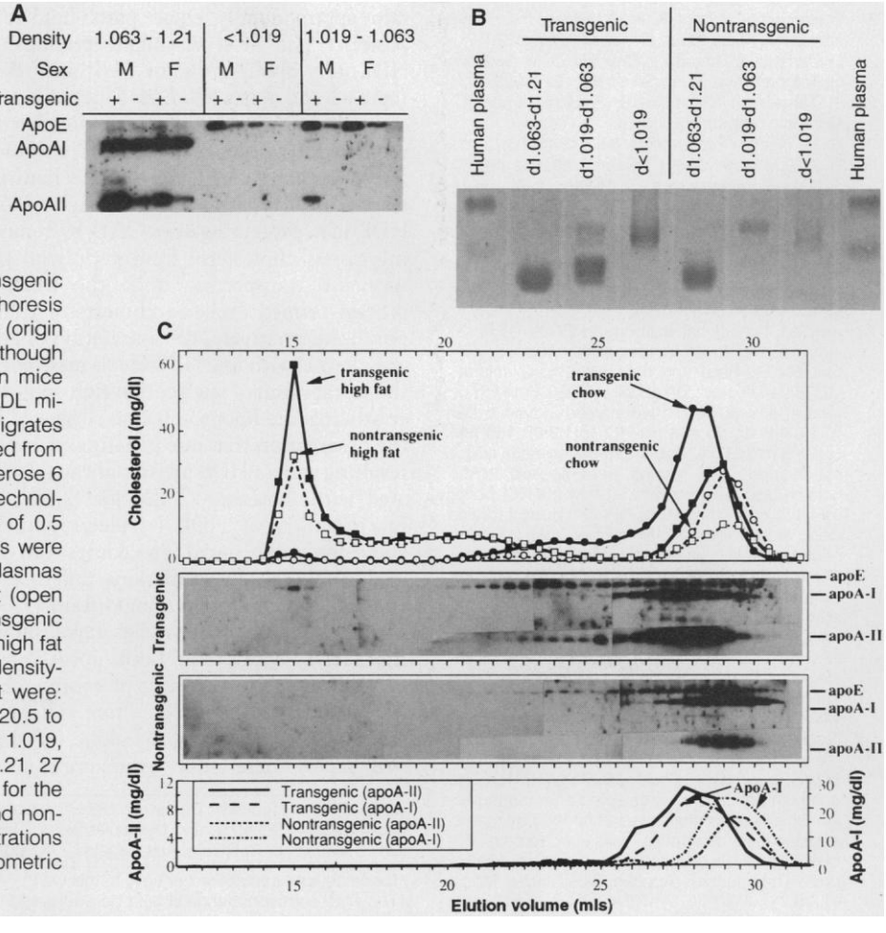


**Fig. 1.** Separation of plasma lipoproteins from apoA-II transgenic mice and nontransgenic littermates. (**A**) Plasma samples pooled from male (M) or female (F) mice, transgenic (+) or nontransgenic (−), were fractionated by density ultracentrifugation (*35*). Aliquots of fractions were then analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblot with antibodies specific for mouse apoE, apoA-I, and apoA-II as described (*18*), except that detection was with a chemiluminescent system. (**B**) Aliquots of density fractions from male transgenic and nontransgenic mice were separated by electrophoresis on agarose gels and stained for lipid with Fat Red 7B (origin is at top). Human plasmas were run as controls. Although absolute mobilities of the lipoproteins differ between mice and humans, relative mobilities were similar; thus, LDL migrates most slowly, slightly behind VLDL, and HDL migrates most rapidly (*37*). (**C**) Plasmas (100-μl aliquots) pooled from four female mice were chromatographed on two Superose 6 columns connected in series (Pharmacia-LKB Biotechnology, Inc.), and 0.5-ml fractions collected at a rate of 0.5 ml/min as described (*38*). Cholesterol concentrations were determined in duplicate in each fraction (*18*) for plasmas from nontransgenic mice maintained on a chow diet (open circles) or a high fat diet (open boxes), and for transgenic mice maintained on a chow diet (closed circles) or a high fat diet (closed boxes). The positions of elution of density-fractionated lipoproteins from mice fed a chow diet were: transgenics (d < 1.019, 14 to 16 ml; 1.019 to 1.063, 20.5 to 25 ml; 1.063 to 1.21, 25 to 30 ml), nontransgenics (d < 1.019, 14 to 16 ml; 1.019 to 1.063, 21 to 23.5 ml; 1.063 to 1.21, 27 to 30.5 ml). Shown below are immunoblots (as in A) for the corresponding fractions obtained from transgenic and nontransgenic mice maintained on a chow diet. Concentrations of apoA-I and apoA-II were estimated by densitometric scanning and comparison with C57BL/6J standards.

