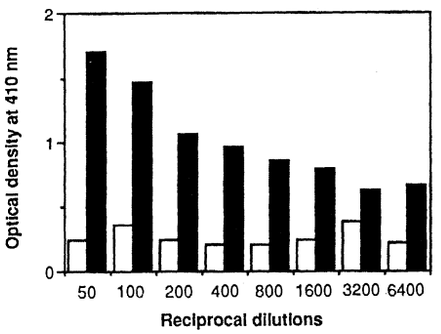

Fig. 4. Antibody response of five mice immunized with SL5929, a *Salmonella dublin* live vaccine strain that expresses the chimeric flagellin genes; □, before immunization, ■, after immunization with SL5929. Reactivity of mouse sera with whole native cholera toxin was measured by solid-phase ELISA (*9*), with peroxidase-conjugated goat antibody to mouse IgG (TAGO). Mice were injected intraperitoneally three times, at weekly intervals, with 5×10^6 Formalin-killed bacteria; sera were collected 7 days after the last injection. The bars represent the mean optical density for sera from five mice (SE <15% for all dilutions).

lar antigen, and the altered *Salmonella* strains, when used as vaccines, can cause the production of antibodies specific for the foreign epitope (*19, 20*). Some inserts three times as long as the 16–amino acid deletion are compatible with flagellar function (*18*). This flexibility indicates that it may be feasible to insert several epitopes of a protective antigen (at one or more sites), to increase the chance of an immune response, despite the probability that a given epitope will be ineffective in some subjects of an outbred population.

Some in-frame inserts did not confer motility on a flagellin-negative host; we are investigating what constraints exist for retention of function. In results to be presented elsewhere, we provide evidence that an epitope placed in the flagellar filament protein can elicit a cellular immune response, as shown by proliferative response of cultured spleen lymphocytes to the relevant peptide (*21*). The ability to (i) synthesize peptides for the identification of antigenic and protective epitopes from pathogens, (ii) insert the corresponding oligonucleotides into flagellin genes, and (iii) use safe and effective live vaccine strains of *Salmonella* that induce cellular and humoral immunity makes possible the construction of vaccines for protection against infections for which immune prophylaxis is presently not feasible.

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12. Strain CL447 is a plasmid-free segregant of a strain (that we named CL402) given to us by T. M. Joys as *E. coli* C600 *hag* harboring plasmid pBR322 with an Eco RI insert that includes *H1-d* of *S. muenchen* ATCC8388 (*5*); we designate this plasmid pLS402.
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14. The anti-*d* (*Salmonella* H) serum used, from P. H. Mäkela, was raised in rabbits by injection of killed whole flagellate bacteria.
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19. The advantage of this approach is exemplified by the fact that although in our experiments CTP3 expressed within the flagellin protein induced antipeptide response, CTP3–β-galactosidase hybrid protein in *E. coli* did not lead to detectable production of antipeptide antibody, although a "priming" immune response could be obtained. Even the presence of the OmpF signal peptide at the amino terminus of the hybrid protein (which would have made the fusion protein a component of the bacterial outer membrane) did not lead to an immune response against CTP3. C. O. Jacob, M. Leitner, A. Zamir, D. Salomon, R. Arnon, *EMBO J.* **4**, 3343 (1985).
20. After the completion of our work we saw the report by G. Kuwajima *et al.* [*Bio/Technology* **6** (no. 9), 1080 (1988)] on the insertion of an oligonucleotide specifying an 11-residue epitope of egg-white lysozyme at the site of a deletion in the central region of the flagellin gene, hag, of *E. coli*; the chimeric flagellin filaments bound antibody to a lysozyme fragment but their injection into guinea pigs did not evoke antibody able to bind the lysozyme fragment.
21. S. M. C. Newton, L. Rosenberg, B. A. D. Stocker, unpublished observations.

22. Supported by grant 000553 from the American Foundation for AIDS Research (to B.A.D.S.), a gift from Praxis Biologics Inc., and a postdoctoral fellowship from Funacao de Amparo a Pesquisa Estado de Sao Paolo, Brazil (to S.N.). We thank T. M. Joys for providing strains and plasmids, P. H. Mäkela for supplying the anti-*d* serum, and M. Hovi and L. Rosenberg for assistance and comments.

