Monoclonal antibodies to human low density lipoprotein identify distinct areas on apolipoprotein B-100 relevant to the low density lipoprotein-receptor interaction

Simona Fantappiè, Alberto Corsini, Alessandro Sidoli,* Patrizia Uboldi, Agnese Granata, Tiziano Zanelli, Paola Rossi,† Santica Marcovina, Remo Fumagalli, and Alberico L. Catapano**[1]

Institute of Pharmacological Sciences, University of Milano, Milano, Italy; Department of Biological and Technological Research,* S. Raffaele Hospital, Milano, Italy; Institute of Physiology,† University of Pavia, Pavia, Italy; and University of Washington,** Harborview Medical Center, Northwest Lipid Research Center, Seattle, WA

Abstract *We have characterized the epitopes for ten murine* monoclonal antibodies (Mabs) to human low density lipoprotein (LDL) and studied their ability to interfere with the LDL-receptor interaction. The epitopes for the antibodies were defined by using the following approaches: *1)* interaction with apoB-48; *2)* interaction with apoB-100 thrombolytic fragments; and *3)* interaction with β-galactosidase–apoB fusion proteins spanning different areas of the apoB-100 sequence. The results obtained are consistent with the following map of epitopes: Mab 6E, amino acids (aa) 1–1297, Mabs 5A and 6B, aa 1480–1693, Mabs 2A, 7A, 3B, and 4B, aa 2152–2377, Mabs 8A and 9A, aa 2657–3248 and 3H, aa 4082–4306. Four Mabs (2A, 5A, 7A, and 9A) whose epitopes are located in three different areas of apoB, dramatically reduced (up to 95%) the LDL-receptor interaction on cultured human fibroblasts; Fab fragments were as effective as the whole antibodies. Mab 3H, on the other hand, increased LDL binding up to threefold. These findings are consistent with the hypothesis that several areas of apoB-100 are involved independently or in concert in modulating the apoprotein B conformation required for interaction with the LDL receptor.—**Fantappiè, S., A. Corsini, A. Sidoli, P. Uboldi, A. Granata, T. Zanelli, P. Rossi, S. Marcovina, R. Fumagalli, and A. L. Catapano.** Monoclonal antibodies to human low density lipoprotein identify distinct areas on apolipoprotein B-100 relevant to the low density lipoprotein-receptor interaction. *J. Lipid Res.* 1992. **33:** 1111–1121.

Supplementary key words β-galactosidase–apoB fusion proteins • apoB-100 thrombolytic fragments • apoB receptor binding domain

Apolipoprotein (apo) B, the main protein constituent of low density lipoproteins (LDL), very low density lipoproteins (VLDL), and chylomicrons (1), is heterogeneous and exists in two forms: apoB-100 and apoB-48 (2). ApoB-100 is synthesized primarily by the liver, is present in LDL and VLDL, and is responsible for the interaction of LDL with their receptor (3, 4). ApoB-48 is mainly associated with chylomicrons and chylomicron remnants; in humans it is believed to be synthesized solely by the intestine (5) and does not interact with the LDL receptor (6). The complete amino acid sequence of human apolipoprotein B was deduced from nucleotide sequence of its mRNA (7–9). ApoB-100 is the largest protein sequenced to date and consists of a single 4536 amino acid (aa) polypeptide chain; apoB-48 represents the amino terminal 47% of apoB-100 (10). With the knowledge of the primary structure of apoB-100, it is now possible to aim at identifying structural and functional domains of this apolipoprotein. To this end a possible approach is the use of monoclonal antibodies (Mabs). Recently a number of Mab libraries to apoB-100 have become available (11–16). Monoclonal antibodies were used to study apoB epitope expression in various lipoproteins (17–21). Some of the antibodies identify genetic variants of apoB (19, 21–25), and others inhibit binding of LDL to their receptor (14, 15, 26–28). The vast majority of the latter antibodies map to a region that encompasses the T2/T3 thrombin cleavage at aa residue 3249 of apoB-100, suggesting that this area may be responsible for the LDL-receptor interaction (14, 17, 28). We have recently

Abbreviations: Mabs, monoclonal antibodies; LDL, low density lipoproteins; VLDL, very low density lipoproteins; aa, amino acid; LPDS, lipoprotein-deficient serum; PBS, phosphate-buffered saline.

[1]To whom correspondence should be addressed at: Institute of Pharmacological Sciences, University of Milano, Via Balzaretti, 9, 20133 Milano, Italy.

reported, however, that Mab 5A, an antibody that recognizes B-48, fully inhibits LDL binding (27). Furthermore, Milne et al. (28) showed partial inhibition of LDL binding by antibodies 1, 3, 10, 14 whose epitopes lie in the amino terminal region of apoB-100. These findings indicate that other areas of apoB may be involved in the recognition of the LDL receptor. To further address this question we have characterized ten murine monoclonal antibodies to human apoB. For initial assignment of epitopes, we used immunoblots of apoB-100 and apoB-48. For a more precise localization of the amino acid sequences that constitute the epitopes recognized by the Mabs, β-galactosidase–apoB fusion proteins, produced in bacterial expression vectors, were used. Four Mabs, whose epitopes were localized in different areas of thrombolytic fragment T3, inhibited the LDL-receptor interaction. Furthermore, a monoclonal antibody, designated 3H, that recognized the COOH terminal region (T2) of apoB, increased LDL uptake and degradation by cultured cells in a dose-dependent manner.

We conclude that several areas of apoB are involved in determining the LDL-receptor interaction; these regions may contribute to the overall apoB structure recognized by the LDL receptor.

MATERIALS AND METHODS

Eagle's minimum essential medium (MEM), fetal calf serum, trypsin-EDTA (1 ×), penicillin (10,000 U/ml), streptomycin (10 mg/ml), tricine buffer (1 M, pH 7.4), and nonessential amino acids (100 ×) were purchased from Gibco (Grand Island, NY); disposable culture flasks and Petri dishes were from Corning Glass Works (Corning, NY), filters from Millipore Corp. (Bedford, MA). Sodium [^{125}I]iodide, carrier-free, was purchased from Amersham (U.K.). Nitrocellulose membranes and all electrophoresis material and equipment were from Bio-Rad Laboratories (Richmond, CA). The Fab preparation kit was from Pierce (Rockford, IL). All other reagents were analytical grade.

Plasma lipoprotein isolation

Lipoproteins were isolated from plasma of clinically healthy normolipidemic volunteers. Chylomicrons + very low density lipoproteins (d < 1.006 g/ml) and low density lipoproteins (d 1.019–1.063 g/ml) were isolated by sequential preparative ultracentrifugation for 16 and 18 h, respectively, at 40,000 rpm in a 60 Ti Beckman rotor (29). Triglyceride-rich lipoproteins were from the blood of a normolipidemic volunteer three h after a fatty meal (250 g of cream). Human lipoprotein-deficient serum (LPDS) was isolated at d 1.25 g/ml by ultracentrifugation for 72 h at 40,000 rpm at 12°C (30). All lipoproteins and LPDS were dialyzed at 4°C against 0.15 M NaCl, 0.3 mM EDTA, pH 7.4. Lipoproteins were stored at 4°C after sterilization through a Millipore 0.22-μm filter. The LPDS was stored at −20°C and thawed just before use. LDL were labeled with ^{125}I according to Bilheimer, Eisenberg, and Levy (31) (sp act 100–200 cpm/ng of protein) and used within 3 days of preparation. Free iodine accounted for 1% of total radioactivity. Lipid-associated radioactivity was less than 5%.

Digestion of LDL with thrombin

LDL (1 mg) was dialyzed for 12 h at 4°C against 10 mM Tris, 0.3 mM EDTA (pH 8.0), filtered (0.45 μm), and incubated with thrombin (enzyme/substrate 1:100, w/w) for 36 h at room temperature in a nitrogen atmosphere. The reaction was stopped either by addition of electrophoresis sample buffer or by freezing the sample (32).