by density (Fig. 1A), agarose gel electrophoresis (*24*), and size (Fig. 1C), this particle contained apoE and apoA-II, but not apoB. Total apoB levels were not elevated in transgenic mice (*24*), although there was an increase in the amount of cholesterol and apoE associated with VLDL (which elutes from Superose 6 columns at 14 to 16 ml of buffer, Fig. 1C). In contrast to these results, when the human apoA-II gene was expressed in transgenic mice, apoA-II–enriched LDL-sized particles were not observed and the effects on HDL-cholesterol concentrations and composition were negligible (*25*). The difference in the two studies may relate to species-specific interactions between apoA-II and other proteins in the mouse. Apparent species-specific interactions have been observed for other apolipoproteins (*26*).

When maintained on an atherogenic diet high in fat (15%) and cholesterol (1.25%), both transgenic and nontransgenic mice exhibited greatly increased amounts of VLDL-cholesterol and cholesteryl-ester rich particles intermediate in size between VLDL and LDL (Fig. 1), and a decrease in plasma apoA-I concentrations (Table 1). The transgenic mice had more apoA-II and HDL-cholesterol and an increased ratio of apoA-II to apoA-I in comparison to nontransgenic littermates (Table 1). On both chow (Fig. 1) and high-fat diets (*24*), the ratio of apoA-II to apoA-I was increased in transgenic mice in all HDL subfractions as judged by quantitation of the two proteins in fractions separated by gel filtration.

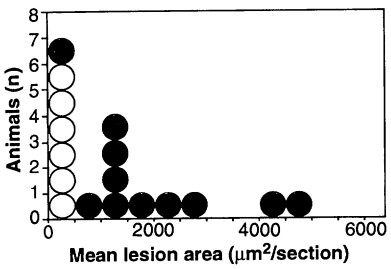


**Fig. 2.** Aortic fatty streak lesions in apoA-II transgenic mice (circles) and nontransgenic littermates (open circles) maintained on a low fat chow diet. Each symbol represents one mouse. The mean lipid-staining lesion area in the proximal aorta is shown. The distribution of lesions in males and females is presented in Table 2.

Laboratory strains of mice do not develop significant aortic fatty streak lesions when maintained on chow diets (*19, 21–23*). ApoA-II transgenic mice developed aortic lesions on a chow diet (Fig. 2 and Table 2) despite the fact that they had high concentrations of HDL and low concentrations of LDL and VLDL (Fig. 1). Lesion development was observed in transgenic mice of both sexes (Table 2). The lesions resembled typical fatty streaks with subendothelial accumulations of lipid and foam cells and positive staining for the macrophage antigen Mac-1 (*19*), such as occur in C57BL/6J mice maintained on an atherogenic diet (*24*).

Overexpression of apoA-II increased the size of aortic lesions in male mice ($P < 0.019$), but not female mice, maintained for 11 to 12 weeks on an atherogenic diet (Table 2). ApoA-II levels were correlated with mean lesion size in transgenic mice on the high fat diet ($r = 0.59$). The explanation for the failure to observe increased lesion size in female mice is unknown, but it may be related to the higher concentrations of apoA-II in male than in female transgenic mice. In females the ratio of apoA-II to apoA-I was only slightly increased in transgenic mice as compared to nontransgenic littermates, whereas in males it was increased about 2.5-fold (Table 1).