29 September 1988; accepted 12 January 1989

Heritable Allele-Specific Differences in Amounts of apoB and Low-Density Lipoproteins in Plasma

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Low-density lipoprotein (LDL) concentrations correlate with risk of coronary heart disease, and genetic variation affecting LDL levels influences atherosclerosis susceptibility. The principal LDL protein is apolipoprotein B (apoB); apoB is not exchangeable between lipoprotein particles and there is only one apoB per LDL particle. Plasma LDL therefore consists of two populations, one containing apoB derived from the maternal and one from the paternal apoB alleles. Products of the apo*b* gene with high or low affinity for the MB-19 monoclonal antibody can be distinguished, and this antibody was used to identify heterozygotes with allele-specific differences in the amount of apoB in their plasma. A family study confirmed that the unequal expression phenotype was inherited in an autosomal dominant manner and was linked to the apo*b* gene locus. Significant apoB genetic variation affecting plasma LDL levels may be more common than previously appreciated.

APOLIPOPROTEIN B (APOB) IS A constituent of several types of lipoprotein particles and exists in two forms, B-100 and B-48. The B-100 protein, which is made primarily in the liver, is virtually the only protein in low-density lipoprotein (LDL) and mediates the interaction of LDL with its receptor (*1–4*). Defects in the apo*b* gene have been described that result in diminished plasma apoB in the disorder hypobetalipoproteinemia (*5–8*). As a consequence, homozygotes have little or no plasma LDL cholesterol, whereas heterozygotes have approximately 30% of normal levels (*5*). These observations suggest that other defects in the apo*b* gene might also influence LDL cholesterol levels.

We now report that there are heritable differences in the contribution of specific apo*b* alleles to plasma apoB concentrations.

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Since apoB does not exchange between lipoprotein particles and there is only one apoB per LDL particle, plasma LDL consists of two populations. One population contains apoB coded for by the paternally derived apo*b* allele and the other from the maternally derived allele. The monoclonal antibody MB-19 recognizes a functionally insignificant genetic variation at the apo*b* locus corresponding to gene products that exhibit high- (+) or low-affinity (−) binding to the antibody (*9–13*). This antibody was used to demonstrate unequal amounts of the (+) and (−) allele gene products in the plasma of some apo*b* heterozygotes defined by MB-19 (designated MB-19 heterozygotes). This implies that in these people, there may be another functionally significant mutation in either the MB-19+ or the MB-19− allele. In one family we showed that the unequal expression phenotype was inherited and linked to the apo*b* locus. Most affected family members had low apoB and LDL cholesterol suggesting that the functionally significant apo*b* gene mutation caused either decreased synthesis or increased catabolism of apoB.

Table 1. LDL affinity for the MB-19 monoclonal antibody in family members analyzed by the Scatchard method. The Scatchard analysis derived high [K_d (h)]- and low [K_d (l)]-affinity dissociation constants and the number of high-affinity [B_{max} (h)] and total [B_{max} (t)] binding sites for 15 family members are shown. The relative contributions of high (h)- and low (l)-affinity apoB to the plasma pool are also shown. The standard errors of the K_d values were less than 11% of the reported values. The dissociation constants and number of binding sites were not determined for five family members whose immunoreactivity phenotypes were determined (pedigree individuals nos. 8, 9, 11, 21, and 23).

Pedigree no.*	Phenotype†	K_d (h) ($10^{-10}M$)	K_d (l) ($10^{-9}M$)	B_{max} (h) (fmol)	B_{max} (t) (fmol)	Allele-1 % (h)	Allele-2 % (l)
5	L		1.9		59		100
10	L		2.6		70		100
14	L		2.7		70		100
7	I	4.2	1.8	39	74	53	47
15	I	2.8	1.3	30	60	50	50
20	I	4.9	1.7	35	70	50	50
22	I	6.2	1.5	38	68	56	44
13	H	6.6		60	60	100	
18	H	6.0		60	60	100	
3	IL	2.8	2.6	18	72	25	75
4	IL	1.9	1.8	15	60	25	75
6	IL	2.4	2.5	15	70	21	79
12	IL	1.2	2.8	13	60	22	78
16	IL	2.1	3.0	15	60	25	75
17	IL	2.2	1.8	15	60	25	75

*Pedigree number refers to Fig. 2. †Immunoaffinity phenotype L, low; I, intermediate; H, high; and IL, between intermediate and low affinity.