Fusion proteins

Different DNA coding sequences of human apolipoprotein B-100 from a human liver cDNA and genomic libraries (33, 34; data not shown) were subcloned in the expression vectors pUR 288, 289, 290 (35) in the correct translational reading frame downstream to the β-galactosidase gene (lacZ) (36).

These plasmids were used to transform *E. coli* strain JM 101. The two clones pISMB3 (PstI) and pISMB4 (BamHI) were derived as follows. Clone pISMB3 (PstI) was obtained from clone pISMB3 by PstI digestion, in order to delete the DNA sequence between position 12241 and the PstI site in the poly-linker, downstream of the 3' end of the coding sequence. After ligation in vector pUR 290 and transformation in *E. coli* strain JM 101, we obtained a new clone coding for apoB sequences from aa 3728 to aa 4081.

Clone pISMB4 (BamHI) was derived from pISMB4 by digestion with BamHI/SalI, subsequently filling-in. After ligation and transformation of *E. coli* strain JM 101, we obtained a clone coding for apoB sequences from aa 1306 to aa 1480, having deleted sequences between position 4440 and 5912 bp.

All clones were tested for expression of the corresponding hybrid proteins by SDS-PAGE and reactivity with commercial monoclonal antibodies (36). Immunochemical integrity of fusion proteins was determined in two ways: *1)* by reaction with polyclonal antibodies to human apoB-100; and *2)* by reaction with polyclonal anti-peptide antibodies kindly provided by Dr. Tom Innerarity (Gladstone Foundation, UCSF, San Francisco, CA). Furthermore, authenticity of the clones used was confirmed by sequencing (data not shown).

Monoclonal antibodies

Monoclonal antibodies against human native LDL (d 1.030–1.050 g/ml) were produced and characterized as described by Marcovina et al. (13). Monoclonal antibody

Mb 47 (26) was a generous gift from Drs. L. Curtiss and S. Young (La Jolla, CA). All antibodies studied were used as ascitic fluids. The antibody concentration was determined as % of total protein after protein A affinity chromatography. Monovalent Fabs were prepared by papain digestion (37) of isolated IgG and Fc fragments were removed by passage on a protein A Sepharose 4B column. SDS-PAGE of the Fab fragments revealed two bands of 25 and 40 kDa, and their authenticity was determined by immunoreaction with anti-Fab immunoglobulins conjugated with horseradish peroxidase.

Cell culture

Human skin fibroblasts were grown from explants of skin biopsies obtained from normolipidemic, clinically healthy individuals and from a patient affected by classical familial homozygous hypercholesterolemia (FH), whose cells were shown to be LDL receptor negative (data not shown). Cells were grown in monolayers and maintained in 75-cm^2 plastic flasks at 37°C in a humidified atmosphere of 95% air, 5% CO_2 in MEM supplemented with 10% fetal calf serum, nonessential amino acid solution (1%, vol/vol), penicillin (100 U/ml), streptomycin (100 μg/ml), tricine buffer (20 mM, pH 7.4), and $NaHCO_3$ (24 mM). For all experiments, cells from the stock flasks were dissociated with 0.05% trypsin–0.02% EDTA at confluency (5–15 passages), seeded in 35-mm dishes (1.2×10^5 cells) and used just before reaching confluency, usually 6 days after plating. The medium was changed every 2–3 days. Cell viability, assessed by Trypan blue exclusion, was always >95%.

On day 6 the medium was changed with one containing 10% LPDS, and confluent monolayers were incubated for 24 h at 37°C. Fresh medium, containing 7.5 μg ^{125}I-labeled LDL protein/ml, and either the indicated amount of monoclonal antibodies or a 100-fold excess of unlabeled LDL, was incubated for 2 h at 37°C and then added to the cells for a further 5 h. Under these conditions all Mabs tested recognized more than 90% of ^{125}I-labeled LDL by liquid phase radioimmunoassay (data not shown).

The uptake and degradation of ^{125}I-labeled LDL were determined as previously described (27, 30).

Polyacrylamide gel electrophoresis and immunoblotting

ApoB, apoB-100 thrombolytic fragment, and β-galactosidase fusion proteins were separated by SDS polyacrylamide (4% and 6%, respectively) gel electrophoresis (38) and transferred onto nitrocellulose according to Towbin, Staehelin, and Gordon (39). After saturation of nitrocellulose free sites with PBS+BSA 3% at 37°C for 1 h, nitrocellulose strips were incubated with monoclonal antibodies (100 μg protein in 5 ml PBS-BSA 0.5%) for 1 h at room temperature. Antibodies were detected with a rabbit antibody against mouse immunoglobulins labeled with ^{125}I by the Iodogen procedure, to a specific activity, of 1500 cpm/ng (40).

RESULTS

The murine monoclonal antibodies used in this study were all raised against freshly isolated LDL (1.030–1.050 g/ml). We selected ten antibodies on the basis of their Ka (> 10^9 l/mol) and maximum binding for LDL (> 90%) (13). All antibodies were IgGs.

Monospecificity of the antibodies was assessed by immunoblotting on nitrocellulose replicas of whole delipidated normolipidemic plasma after SDS gel electrophoresis. Only a single band at a molecular mass of 500 kDa was detected. This band was absent in lipoprotein-deficient serum obtained by ultracentrifugation (d 1.25 g/ml) (data not shown).

Immunoblots of apoB-48 and apoB-100 thrombolytic fragments

The amino acid map of apoB-100 and apoB-48 is shown in **Fig. 1**. Thrombin cleavage sites are indicated as T4/T3 and T3/T2 giving rise to T2, T3, and T4 fragments. Immunoblots of apoB-100, and apolipoproteins from d < 1.006 g/ml lipoproteins from the postprandial plasma of a normal subject are presented in **Fig. 2**. All antibodies reacted with apoB-100 but only three, 5A, 6B, and 6E, recognized a lower molecular weight apolipoprotein that we identified as apoB-48 as judged by the following: *a)* relative electrophoretical mobility; *b)* presence in postprandial lipoproteins; *c)* immunoreactivity with antibody previously shown to interact with apoB-48 (Mb19 and 5A), (23, 27); and *d)* immunoreactivity with polyclonal antibodies raised against synthetic peptides spanning different regions of apoB-48 (kindly provided by Dr. Tom Innerarity).

The interaction of Mabs with thrombin-generated apoB-100 fragments is shown in **Fig. 3.** The epitopes for eight of these antibodies were on fragment T3. Antibody 6E interacted with fragment T4; antibody 3H with fragment T2. T2 and T4 fragments were identified by their interaction with Mabs (Mb47 and Mb19, respectively) and with the relevant polyclonal antibodies against synthetic peptides.

Immunoblots of β-galactosidase fusion proteins

For a more precise localization of the amino acid sequence of the epitopes for our Mabs, β-galactosidase–apoB fusion proteins were used.

The restriction map and the localization of the cDNA probes directing the transcription of the β-galactosidase–apoB-100 fusion proteins along the linear and mRNA sequences are shown in Fig. 1.