These results suggest that the composition of HDL influences the development of atherosclerosis. Despite increasing HDL-cholesterol concentrations, overexpression of mouse apoA-II increased aortic fatty streak development, even in animals maintained on a low-fat chow diet. The results are consistent with certain studies in vitro indicating that HDL containing both apoA-II and apoA-I are less efficient in promoting sterol efflux from cells than HDL containing apoA-I alone (*10–14*). The apoA-II content of HDL also affects interactions with hepatic lipase, lecithin cholesterol acyl transferase and other proteins that transport lipids (*27–31*). ApoA-II enriched particles are, therefore, likely to be functionally altered, and this may influence not only lipid transport but interactions with the artery wall (*32, 33*). Human epidemiological and family studies of the effect of apoA-II on premature atherosclerosis are complicated by multifactorial inheritance and genetic heterogeneity, but some results suggest that HDL particles containing both apoA-I and apoA-II do not provide the same protection as would be expected from particles containing only apoA-I (*34*). In conclusion, the interactions of plasma lipoproteins in atherosclerosis are likely to be more complex than previously recognized.

**Table 2.** Aortic lesions in apoA-II transgenic mice and nontransgenic littermates. Mice 4 to 6 months of age were maintained on either Purina chow containing 4% fat or an atherogenic diet containing 1.25% cholesterol, 7.5% cocoa butter, and 0.5% cholic acid (with a total fat content of 15%) as described (*21, 22*). Following the dissection of the heart and proximal aorta, the size of oil red O–staining lesions was estimated by examining 10-μm-thick sequential sections covering 400 μm of the proximal aorta (*19, 21–23*). Results are presented as the mean stained area per section. Statistics were calculated with the use of the Macintosh Statview nonparametric Mann-Whitney U test.

| Diet | Sex | Mean lesion area [$\mu m^2 \pm$ SE ($n$)] | | |
|---|---|---|---|---|
| | | Transgenic | Nontransgenic | $P$ |
| Chow | Males | 2200 ± 610 (7) | 120 ± 120 (4) | <0.008 |
| | Females | 1660 ± 940 (4) | 250 ± 250 (2) | <0.24 |
| | Total | 2020 ± 500 (11) | 170 ± 100 (6) | <0.003 |
| Atherogenic | Males | 8603 ± 2900 (7) | 2400 ± 3102 (10) | <0.019 |
| | Females | 13700 ± 2950 (11) | 15300 ± 3200 (9) | <0.72 |
| | Total | 11700 ± 2160 (18) | 8500 ± 2100 (19) | <0.29 |

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# Interleukin-1 Type II Receptor: A Decoy Target for IL-1 That Is Regulated by IL-4

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**Interleukin-1 (IL-1) interacts with cells through two types of binding molecules, IL-1 type I receptor (IL-1R I) and IL-1R II. The function of IL-1R II is unknown. In studies using monoclonal antibodies, IL-1 prolonged the in vitro survival of polymorphonuclear cells (PMN) through IL-1R I, and IL-4 antagonized the action of IL-1 by inducing expression and release of IL-1R II. Dexamethasone also induced expression and release of the IL-1R II in PMN. These results, together with the effect of antibodies to IL-1R on IL-1–induced production of cytokines in monocytes, indicate that IL-1 acts on myelomonocytic cells through IL-1R I and that IL-1R II inhibits IL-1 activity by acting as a decoy target for IL-1. The existence of multiple pathways of regulation emphasizes the need for tight control of IL-1 action.**

IL-1α and IL-1β are pleiotropic cytokines that mediate a wide range of biological activities on different cell types (*1*). Two molecules in the cell membrane that bind IL-1, referred to as IL-1R I and IL-1R II, have been identified and are expressed in different amounts in cells of different lineages. IL-1R I is an 80-kD transmembrane protein, and its signaling function has been demonstrated (*2*). It represents the main form of IL-1 receptor found in fibroblasts and T lymphocytes. IL-1R II is a 68-kD molecule (*3–5*) with a relatively short (29 amino acids) cytoplasmic tail (*6*) and is predominantly expressed in B lymphocytes, monocytes, and PMN. The biological role of IL-1R II is unknown.