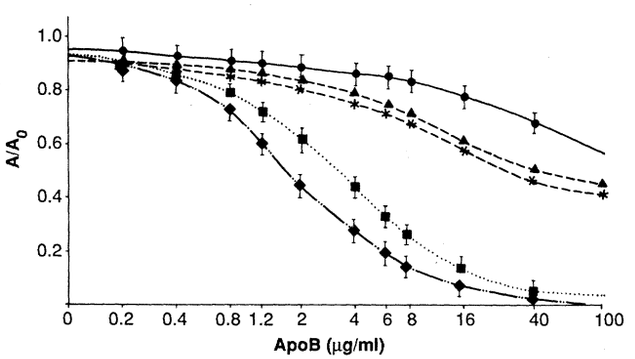

Fig. 1. LDL reactivity with the MB-19 monoclonal antibody assessed by competitive displacement ELISA. Twenty normal volunteers with plasma LDL cholesterol below 85 mg/dl were selected for study. Blood was collected and LDL with a density of 1.025 to 1.050 g/ml was prepared by sequential isopycnic ultracentrifugation (*23*). The apoB was quantified by direct ELISA with a monospecific polyclonal antibody (*19*). The ability of the various LDL samples to compete with a standard LDL for binding to the MB-19 monoclonal antibody was assessed by competitive displacement ELISA. Flat bottom microtiter plates (NUNC, Denmark) were coated by incubating overnight at 4°C with 200 μl of standard LDL in phosphate-buffered saline (PBS) (1 μg/μl) from an individual of the MB-19 +/+ genotype. The plates were then washed three times in a mixture of Tween 20 and PBS (1:2000), and nonspecific binding sites were blocked by incubating the well for 2 hours at 37°C with bovine serum albumin (BSA) (fraction V, Sigma) at a concentration of 3 g/dl in PBS. Wells were again rinsed three times with the Tween 20 and PBS. To each well was then added 60 μl of the MB-19 antibody (1:15,000 dilution in BSA-PBS) and 60 μl of serial dilutions in the BSA-PBS of each LDL preparation containing 0.2, 0.4, 0.8, 1.2, 2, 4, 6, 8, 16, 40, and 100 μg of total apoB per milliliter as quantitated by the direct ELISA. The wells were incubated for 2 hours at 37°C and then rinsed three times with Tween 20 and PBS. Wells were incubated for a further 2 hours at 37°C with a 1:1000 dilution of alkaline phosphatase conjugated antiserum to mouse immunoglobulin G (Cappel) in 1% BSA in PBS. The color reagent, 0.2% disodium *p*-nitrophenyl phosphate (Sigma), was then added to each well. The reaction was stopped after 40 min by addition of 20 μl of 1*M* NaOH to each well. Color development was measured at 405 nm (Dynatech microplate reader). Each dilution for every LDL sample was run in duplicate. The results are expressed as a fraction of the average absorbancy (*A*) divided by the average absorbancy in three wells on the same plate incubated in the absence of competing apoB (A_o). The results are expressed as A/A_o. Three previously recognized patterns of reactivity were found: low affinity (●—●), intermediate affinity (■ · · ■), and high affinity (◆— · · —◆) in eight, eight, and two individuals, respectively. The points are the mean value for all individuals in that group and the bars are the range. A new pattern between intermediate and low affinity was found in two individuals (* – – *) and ▲– –▲).

LDL was prepared from 20 normal volunteers with LDL cholesterol less than 85 mg/dl (*14*). The ability of LDL from these volunteers to compete with a standard LDL from a person with the MB-19 +/+ genotype for binding to the MB-19 antibody was assessed by a competitive displacement enzyme-linked immunosorbent assay (ELISA) (Fig. 1). Serial dilutions of each LDL sample were used, and the three typical patterns of displacement previously reported (*9–13*) were observed. High-, intermediate-, and low-affinity binding, corresponding to the MB-19 +/+, +/−, and −/− genotypes were seen in two, eight, and eight individuals, respectively. However, in two subjects a previously unknown pattern falling between intermediate- and low-affinity binding was observed, this pattern being particularly obvious when relatively large amounts of LDL apoB competitor were used. LDL was prepared from the plasma of 16 relatives of one of these two people and also tested in the competitive displacement assay (Fig. 2). The LDL from six relatives had the new immunoreactivity phenotype, suggesting that it was inherited and had been transmitted through three generations of this family.