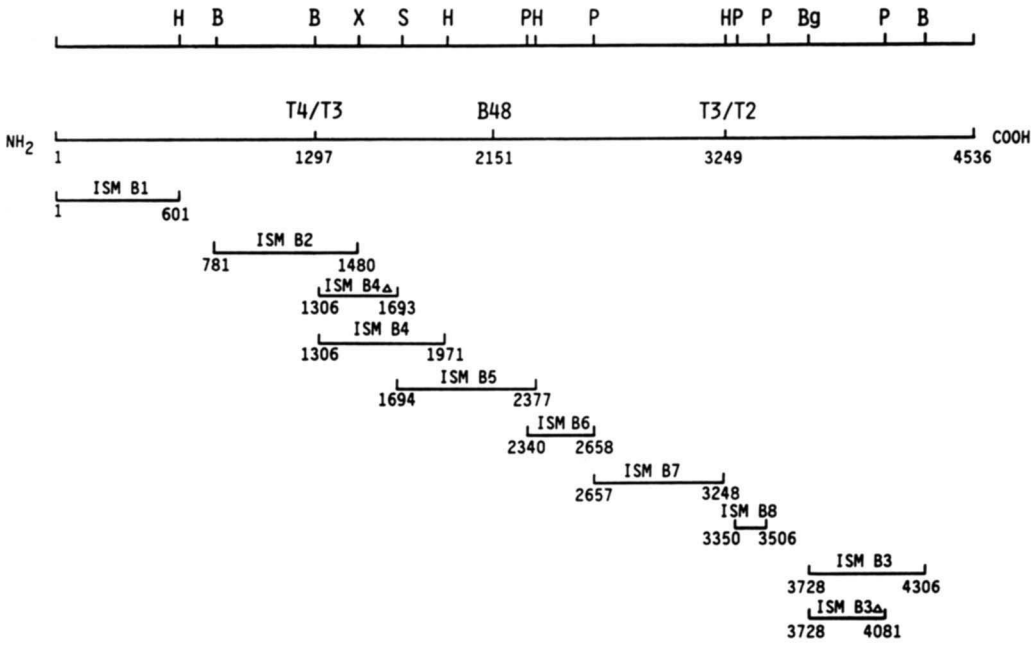

Fig. 1. Linear map of apoB-100. Amino acids starting at the major thrombin cleavage sites of apoB-100 and for the COOH-terminal of apoB-48 are indicated. Regions of apoB-100 coded for by the fusion proteins used in this study are indicated below the apoB-100 chain. A partial restriction map of apoB-100 cDNA (indicating those sites relevant to the fusion proteins) is shown at the top of the figure. Abbreviations: B, BamHI; H, HindIII; X, XhOI; S, SalI; P, PstI; Bg, BglII.

A representative blot with fusion proteins is given in **Fig. 4.** for antibody 3B. The monoclonal antibodies reacted neither with the expression vector nor with more than one fusion protein covering distinct segments of apoB. The presence of multiple bands in the immunoblotting is probably due to partial degradation of the fusion protein; this is a common finding using this approach (15, 41).

The immunoblots for antibodies 2A, 5A, 7A, and 9A are shown in **Fig. 5**.

Table 1 summarizes findings of the immunoblot experiments using apoB-48, apoB-100 thrombolytic fragments, and the fusion proteins. From all these data a map of the location of the epitopes for the ten antibodies is proposed (**Fig. 6**).

In all cases but one, binding to thrombin fragments and to one or more fusion proteins produced consistent results (Table 1), mutually confirming the assignment of epitopes. Antibody 6E, however, recognized B-48 and thrombolytic fragment T4, but failed to recognize fusion protein B1 or B2 which encompass most of the T4 amino acid sequence. We cannot exclude, however, that the epitope for this antibody lies between aa 602 and 781, a sequence not covered by fusion proteins. Therefore, the epitope for Mab 6E cannot be precisely located within fragment T4.

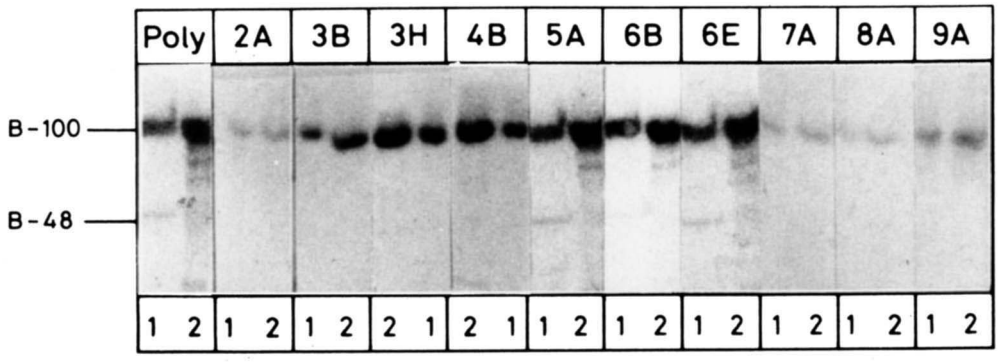

Fig. 2. Autoradiographic analysis of the immunoblots showing the interaction of Mabs to LDL with apolipoprotein B-100 (lane 2) and B-48 (lane 1). Proteins (20 μg) were separated by SDS electrophoresis on 4% acrylamide gels and transferred onto nitrocellulose as described in Methods. The antibodies were detected with a ^{125}I-labeled rabbit anti-mouse immunoglobulin (monoclonal antibodies) and ^{125}I-labeled goat anti-rabbit immunoglobulin (polyclonal antibody).

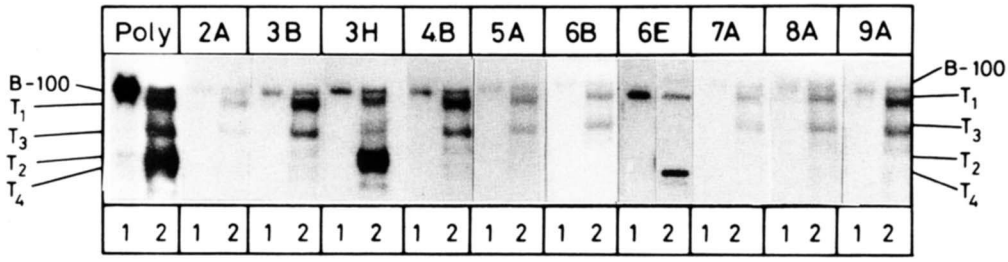

Fig. 3. Autoradiographic analysis of the immunoblots showing the interaction of Mabs to LDL apolipoprotein B-100 (lane 1) and apoB-100 (lane 2) thrombolytic fragments. Conditions as in Fig. 2 with the exception that electrophoresis was performed using 6% acrylamide gels. Nomenclature of fragments as reported by Cardin et al. (32).

Effects of Mabs on the LDL-receptor interaction

Monoclonal antibodies to apoB have been used to identify areas of apolipoprotein B involved in the LDL-receptor interaction (14, 15, 26–28). Having determined the epitope localization of our antibodies, we next explored their ability to interfere with this parameter using an in vitro cell-binding assay. Data are depicted in **Fig. 7**. We confirmed our previous report that antibody 5A inhibits the LDL-receptor interaction in a dose-dependent manner, on cultured cells (27). In addition, we have identified three other antibodies designated 2A, 7A, and 9A, that also inhibited cellular uptake and degradation of LDL (Fig. 7). The epitopes for the inhibitory antibodies lie on distinct sites of apoB thrombolytic fragment T3. Mab 5A interacted with the NH_2 terminal, Mab 2A and 7A with the middle portion, and Mab 9A with the COOH terminal of thrombolytic fragment T3 (Fig. 5).

Amongst the other antibodies, we consistently found (four experiments) that antibody 3H stimulated the LDL uptake and degradation by human skin fibroblasts in a dose-dependent manner (Fig. 7). The epitope for this antibody lies between aa 4082 and 4306 in the T2 fragment (Fig. 6).

To address the question of whether the effects of these antibodies on the LDL-receptor interaction were not due to steric hindrance, we prepared Fab monovalent fragments. As shown in **Fig. 8**, in all cases the activity of Fab was comparable to that of the parent antibody, thus demonstrating authenticity of the effect. Furthermore, the potency of our inhibitory Mabs was similar to that of Mb 47, a previously described antibody that effectively interferes with the LDL-receptor interaction (26).