Human PMN have a limited life-span in vitro and in vivo. A series of cytokines and bacterial products promote survival of PMN in vitro by inhibiting the spontaneous process of programmed cell death (apoptosis) (*7, 8*). In particular, IL-1 strongly promotes survival of PMN (*7*). IL-4, a cytokine that inhibits various functions related to inflammation in myelomonocytic cells (*9*), almost completely abolishes the effect of IL-1 in promoting the survival of PMN. In searching for the mechanism underlying this phenomenon, we have examined the biological role of IL-1 receptors in PMN. Our results indicate that, although the biological activity of IL-1 in PMN is exerted through IL-1R I, IL-1R II, either membrane-bound or secreted, acts as a molecular trap for IL-1, inhibiting its activity.

Although IL-4 alone did not affect the in vitro life-span of PMN, it almost completely abolished the prolongation of survival induced by IL-1β (Fig. 1A). In results from 15 donors, the inhibitory effect of IL-4 on IL-1β–induced survival of PMN ranged from 85 to 100%. The effect of IL-4 was dose-dependent between 0.1 and 10 ng/ml (*10*) and specific for IL-1β, in that the effects of other cytokines, such as interferon-γ (IFN-γ), granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor (GM-CSF and G-CSF), IL-6, and tumor necrosis factor (TNF), that prolong the survival of PMN (*7, 8*) were unaffected (Fig. 1B shows results for GM-CSF). IL-4 also partially inhibited the prolongation of survival induced by lipopolysaccharide (LPS) (Fig. 1B), an inducer of IL-1 in these cells (*11*).

PMN express IL-1R II (*4, 5*). We therefore investigated the role of IL-1 receptors in the contrasting activities of IL-1 and IL-4 on PMN life-span. PMN had high-affinity binding sites for IL-1β [217 ± 62 sites per cell, dissociation constant ($K_d$ = 8.8 × $10^{-10}$ mol liter$^{-1}$) (Fig. 2A). By Northern (RNA) analysis, it was found that PMN expressed predominantly IL-1R II mRNA transcripts and a barely visible IL-1R I band (Fig. 2B). Cross-linking revealed the presence of a 68-kD molecule (after subtraction of the size of the ligand; Fig. 2C), a size consistent with that of IL-1R II.

Exposure of PMN to IL-4 increased expression of IL-1R II. IL-4–treated PMN showed 553 ± 105 sites per cell, whereas the $K_d$ was unchanged (7.8 × $10^{-10}$ mol liter$^{-1}$) (Fig. 2A). IL-1β did not affect the number or affinity of binding sites on PMN, nor did it affect the action of IL-4 (*10*). Treatment of cells with IL-4 also augmented the expression of IL-1R II mRNA (Fig. 2B), and cross-linking revealed a prominent 68-kD molecule that bound IL-1β on the surface of PMN (Fig. 2C). Treatment of PMN with IL-4 also increased the steady-state amount of IL-1R I mRNA. Cross-linking with $^{125}$I-labeled IL-1β of proteins released into the culture medium by PMN treated with IL-4 revealed the presence of an IL-1β–binding protein (Fig. 2D). A small amount of this cross-linked product was also released from untreated PMN (Fig. 2D). Cross-linking of the protein was inhibited by an excess of unlabeled IL-1β (Fig. 2D).

After taking into account the molecular size of the ligand, we determined that the apparent molecular size of IL-1–binding protein released by IL-4–treated PMN was ~45 kD. Soluble IL-1–binding proteins of similar molecular size have been identified in conditioned media from human mononuclear cells and from the Raji B cell line (*12*) and probably represent a soluble form of IL-1R II. Treatment of cells with IL-4

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