In order to determine whether the new immunoreactivity phenotype resulted from genetic variation at or near the apo*b* locus, a formal genetic analysis was performed. DNA was prepared from 18 members of the kindred, and apo*b* allele haplotypes were defined by the Xba I site restriction fragment length polymorphism (RFLP) located 6.5 kb upstream of the 3′ end of the apo*b* gene and a variable number of tandem repeat (VNTR) polymorphism just 3′ to the end of the gene. A DNA haplotype defined by the presence of the Xba I site and the VNTR allele of 1.2 kb (between the flanking Xba I sites) was coinherited with the variant affinity pattern. The LIPED program indicated a logarithm for the likelihood ratio for linkage (lod score) of 4.5 at a recombination fraction of 0, if we assume a dominant mode of transmission (*15*). A recessive mode of inheritance was also compatible with the observed phenotypes, but less likely with a lod score of 3.4 at a

recombination fraction of 0. We considered the recessive mode to be improbable, since it implies that there is an interaction between the two apo*b* alleles that causes an alteration in the plasma concentration of the gene product of just one of them. Thus, our data suggest that the new immunoreactivity pattern is due to a mutation in the apo*b* gene probably transmitted in an autosomal dominant fashion.

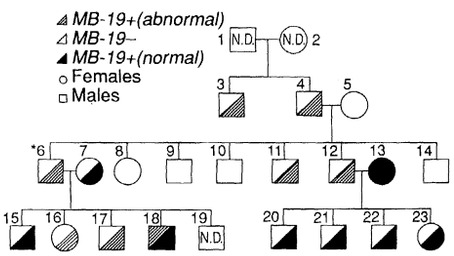

Fig. 2. Pedigree of an individual with the new MB-19 immunoaffinity phenotype. The proband with the new phenotype is individual 6 (*). This phenotype was also found in his father (individual 4), uncle (individual 3), two siblings (individuals 11 and 12), and two children (individuals 16 and 17). These individuals were heterozygotes for the abnormal MB-19+ allele. It was inferred by linkage analysis that another child of the proband (individual 18) was a heterozygote for the abnormal MB-19+ allele and a normal MB-19+ allele. This could not be demonstrated by the immunoreactivity method, since all of the LDL apoB of this individual was of the high-affinity phenotype.

There are two possibilities as to the nature of the new immunoreactivity phenotype. It could have originated at the MB-19 epitope and given rise to a new class of binding sites with a reactivity falling between intermediate and low affinity. Alternatively, individuals with the new pattern could have the MB-19 +/− genotype with less of the (+) or more of the (−) allele gene product in plasma. To test these possibilities another immunoassay was devised to measure the affinity with which LDL from different subjects binds to MB-19 (Fig. 3). The LDL from a subject with high-affinity binding (MB-19 +/+) showed a single class of high-affinity binding site with a dissociation constant (K_d) of $6 \times 10^{-10} M$ (Fig. 3A). LDL from a person with low-affinity binding (MB-19 −/−) also showed a single class of binding site, but with a lower affinity ($K_d = 2.5 \times 10^{-9} M$) (Fig. 3B). LDL from a person with intermediate-affinity binding (MB-19 +/−) showed two classes of binding site. Approximately half the sites were high affinity ($K_d = 4.2 \times 10^{-10} M$) and the other half low affinity ($K_d = 1.8 \times 10^{-9} M$) (Fig. 3C). When a person with the new pattern of reactivity was studied, both high- and low-affinity binding sites were found (Fig. 3D), but there was a relative decrease in the number of high-affinity sites or a relative increase in the number of low-affinity sites. There was no evidence for a new class of binding site with reactivity falling between intermediate and low affinity. Thus, the new phenotype appears to be due to an inherited allele-specific difference in the relative amounts of the two parental types of LDL apoB in plasma.