The possibility that Mab 3H stimulated LDL uptake and degradation via a non-LDL-receptor pathway was ruled out by experiments with human skin fibroblasts from a homozygous familial hypercholesterolemic patient with a receptor-negative phenotype. Mab 3H Fab fragments at 5 μg/ml stimulated specific uptake and degradation of LDL by 42% in control but had no effect on FH fibroblasts, indicating that the antibody stimulatory effect requires the presence of functional LDL receptors.

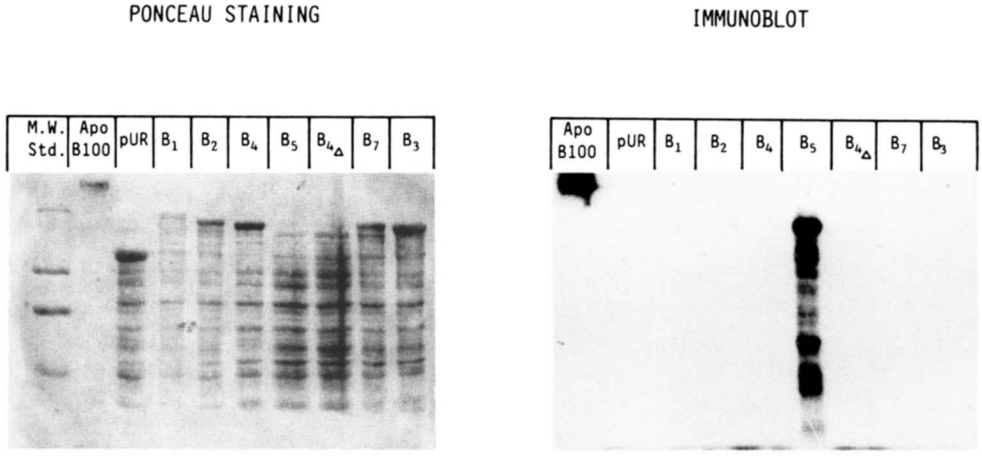

Fig. 4. Immunoreactivy of β-galactosidase–apoB-100 fusion proteins with Mab 3B. *E. coli* lysates were electrophoresed in 6% acrylamide-SDS gels and transferred onto nitrocellulose. The protein staining is shown on the left panel (Ponceau staining); on the right the immunoreactivity of these proteins with Mab 3B. Experimental details as in Fig. 2. Molecular weight markers from the top are: 200, 116, 96, 60 kDa; pUR, expression vector; B_1–B_7, β-galactosidase fusion proteins.

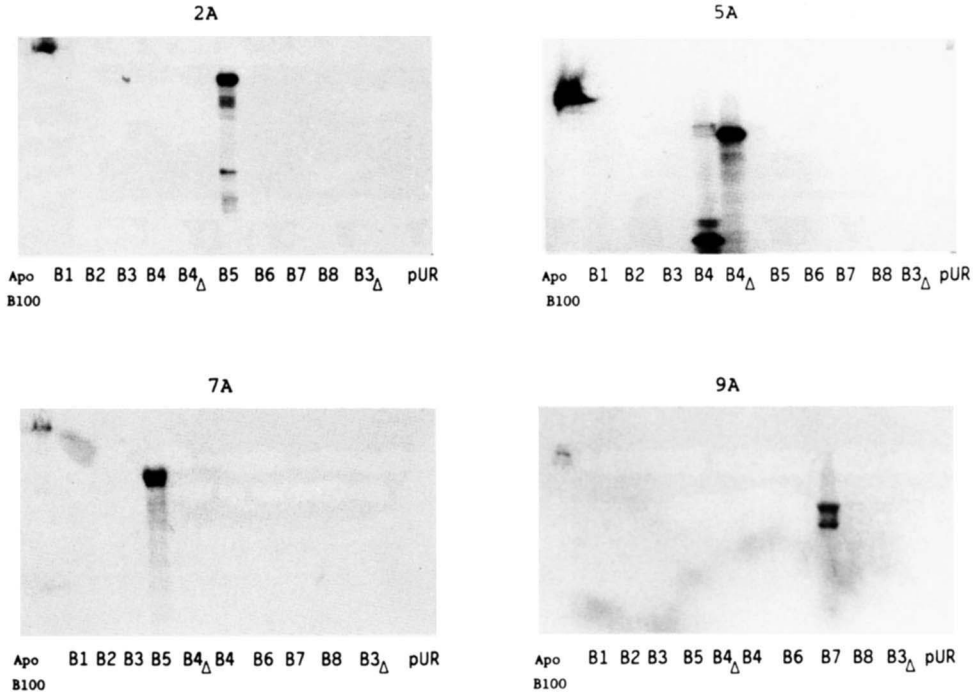

Fig. 5. Immunoreactivity of β-galactosidase–apoB fusion proteins with Mab 2A, 5A, 7A, and 9A. Experimental details as in Fig. 2. pUR, expression vector; B1–B8, β-galactosidase fusion proteins.

DISCUSSION

Aims of the present study were: *1)* to map the epitopes of anti-human apoB monoclonal antibodies raised against human LDL; *2)* to assign more precisely the epitopes for our previously reported antibodies; and *3)* to assess the ability of these antibodies to interfere with the LDL-receptor interaction, thus identifying areas of apoB potentially involved in the binding process.

Antibodies 2A, 5A, 6B, 6E, 7A, and 9A have been reported previously (13, 16, 27, 42). We have mapped their epitopes to thrombolytic fragments T3 with the exception of Mab 6E which interacted with thrombolytic fragment T4 (16). Antibodies 6B and 9A have been previously mapped to apoB-48, while 2A recognized only apoB-100 (42). Our data are in agreement with these findings with the exception of Mab 9A which fails to react with apoB-48. We have repeatedly observed a lack of reaction using different preparations of apoB-48, and confirmed this finding by the interaction of Mab 9A with fusion protein B7 which encompasses aa 2657–3288 of apoB-100 (Fig. 1 and Fig. 5). Furthermore, antibodies 5A and 6E were reported also to interact with apoB-48 (42). In this study we confirmed this epitope assignment.

TABLE 1. Immunological reactivities of monoclonal antibodies to apoB-100 with apoB-48, apoB-100 thrombolytic fragments and β-galactosidase fusion proteins

Antibody	ApoB-48	Thrombolytic Fragments				Fusion Proteins									
		T1	T2	T3	T4	B1	B2	B3	B3Δ	B4	B4Δ	B5	B6	B7	B8
2A	−	+	−	+	−	−	−	−	−	−	−	+	−	−	−
3B	−	+	−	+	−	−	−	−	−	−	−	+	−	−	−
3H	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
4B	−	+	−	+	−	−	−	−	−	−	−	+	−	−	−
5A	+	+	−	+	−	−	−	−	−	+	+	−	−	−	−
6B	+	+	−	+	−	−	−	−	−	+	+	−	−	−	−
6E	+	+	−	−	+	−	−	−	−	−	−	−	−	−	−
7A	−	+	−	+	−	−	−	−	−	−	−	+	−	−	−
8A	−	+	−	+	−	−	−	−	−	−	−	−	−	+	−
9A	−	+	−	+	−	−	−	−	−	−	−	−	−	+	−

ApoB-100, apoB-48, and β-galactosidase fusion proteins were separated by SDS-PAGE and transferred onto nitrocellulose. The interaction of different antibodies with different proteins was determined as described in Methods.

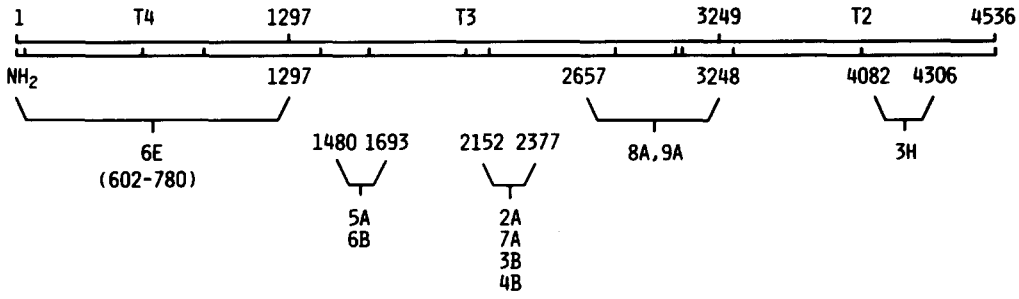

Fig. 6. Linear map of the epitopes for monoclonal antibodies to human LDL on apoB-100. The sites of cleavage by thrombin giving raise to fragments T2, T3, and T4 are indicated. Bars along the line below the linear sequence of apoB-100 indicate the basic regions (45). The epitopes for the various antibodies are indicated by brackets.

We further restricted the apolipoprotein B sequence that contains the antigenic determinants for these antibodies by using β-galactosidase–apoB fusion proteins that span almost the entire aa sequence of apolipoprotein B-100 (see Fig. 1). This approach proved effective for all antibodies but 6E. This Mab interacted with thrombolytic fragment T4 but failed to recognize fusion proteins B1 and B2 which encompass almost the entire T4 sequence. An obvious explanation for this finding is that the epitope for this antibody lies between aa 602 and 780, a sequence not covered by these fusion proteins. Alternatively, we may consider that the use of recombinant proteins for epitope mapping could be hampered by the absence of glycosylation and disulfide bridge formation (41). Fragment T4 is particularly enriched in cysteine residues (7). This may imply complex folding of this region. Recent investigations of crystal structure of protein–Fab complexes show that antigenic determinants are usually located in several discrete segments of the polypeptide chain brought into contiguity on the surface of the folded protein (43). This may be the case for Mab 6E if the epitope determinants were to be located in different areas of T4. This complex structure may not be reproduced by recombinant proteins. This concept is further strengthened by the high sensitivity to reduction with 2-β-mercaptoethanol of the reaction of Mab 6E with apoB-100, apoB-48, and thrombolytic fragment T4. Similar results have been recently reported by Pease et al. (41) for antibody Mb19; we have confirmed these findings using the same antibody (data not shown). Krul et al. (15) have recently reported that antibody B1B3 fails to recognize a fusion protein containing the epitopes for this antibody, suggesting that in some instances inappropriate conformation of the epitope on a linear protein may hamper the antibody–antigen interaction. However, our B1 and B2 fusion proteins were readily recognized by rabbit polyclonal antibodies against synthetic peptides 12–27, 259–279, and 890–908 of apoB-100 sequence, respectively

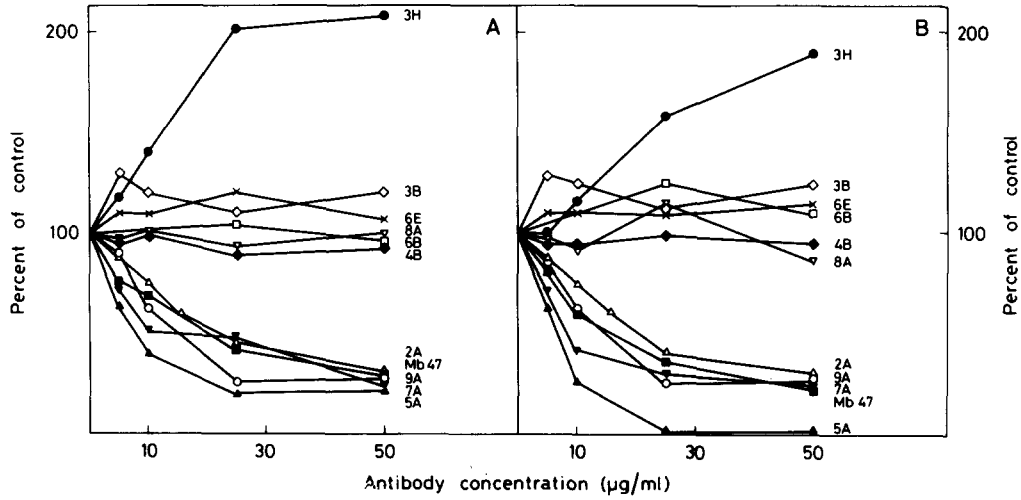

Fig. 7. Effect of monoclonal antibodies on the uptake and degradation of ^{125}I-labeled LDL by human skin fibroblasts. Mabs were used as ascitic fluids. The antibody content of each ascitic fluid was determined after protein A affinity chromatography and were, as % of total protein, 2A, 12%; 6E, 13%; 8A, 14%; 6B, 7A, and 3H, 15%; 5A, 16%; 9A, Mb 47, and 4B, 17%; 3B, 18%. ^{125}I-labeled LDL (7.5 μg) were preincubated for 2 h at 37°C with the indicated amount of Mabs and tested for uptake (panel A) and degradation (panel B) as described in Methods. Data are expressed as % of control values (absolute values ranged from 200 to 350 ng LDL protein/mg cell protein and from 350 to 560 ng LDL protein/mg cell protein for uptake and degradation, respectively).

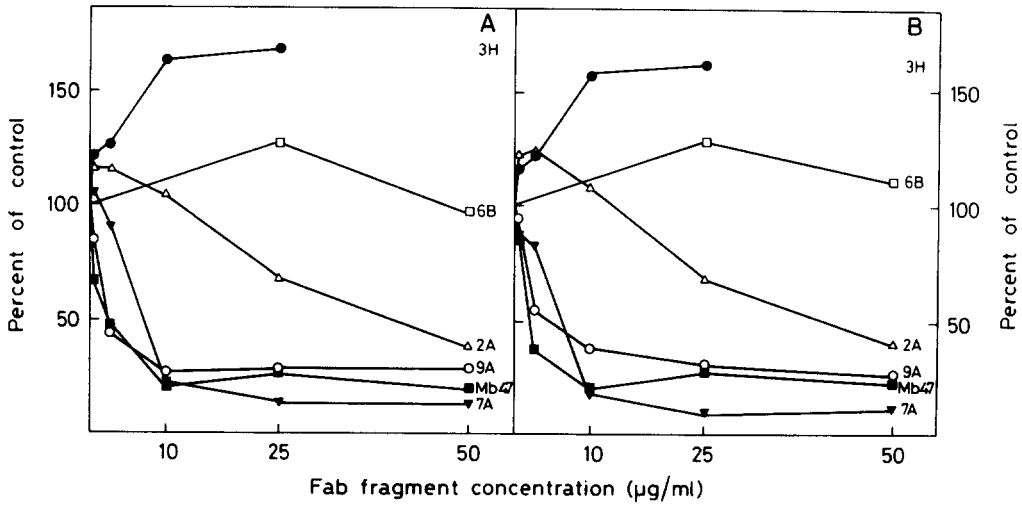

Fig. 8. Effect of Fab fragments on the uptake (panel A) and degradation (panel B) of ^{125}I-labeled LDL by human skin fibroblasts. Experimental conditions as described in Fig. 6. Data are expressed as % of control values (control range 150–250 ng LDL protein/mg cell protein and 300–500 ng LDL protein/mg cell protein for uptake and degradation, respectively).

(44), suggesting that antigenic determinants that lie on a linear sequence were spared. Glycosylation might also have played a role in the antigenicity of apoB-100; however the ability of several antibodies to react with fusion proteins is not in agreement with this concept.