All persons in the family studied with the new immunoreactivity phenotype showed both high- and low-affinity binding sites (Table 1). The average fraction of the total binding sites with high affinity was 25% in these subjects compared to 50% in subjects with the intermediate affinity pattern. For all the immunoreactivity phenotypes the total number of binding sites did not change, since the same total amount of apoB (detected with a polyclonal antibody) was used for each subject regardless of their MB-19 reactivity.

Unequal amounts of LDL apoB derived from the two parental alleles could result from differential synthesis or catabolism of LDL apoB derived from either of the two parental alleles. In the family studied, the unusual reactivity pattern cosegregated with the MB-19+ allele inherited from the proband's father and showed the same expression regardless of which apo*b* haplotype was inherited from the mother. Six of the seven family members showing the new phenotype (Table 2) had LDL cholesterol levels below the 15th percentile for age and sex. These findings are consistent with the possibility that the variant MB-19+ allele is associated with decreased synthesis or increased catabolism of LDL (or both).

Our results show that quantitative differences in the amount of apo*b* allele gene products can be recognized in situations where the two gene products can be identified. Such differences can only arise from cis-acting apo*b* mutations, because trans-acting mutations would be expected to affect both alleles in an equivalent manner. Our method, with the MB-19 monoclonal antibody to distinguish the parental allele gene products, is only applicable to MB-19 heterozygotes. The MB-19+ and (−) allele frequencies (0.4 and 0.6, respectively) are such that approximately 48% of the population are heterozygotes. However, from the shape of the competitive binding curves (Fig. 1), it is only possible to reliably distinguish those phenotypes falling between intermediate and low affinity. Phenotypes falling between intermediate and high affinity cannot be distinguished. Thus, if the latter phenotypes are equally common, the MB-19 assay de-

Table 2. Lipoprotein profile and apoB allele haplotypes in family members. Blood specimens from fasting patients were analyzed for lipid, lipoprotein, and apolipoprotein (*19*). LDL cholesterol percentile, for age and sex, are derived from the Lipid Research Clinic database (*20*). (Chol, cholesterol; TG, triglyceride; VLDL, very low density lipoprotein; HDL, high-density lipoprotein.)

Pedigree no.*	Phenotype*	Age (years)	Concentrations (mg/dl plasma)						LDL Chol percentile	ApoB allele haplotypes†
			Chol	TG	VLDL Chol	LDL Chol	HDL Chol	ApoB		
5	L	70	209	87	37	131	41	92	30	AC
8	L	43	144	127	40	75	29	55	6	BC
9	L	39	169	94	28	102	39	84	15	
10	L	38	157	142	41	76	40	61	3	AB
14	L	28	174	92	51	77	46	62	12	AB
7	I	32	172	45	10	106	54	74	46	AB
15	I	5	164	51	19	94	51	75	58	BC
20	I	10	185	75	28	117	40	97	85	AC
21	I	9	159	41	12	107	40	91	80	AC
22	I	8	162	57	17	113	32	80	86	AC
23	I	5	195	75	24	142	29	108	96	
13	H	35	176	136	39	102	35	80	33	AB
18	H	2	113	52	15	54	44	40	1	AB
3	IL	68	192	87	26	108	58	85	13	AA
4	IL	70	147	82	19	87	41	62	5	AB
6	IL	35	137	100	30	70	38	63	1	AC
11	IL	32	155	103	28	88	39	73	10	AA
12	IL	37	218	118	42	145	31	100	65	AC
16	IL	9	126	56	8	75	43	48	14	AA
17	IL	7	120	48	10	56	54	30	1	AA

*The pedigree numbers and immunoaffinity phenotypes are as in Table 1. †For linkage analysis, DNA was prepared by standard methods from the buffy coat of 30 ml of venous blood, completely digested with Xba I, fractionated by electrophoresis through 0.8% agarose, and transferred to nylon membrane filters. The filters were then hybridized with the pB27 and pB24 probes, which detect an Xba I site RFLP 6.5 kb 5′ to the 3′ end of the apo*b* gene and a VNTR polymorphism just 3′ to the end of the gene, respectively (*21, 22*). For the family study, these two RFLPs were used to establish apoB allele haplotypes, and linkage with the immunological phenotype was assessed by the LIPED computer program (*15*). Four apoB allele haplotypes were defined. A, Xba I 5-kb– and VNTR 1.2-kb–fragments; B, Xba I 8.6-kb– and VNTR 1.2-kb–fragments; C, Xba I 8.6-kb– and VNTR 1.4-kb–fragments; D, XbaI 5-kb– and VNTR 1.4-kb–fragments. For the purposes of running the program, the A to D allele frequencies were assumed to be 0.05.