Using the same approach we also assigned the epitopes for four new antibodies designated 3H, 8A, 3B, and 4B. All but 3H interacted with thrombolytic fragment T3. This latter antibody recognized fragment T2. The epitopes for these antibodies were more precisely located using fusion proteins (Figs. 1 and 6). In these studies antibody Mb 47, used as control, interacted with thrombolytic fragment T2 and fusion protein B8 (3351–3504), as expected (41). The epitope distribution for our antibodies on apoB-100 linear sequence indicates a high frequency of immunogenic sites on fragment T3. Based on the intramolecular specificities of the murine Mabs described to date (11–16), it would appear that we have a relatively high frequency of antibodies for T3 as compared to T2 and T4. These differences may depend upon several variables including immunization and screening procedures. Furthermore, Fievet et al. (16) have recently reported a high frequency of rat Mabs to human apoB-100 fragment T3.

Using our panel of Mabs, three segments of fragment T3 appear to be highly antigenic. The area encompassing aa 1480–1693 contains the epitope for antibodies 6B and 5A; competition studies indicate that these antibodies recognize different areas (data not shown). The epitopes for four antibodies (2A, 3B, 4B, 7A) are located in the area encompassing aa 2152–2377. Those for the remaining two antibodies lie in the sequence 2657–3249 near the T3/T2 junction.

There has been some controversy as to whether there are one or more receptor recognition sites per apoB-100 molecule (7, 9, 14, 18, 26–28, 41, 45, 46). Using monoclonal antibodies, several groups concluded that the apoB receptor-binding domain is located near the T2/T3 junction. Two putative areas have been identified at residues 3120–3156 and 3352–3371 of the apoB sequence (7, 9, 14, 15, 26). Furthermore, a mutation at residue 3500 leading to an Arg → Gln substitution is related to a dramatic reduction of LDL receptor binding ability (47, 48). Recently, using a large panel of Mabs, Pease et al. (41) concluded that the receptor binding region of apoB lies between aa 2835 and 4081. However, recent data from our laboratory show that antibody 5A, a Mab that recognizes both apoB-100 and B-48, effectively inhibits the LDL-receptor interaction (27). In this report we have confirmed this finding and extended our observations. Among the ten Mabs tested, four inhibited the LDL binding in a dose-dependent manner. Epitope mapping for these Mabs using β-galactosidase fusion proteins revealed at least three different areas encompassing aa 1480–1693 (5A), 2152–2377 (2A and 7A), and 2657–3288 (9A). To exclude the possibility that steric hindrance might have played a role in determining the effect of the inhibitory antibodies, we prepared Fab fragments. In all instances these fragments were as effective as the parent antibodies. This evidence argues against a nonspecific effect of the inhibitory antibodies and is supported by the epitope localization for Mab 3B, 4B, 6B and 8A, which span the same regions recognized by antibody 5A, 2A, 7A, and 9A, respectively, and yet do not affect LDL binding (see Fig. 6). Furthermore, all inhibitory antibodies failed to interfere with the interaction of oxidized LDL with scavenger

receptors (A. L. Catapano, P. Roma, and S. Negri, unpublished data).

Altogether our findings are consistent with the presence of multiple receptor binding sites on apoB-100. Hospattankar et al. (45) identified several areas of the apoB sequence by computer-assisted analysis, distributed all along the protein and enriched in basic amino acids that could potentially interact with the negatively charged amino acids of the LDL receptor. Furthermore, Corsini et al. (46) have shown that recombinant lipoprotein particles, each containing a thrombolytic fragment of apoB-100, bound to the LDL receptor to the same extent although less efficiently than native lipoproteins. While the binding of the recombinant lipoprotein may not truly reflect the binding of native LDL, it is nevertheless an observation that is at least consistent with the presence of multiple binding domains on apoB-100 that interact with the LDL receptor either independently or in concert.

Alternatively, our findings can be interpreted as indicating that the entire apoB-100 sequence is required for appropriate expression of the apolipoprotein area responsible for binding activity. It is reasonable to assume that in apoB-100 long-range conformational interactions may occur. It has been shown that binding to the enzymes of monoclonal and polyclonal antibodies with epitopes distant from the catalytic site may modulate enzymatic activity (49). Furthermore, phosphorylation of the NH_2-terminal region of glycogen phosphorylase induces conformational changes of the COOH-terminal region and alters the binding sites for substrates (50). Antibodies 5A, 2A, 7A, and 9A may interact with areas of apolipoprotein B-100 that dictate the conformation of a distant site(s) involved in the LDL-receptor interaction. Recently, Kinoshita, Krul, and Schonfeld (51) have reported that modification of the core lipids of LDL reduces the lipoprotein-receptor interaction with a concomitant decrease of the expression of epitopes for Mabs in the NH_2- and COOH-terminal regions of apoB-100. These authors conclude that regions at a distance from a putative LDL receptor recognition domain of apoB-100 may modulate the interaction of LDL with their receptor. These antibodies, however, do not affect the LDL-receptor interaction (15). Furthermore, in familial defective apoB-100 (47, 48, 52), the Arg → Gln substitution at aa 3500 is also linked to an overall change of the apolipoprotein B conformation (53).

Very recently Peterson and Schachman (54) have shown that single amino acid substitution in one element of the secondary structure of aspartate transcarbamoylase causes structural alterations that propagate throughout the molecule to distant sites.

The concept that different regions of apoB-100 may modulate the LDL-receptor interaction is further stressed by the findings with antibody 3H. This antibody stimulates the binding of ^{125}I-labeled LDL to the LDL-receptor in a dose-dependent manner; Fab fragments were also effective. Furthermore, the specificity of the stimulatory effect was demonstrated by the experiments with receptor-negative fibroblasts. Antibody 3H failed to stimulate LDL uptake, thus ruling out a role for non-LDL receptor-mediated pathways in determining the antibody stimulatory effect.

The epitope for Mab 3H lies in the COOH-terminal region between aa 4082 and 4306, an area of apoB that may play a "suppressive" role on the LDL-receptor interaction. Recent data with LDL containing an apoB-100 truncated form of 4039 aa that lacks the COOH-terminal end show that these lipoproteins interact with LDL receptor with greater affinity than normal (54–56). Similar findings have been reported by Gabelli et al. (57) with apoB-90 and by Krul et al. (58) with apoB-75. Furthermore, a point mutation at aa 4019 (Arg → Trp) is linked to a moderate increase of the LDL-receptor interaction (59). This is in agreement with results obtained with antibody 3H and suggest that the COOH-terminal region of apoB-100 may play a "suppressive" role in the LDL-receptor interaction.

In summary, we have characterized the epitopes for ten monoclonal antibodies to apolipoprotein B-100. Among them four, recognizing distinct epitopes (2A, 5A, 7A, and 9A), inhibited and one (3H) increased the LDL-receptor interaction. These findings are consistent with the hypothesis that different areas of apoB-100 are involved in the modulation of the LDL-receptor interaction.

We wish to thank Dr. Tom Innerarity (Gladstone Foundation Laboratories) for providing us with the antisera against apoB synthetic peptides, and Drs. Linda Curtiss and Joseph Witztum (La Jolla, CA) and Dr. Steve Young (Gladstone Foundation Laboratories) for kindly supplying us with antibodies Mb47 and Mb19. The authors wish to thank Miss Maddalena Marazzini for secretarial help. Part of the work was supported by grants from CNR (Progetto Finalizzato Biotecnologie e Biostrumentazione and Progetto Finalizzato Ingegneria Genetica) and from Ministero Pubblica Istruzione.

Manuscript received received 7 December 1990 and in revised form 19 March 1992.

REFERENCES

1. Kane, J. P. 1983. Apolipoprotein B: structural and metabolic heterogeneity. *Annu. Rev. Physiol.* **45:** 637–650.
2. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
3. Edge, S. B., J. M., Hoeg, P. E. Schneider, and H. B. Brewer, Jr. 1985. Apolipoprotein B synthesis in humans: liver synthesizes only apolipoprotein B-100. *Metabolism.* **34:** 726–729.
4. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.

5. Glickman, R. M., M. Rogers, and J. M. Glickman. 1986. Apolipoprotein B synthesis by human liver and intestine in vitro. *Proc. Natl. Acad Sci. USA.* **83:** 5296–5300.
6. Hui, D. Y., T. L. Innerarity, R. W. Milne, Y. L. Marcel, and R. W. Mahley. 1984. Binding of chylomicron remnants and β-very low density lipoproteins to hepatic and extrahepatic lipoprotein receptors: a process independent of apolipoprotein B-48. *J. Biol. Chem.* **259:** 15060–15068.
7. Knott, T. J., R. J. Pease, L. M. Powell, S. C. Wallis, S. C. Rall, Jr., T. L. Innerarity, B. Blackhart, W. H. Taylor, Y. Marcel, R. Milne, D. Johnson, M. Fuller, A. J. Lusis, B. J. McCarthy, R. W. Mahley, B. Levy-Wilson, and J. Scott. 1986. Complete protein sequence and identification of structural domains of human apolipoprotein B. *Nature.* **323:** 734–738.
8. Law, S. W., S. M. Grant, K. Higuchi, A. Hospattankar, K. Lackner, N. Lee, and H. B. Brewer, Jr. 1986. Human liver apolipoprotein B-100 cDNA: complete nucleic acid and derived amino acid sequence. *Proc. Natl. Acad. Sci. USA.* **83:** 8142–8146.
9. Yang, C. Y., S. H. Chen, S. H. Gianturco, W. A. Bradley, J. T. Sparrow, M. Tanimura, W. H. Li, D. A. Sparrow, H. De Loof, M. Rosseneu, F. S. Lee, Z. W. Gu, A. M. Gotto, Jr., and L. Chan. 1986. Sequence, structure, receptor-binding domains and internal repeats of human apolipoprotein B-100. *Nature.* **323:** 738–742.
10. Powell, L. M., S. C. Wallis, R. J. Pease, Y. H. Edwards, T. J. Knott, and J. Scott. 1987. A novel form of tissue-specific RNA processing produces apolipoprotein B-48 in intestine. *Cell.* **50:** 831–840.
11. Curtiss, L. K., and T. S. Edgington. 1982. Immunochemical heterogeneity of human plasma apolipoprotein B. I. Apolipoprotein B binding of mouse hybridoma antibodies. *J. Biol. Chem.* **257:** 15213–15221.
12. Mao, S. J. T., R. E. Kazmar, J. C. Silverfield, M. C. Alley, K. Kluge, and C. G. Fathman. 1982. Immunochemical properties of human low density lipoproteins as explored by monoclonal antibodies: binding characteristics distinct from those of conventional serum antibodies. *Biochim. Biophys. Acta.* **713:** 365–374.
13. Marcovina, S., D. France, R. A. Phillips, and S. J. T. Mao. 1985. Monoclonal antibodies can precipitate low-density lipoprotein. I. Characterization and use in determining apolipoprotein B. *Clin. Chem.* **31:** 1654–1658.
14. Marcel, Y. L., T. L. Innerarity, C. Spilman, R. W. Mahley, A. Protter, and R. W. Milne. 1987. Mapping of human apolipoprotein B antigenic determinants. *Arteriosclerosis.* **7:** 166–175.
15. Krul, E. S., Y. Kleinman, M. Kinoshita, B. Pfleger, K. Oida, A. Law, J. Scott, R. Pease, and G. Schonfeld. 1988. Regional specificities of monoclonal anti-human apolipoprotein B antibodies. *J. Lipid Res.* **29:** 937–947.
16. Fievet, C., C. Durieux, R. Milne, T. Delaunay, G. Agnani, H. Bazin, Y. Marcel, and J. C. Fruchart. 1989. Rat monoclonal antibodies to human apolipoprotein B: advantages and applications. *J. Lipid Res.* **30:** 1015–1024.
17. Tsao, B. P., L. K. Curtiss, and T. S. Edgington. 1982. Immunochemical heterogeneity of human plasma apolipoprotein B. II. Expression of apolipoprotein B epitopes on native lipoproteins. *J. Biol. Chem.* **257:** 15222–15228.
18. Tikkanen, M. J., T. G. Cole, and G. Schonfeld. 1983. Differential reactivity of human low density lipoproteins with monoclonal antibodies. *J. Lipid Res.* **24:** 1494–1499.
19. Schumaker, V. N., M. T. Robinson, L. K. Curtiss, R. Butler, and R. S. Sparkes. 1984. Anti-apoprotein B monoclonal antibodies detect human low density lipoprotein polymorphism. *J. Biol. Chem.* **259:** 6423–6430.
20. Teng, B., A. Sniderman, R. M. Krauss, P. O. Kwiterowich, Jr., R. W. Milne, and Y. L. Marcel. 1985. Modulation of apolipoprotein B antigenic determinants in human low density lipoprotein subclasses. *J. Biol. Chem.* **260:** 5067–5072.
21. Young, S. G., S. J. Bertics, T. M. Scott, B. W. Dubois, W. F. Belts, L. K. Curtiss, and J. L. Witztum. 1987. Apolipoprotein B allotypes $MB19_1$ and $MB19_2$ in subjects with coronary artery disease and hypercholesterolemia. *Arteriosclerosis.* **7:** 61–65.
22. Tikkanen, M. J., C. Ehnholm, R. Butler, S. G. Young, L. K. Curtiss, and J. L. Witztum. 1986. Monoclonal antibody detects Ag polymorphism of apolipoprotein B. *FEBS Lett.* **202:** 54–58.
23. Young, S. G., S. J. Bertics, L. K. Curtiss, D. C. Casal, and J. L. Witztum. 1986. Monoclonal antibody MB19 detects genetic polymorphism in human apolipoprotein B. *Proc. Natl. Acad. Sci. USA.* **83:** 1101–1105.
24. Weisgraber, K. H., T. L. Innerarity, Y. M. Newhouse, S. G. Young, K. S. Arnold, R. M. Krauss, G. L. Vega, S. M. Grundy, and R. M. Mahley. 1988. Familial defective apolipoprotein B-100: enhanced binding of monoclonal antibody Mb47 to abnormal low density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **85:** 9758–9762.
25. Gavish, D., E. A. Brinton, and J. L. Breslow. 1989. Heritable allele-specific differences in amounts of apoB and low-density lipoproteins in plasma. *Science.* **244:** 72–76.
26. Young, S. G., J. L. Witztum, D. C. Casal, L. K. Curtiss, and S. Bernstein. 1986. Conservation of the low density lipoprotein receptor-binding domain of apopoprotein B. Demonstration by a new monoclonal antibody, Mb47. *Arteriosclerosis.* **6:** 178–188.
27. Corsini, A., S. Fantappiè, S. Marcovina, A. Granata, R. Fumagalli, and A. L. Catapano. 1989. Monoclonal antibody 5A binds apolipoprotein B-48 and inhibits the low density lipoprotein-receptor interaction. *Biochem. Biophys. Res. Commun.* **162:** 908–915.
28. Milne, R., R. Theolis, Jr., R. Maurice, R. J. Pease, P. K. Weech, E. Rassart, J. C. Fruchart, J. Scott, and Y. L. Marcel. 1989. The use of monoclonal antibodies to localize the low density lipoprotein receptor-binding domain of apolipoprotein B. *J. Biol. Chem.* **264:** 19754–19760.
29. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
30. Goldstein, J. L., S. K. Basu, and M. S. Brown. 1983. Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. *Methods Enzymol.* **98:** 241–260.
31. Bilheimer, W. D., S. Eisenberg, and R. I. Levy. 1972. Metabolism of very low density lipoproteins. I. Preliminary in vivo and in vitro observations. *Biochim. Biophys. Acta.* **260:** 212–221.
32. Cardin, A. D., K. R. Witt, J. Chao, H. S. Margolius, V. H. Donaldson, and R. L. Jackson. 1984. Degradation of apolipoprotein B-100 of human plasma low density lipoproteins by tissue and plasma kallikreins. *J. Biol. Chem.* **259:** 8522–8528.
33. Shoulders, C. C., N. B. Myant, A. Sidoli, J. C. Rodriguez, C. Cortese, F. E. Baralle, and R. Cortese. 1985. Molecular cloning of human LDL apolipoprotein B cDNA. *Atherosclerosis.* **58:** 277–289.
34. Jenner, K., A. Sidoli, M. Ball, J. R. Rodriguez, F. Pagani, G. Giudici, C. Vergani, J. Mann, F. E. Baralle, and C. C.