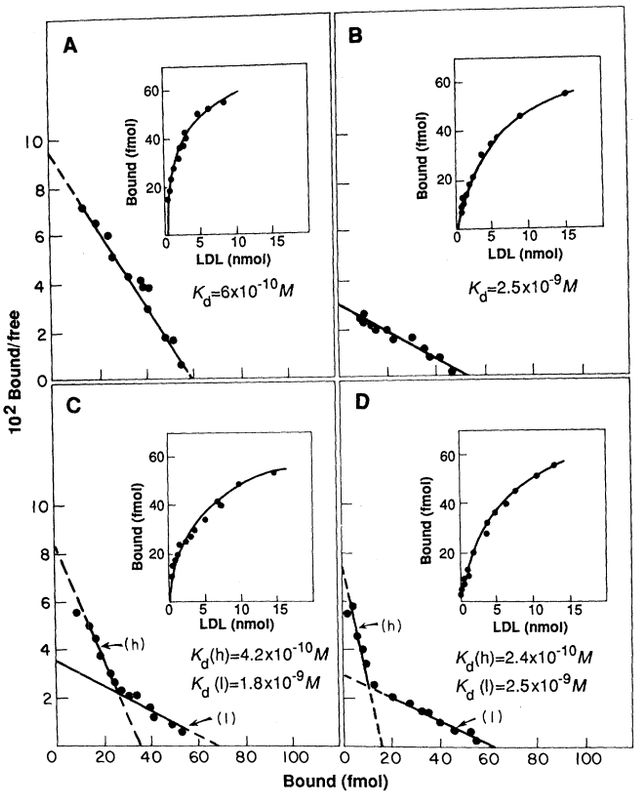

Fig. 3. Affinity of LDL from different subjects for the MB-19 monoclonal antibody. Microtiter wells were coated by incubating overnight at 4°C with 200 μl of the MB-19 antibody (1:5000 dilution in PBS), washed three times in Tween 20 and PBS (1:2000), blocked by incubating with 3% BSA in PBS for 2 hours at 37°C, and rinsed again three times with Tween 20 and PBS. The adhered antibody was exposed for 3 hours at 37°C to increasing concentrations of ^{125}I-labeled LDL (in 1% BSA in PBS) in a volume of 100 μl. The wells were then washed seven times with Tween 20 and PBS. LDL was radiolabeled with ^{125}I as described (*24*). Three wells were exposed to each concentration of ^{125}I-labeled LDL alone to measure total binding, and a fourth well received the ^{125}I-labeled LDL and a 200-fold excess of unlabeled LDL (from an MB-19 +/+ individual) to measure nonspecific binding. Specific binding was the difference between them. Dry wells were cut from the plates and the amount of radioactivity present determined with a gamma counter. Specific binding curves were subjected to Scatchard analysis (*25*) with the LIGAND program for fitting multiple binding sites (*26*). The binding patterns and Scatchard analyses of LDL from individuals with high affinity (**A**), low affinity (**B**), intermediate affinity (**C**), and the new affinity pattern (**D**) are shown. The new affinity phenotype includes both high- and low-affinity components. The standard errors of the K_d values were 6 and 7% of the reported values for (A) and (B), and between 8 and 11% of the reported values for (C) and (D).

scribed can only be used to distinguish unequal expression of the parental alleles in half of the MB-19 heterozygotes or 24% of the population. The discovery of other monoclonal antibodies that recognize different sites of genetic variation in apo*b* would allow further examination of the population for this phenomenon.

Linkage analysis indicates that the defect in the family studied is in the apo*b* gene, but, the exact mutation is unknown. The apo*b* gene is 43 kb in length and significant mutations anywhere in this gene affecting transcription, mRNA processing, translation, secretion, or metabolism could be responsible. The defective MB-19+ apo*b* allele in the family studied was not totally dysfunctional, but resulted in a plasma apoB concentration of 33% of that of the normal allele. In agreement with these data, the LDL cholesterol levels of six of seven affected family members were low. Specific mutations affecting the amount of apoB in plasma have been documented. For example, in hypobetalipoproteinemia essentially nonfunctional alleles due to several types of mutations have been demonstrated, including deletions of 4 bp and 1 bp resulting in frame shifts and new stop codons (*6, 7*), a nonsense mutation (*7*), and an exon deletion (*8*). A disorder called familial defective apoB-100 has been described in which a mutation involving the binding site for the LDL receptor (Arg^{3500} → Gln) results in an increase in plasma LDL (*16, 17*). In affected heterozygotes there is a selective accumulation of the population of LDL with the defective apo*b* allele gene product on its surface. This is analogous to the phenomenon we have observed.

The plasma LDL concentration is a potent risk factor for coronary heart disease (*1–4*), and the extent to which genetic factors can alter concentrations of LDL is fundamental to our understanding of the differential susceptibility to atherosclerosis that exists within human populations. Heterozygous carriers of a single gene for familial hypercholesterolemia occur with a frequency of 1:500 in the general population, and account for 5% of patients that have had a heart attack before age 60 (*4*). Affected individuals have twice the normal plasma LDL concentration, and the responsible mutations involve the LDL receptor locus, whose gene product recognizes a specific binding site on apoB and mediates LDL removal from plasma. In hypobetalipoproteinemia and familial defective apoB-100, each of which occur with a frequency of about 1:1000 in the general population, mutations in the apo*b* gene can have a profound effect on plasma LDL concentrations and, presumably, susceptibility to atherosclerosis (*5–8, 16, 17*). The hypothesis that other mutations involving the apo*b* gene may account, at least in part, for the lesser deviations in LDL cholesterol concentrations that are encountered is strengthened by our findings. Just how common allelic variation of this type is in the population remains to be determined. We have shown that the variant MB-19 immunoreactivity phenotype is present in 5% of persons unselected for lipid levels (*18*). Since this test is only applicable in half of the MB-19 heterozygotes, who in turn are only half of the population, this implies a frequency of variant apo*b* alleles of 20%. If this frequency of variants is confirmed by studies of larger population samples, variation at the apo*b* locus may emerge as an important cause of genetic variation in plasma LDL concentrations and presumably atherosclerosis susceptibility.

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27. We thank L. K. Curtiss for the MB-19 antibody, W. E. Nance for helpful criticisms, and L. Duda for preparing the manuscript. Supported in part by NIH grants (HL33714, HL32435, HL36461, and CA29502), a Clinical Investigator Award (HL02034), a Fogarty International Research Fellowship (TWO3960), a General Clinical Research Center grant (RR00102), and general support from the Pew Trusts. J.L.B. is an Established Investigator of the American Heart Association.

29 July 1988; accepted 23 December 1988

Identification of a Thyroid Hormone Receptor That Is Pituitary-Specific

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Three cellular homologs of the v-*erbA* oncogene were previously identified in the rat; two of them encode high affinity receptors for the thyroid hormone triiodothyronine (T_3). A rat complementary DNA clone encoding a T_3 receptor form of the ErbA protein, called r-ErbAβ-2, was isolated. The r-ErbAβ-2 protein differs at its amino terminus from the previously described rat protein encoded by c-*erbA*β and referred to as r-ErbAβ-1. Unlike the other members of the c-*erbA* proto-oncogene family, which have a wide tissue distribution, r-*erbA*β-2 appears to be expressed only in the anterior pituitary gland. In addition, thyroid hormone downregulates r-*erbA*β-2 messenger RNA but not r-*erbA*β-1 messenger RNA in a pituitary tumor–derived cell line. The presence of a pituitary-specific form of the thyroid hormone receptor that may be selectively regulated by thyroid hormone could be important for the differential regulation of gene expression by T_3 in the pituitary gland.