Shoulders. 1988. Characterization of genetic markers in the 3' end of the apoB gene and their use in family and population studies. *Atherosclerosis.* **69:** 39–49.
35. Ruther, U., and B. Muller-Hill. 1983. Easy identification of cDNA. *EMBO J.* **10:** 1791–1794.
36. Sidoli, A., S. Galliani, C. Tosi, C. Mariani, C. De Lalla, B. Da Ros, P. Rossi, and F. E. Baralle. 1990. Recombinant bifunctional polypeptides as a tool to provide innovative tracers for immunoenzymatic apolipoprotein determination. *Atheroscler. Rev.* **20:** 239–244.
37. Medgyesi, G. A., G. Gorini, and G. Doria. 1969. Structural localization of isotypic markers on mouse myeloma IgG globulins. *J. Immunol.* **103:** 1125–1131.
38. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227:** 680–685.
39. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* **76:** 4350–4354.
40. Fraker, P. J., and J. C. Speck, Jr. 1978. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril. *Biochem. Biophys. Res. Commun.* **80:** 849–857.
41. Pease, R. J., R. W. Milne, W. K. Jessup, A. Law, P. Provost, J. C. Fruchart, R. T. Dean, Y. L. Marcel, and J. Scott. 1990. Use of bacterial expression cloning to localize the epitopes for a series of monoclonal antibodies against apolipoprotein B-100. *J. Biol. Chem.* **265:** 553–568.
42. Cardin, A. D., C. A. Price, N. Hirose, M. A. Krivanek, D. T. Blankenship, J. Chao, and S. J. T. Mao. 1986. Structural organization of apolipoprotein B-100 of human plasma low density lipoproteins: comparison to B-48 of chylomicrons and very low density lipoproteins. *J. Biol. Chem.* **261:** 6744–6748.
43. Davies, D. R., S. Sheriff, and E. A. Padlan. 1988. Antibody–antigen complexes. *J. Biol. Chem.* **263:** 10541–10544.
44. Innerarity, T. L., S. G. Young, K. S. Poksay, R. W. Mahley, R. S. Smith, R. W. Milne, Y. L. Marcel, and K. H. Weisgraber. 1987. Structural relationship of human apolipoprotein B-48 to apolipoprotein B-100. *J. Clin. Invest.* **80:** 1794–1798.
45. Hospattankar, A. V., S. W. Law, K. Lackner, and H. B. Brewer, Jr. 1986. Identification of low density lipoprotein receptor binding domains of human apolipoprotein B-100. A proposed consensus LDL receptor binding sequence of apoB-100. *Biochem. Biophys. Res. Commun.* **139:** 1078–1085.
46. Corsini, A., C. H. Spilman, T. L. Innerarity, K. S. Arnold, S. C. Rall, Jr., J. K. Boyles, and R. W. Mahley. 1987. Receptor binding activity of lipid recombinants of apolipoprotein B-100 thrombolytic fragments. *J. Lipid Res.* **28:** 1410–1423.
47. Innerarity, T. L., K. H. Weisgraber, K. S. Arnold, R. W. Mahley, R. M. Krauss, G. L. Vega, and S. M. Grundy. 1987. Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding. *Proc. Natl. Acad. Sci. USA.* **84:** 6919–6923.
48. Soria, L. F., E. H., Ludwig, H. R. G. Clarke, G. L. Vega, S. M. Grundy, and B. J. McCarthy. 1989. Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. *Proc. Natl. Acad. Sci. USA.* **86:** 587–591.
49. Accolla, R. S., R. Cina, E. Montesoro, and F. Celada. 1981. Antibody-mediated activation of genetically defective *Escherichia coli* β-galactosidases by monoclonal antibodies produced by somatic cell hybrids. *Proc. Natl. Acad. Sci. USA.* **78:** 2478–2482.
50. Sprang, S. R., K. R. Acharya, E. J. Goldsmith, D. I. Stuart, K. Varvill, R. J. Fletterick, N. B. Madsen, and L. N. Johnson. 1988. Structural changes in glycogen phosphorylase induced by phosphorylation. *Nature.* **336:** 215–221.
51. Kinoshita, M., E. S. Krul, and G. Schonfeld. 1990. Modification of the core lipids of low density lipoproteins produces selective alterations in the expression of apoB-100 epitopes. *J. Lipid Res.* **31:** 701–708.
52. Corsini, A., B. J. McCarthy, A. Granata, L. F. Soria, S. Fantappiè, F. Bernini, C. Romano, L. Romano, R. Fumagalli, and A. L. Catapano. 1991. Familial defective apoB-100, characterization of an Italian family. *Eur. J. Clin. Invest.* **21:** 389–397.
53. Lund-Katz, S., T. L. Innerarity, K. S. Arnold, L. K. Curtiss, and M. C. Phillips. 1991. ^{13}C NMR evidence that substitution of glutamine for arginine 3500 in familial defective apolipoprotein B-100 disrupts the conformation of the receptor-binding domain. *J. Biol. Chem.* **266:** 2701–2704.
54. Peterson, C. B., and H. K. Schachman. 1992. Long-range effects of amino acid substitutions in the catalytic chain of aspartate transcarbamoylase. *J. Biol. Chem.* **267:** 2443–2450.
55. Krul, E. S., M. Kinoshita, P. Talmud, S. E. Humphries, S. Turner, A. C. Goldberg, K. Cook, E. Boerwinkle, and G. Schonfeld. 1989. Two distinct truncated apolipoprotein B species in a kindred with hypobetalipoproteinemia. *Arteriosclerosis.* **9:** 856–868.
56. Talmud, P., L. King-Underwood, E. S. Krul, G. Schonfeld, and S. Humphries. 1989. The molecular basis of truncated forms of apolipoprotein B in a kindred with compound heterozygous hypobetalipoproteinemia. *J. Lipid Res.* **30:** 1773–1779.
57. Gabelli, C., G. Baggio, C. Bilato, S. Martini, L. Previato, Y. Marcel, A. Corsini, and G. Crepaldi. 1989. Identification of a new apoB variant associated with hypobetalipoproteinemia. *Arteriosclerosis.* **9:** 706a.
58. Krul, E. S., R. D. Wagner, K. G. Parhofer, H. Barrett, and G. Schonfeld. 1991. Apolipoprotein B-75: a truncated form of apolipoprotein B with increased affinity for the LDL receptor. 9th International Symposium on Atherosclerosis, Rosemont, Chicago, IL. Abstract Book; pp. 166.
59. Ladias, J. A. A., P. O. Kwiterovich Jr., H. H. Smith, M. Miller, P. S. Bachorick, T. Forte, A. J. Lusis, and S. E. Antonarakis. 1989. Apolipoprotein B-100 Hopkins ($Arginine_{4019} \rightarrow Tryptophan$). A new apolipoprotein B-100 variant in a family with premature atherosclerosis and hyperapobetalipoproteinemia. *JAMA.* **262:** 1980–1988.