THE NUMEROUS EFFECTS OF THYROID hormone on vertebrate tissues are thought to be mediated through binding to a nuclear receptor protein (*1*). The protein products of the c-*erbA* proto-oncogene have been identified as nuclear thyroid hormone receptors (*2, 3*). In the rat, three c-*erbA*–related cDNAs have been described and their encoded proteins divided into α and β forms on the basis of their predicted amino acid sequences (*4–8*). The r-*erbA*α-1 and r-*erbA*α-2 mRNAs represent alternative splice products of a single r-*erbA*α gene, which encodes proteins that are identical for the first 370 amino acids and then diverge completely (*5–7*). This divergent region spans the putative ligand-binding domain, probably accounting for the fact that the protein encoded by r-*erbA*α-1 binds T_3, whereas the protein encoded by r-*erbA*α-2 does not bind T_3. Like r-ErbAα-1, r-ErbAβ-1 binds T_3 with high affinity (*8*). The c-*erbA*–related mRNAs are expressed with differing abundance in virtually all rat tissues. The r-*erbA*α-1 mRNA (5 kb in size) is most abundant in skeletal muscle and brown fat, whereas the r-*erbA*α-2 mRNA (2.6 kb) is most highly expressed in brain and hypothalamus (*5*). The r-*erbA*β-1 mRNA (6.2 kb) is highly expressed in kidney and liver (*8*). We now report the isolation of a cDNA encoding a novel thyroid hormone receptor, r-ErbAβ-2, whose mRNA is detected only in the anterior pituitary gland. In addition, we find that the two r-*erbA*β mRNAs are differentially regulated by T_3 in GH_3 cells, a pituitary tumor–derived cell line.

The nucleotide and deduced amino acid sequences of the r-*erbA*β-2 cDNA are shown in Fig. 1A. The protein is schematized in Fig. 1B and compared to the other three rat c-ErbAs. The r-ErbAβ-2 protein has no similarity to r-ErbAβ-1 or either of the rErbA-α proteins at its amino terminus, but is identical to r-ErbAβ-1 from amino acid 147 to the carboxyl end, including the putative DNA- and ligand-binding regions. The identity between r-ErbAβ-1 and r-ErbAβ-2 begins one amino acid on the amino-terminal side of the point where the similarity between r-ErbAβ-1 and the r-ErbAα species begins—13 amino acids on the amino-terminal side of the first cysteine residue in the putative DNA-binding region.

The r-*erbA*β-2 cDNA insert was subcloned into the vector Bluescript and transcribed into RNA, which was translated in reticulocyte lysates. A protein of approximately 62 kD was produced, in agreement with that predicted from the nucleotide sequence (Fig. 2A). The r-ErbAβ-2 protein bound T_3 with an affinity constant (K_d) of 1.1 ± 0.4 n*M* (mean ± SEM) (Fig. 2B), which is similar to that of the endogenous T_3 receptor (*9*). In addition, competition experiments with thyroid hormone analogs showed that the affinity of the r-ErbAβ-2 protein was greatest for 3,5,3′-triiodothyrocetic acid (triac), followed by T_3, T_4, and rT_3 (*10*), the same as that of the r-ErbAβ-1 protein (*8*). These data are consistent with r-*erbA*β-2 encoding a functional T_3 receptor.

We used a transient transfection system to evaluate further the ability of r-ErbAβ-2 to function as a T_3 receptor (*11*). In the choriocarcinoma JEG-3 cell line, expression of a CAT reporter plasmid containing a rat growth hormone thyroid hormone response element was unchanged by T_3 treatment in the absence of a cotransfected *erbA*-containing plasmid. However, when cotransfected with 2 μg of an r-*erbA*β-2–containing plasmid (pCDMerbAβ-2), CAT expression was increased by a factor of 7.1 ± 2.1 (mean ± SEM) by treatment with 10 n*M* T_3. Identical experiments with an r-*erbA*β-1–containing plasmid (pCDMerb62) resulted in similar levels of T_3 induction; CAT expression was increased by a factor of 7.3 ± 1.1. When the vector plasmid alone (PCDM8) was cotransfected, no change in CAT expression was caused by T_3 treatment. Thus, as has been shown for r-ErbAβ-1 (*8*) and r-ErbAα-1 (*6*), r-ErbAβ-2 appears to represent a functional T_3 receptor.

Hybridization analyses of RNA from various rat tissues were performed with probes specific for the r-*erbA*β-1 or r-*erbA*β-2 mRNAs as well as a probe derived from the sequences common to the two cDNAs (r-*erbA*β-common). The r-*erbA*β-1 mRNA is 6.2 kb and is most abundant in brown fat and heart (Fig. 3). The relative intensities of the hybridization signals detected with the r-*erbA*β-common probe were similar to those

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