Definition of a nonlinear conformational epitope for the apolipoprotein B-100-specific monoclonal antibody, MB47

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Abstract The apolipoprotein (apo) B-100-specific monoclonal antibody MB47 has been widely used in lipoprotein metabolism and atherosclerosis research. When bound to apoB-100 on low density lipoproteins (LDL), antibody MB47 completely blocks the binding of LDL to the LDL receptor. The epitope for antibody MB47 has previously been mapped to the vicinity of apoB-100 amino acid (aa) residue 3500. To map the epitope for antibody MB47 more precisely, we used recombinant bacterial fusion proteins. Antibody MB47 bound strongly to a fusion protein containing apoB-100 aa 3214–3728, but no specific binding was observed to fusion proteins containing aa 3214–3351, 3214–3506, 3351–3506, or a fusion protein containing aa 3214–3351 and 3506–3728. Although antibody MB47 did not bind to aa 3214–3506, it did bind to aa 3214–3510. Further fusion protein studies revealed that antibody MB47 bound to aa 3429–3510, but bound only very weakly to aa 3453–3510, indicating that aa 3429–3453 constitute an important part of the MB47 epitope. Subsequent fusion protein studies revealed that MB47 bound much more strongly to aa 3429–3523, 3429–3544, 3429–3565, and 3429–3590 than to aa 3429–3510. Thus, aa 3507–3523 also constitute an important part of the MB47 epitope. In summary, the fusion protein data indicated that two nonlinear domains of apoB-100 separated by ~53 aa (the 25 residues from aa 3429 to 3453 and the 17 residues from aa 3507 to 3523) form key parts of the MB47 epitope. Antibody MB47 failed to bind to any of 15 different synthetic apoB peptides that spanned aa 3415–3510, a finding that is consistent with the fusion protein data indicating that the MB47 epitope is formed by the conformational alignment of discontinuous amino acid sequences.—**Young, S. G., R. K. Koduri, R. K. Austin, D. J. Bonnet, R. S. Smith, and L. K. Curtiss.** Definition of a nonlinear conformational epitope for the apolipoprotein B-100-specific monoclonal antibody, MB47. *J. Lipid Res.* 1994. **35:** 399–407.

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In 1986, Young et al. (1) characterized a novel apoB-100-specific monoclonal antibody, MB47. Antibody MB47 binds to apoB-100 with high affinity, and, when bound to the apoB-100 of low density lipoproteins (LDL), blocks the binding of LDL to the LDL receptor. Unlike many other apoB-specific monoclonal antibodies (2–4), antibody MB47 binds to virtually 100% of apoB-100-containing particles, indicating that the MB47 epitope is exposed on almost all LDL particles (1). Antibody MB47 binds with high affinity to the apoB of every mammalian species that has been tested except for rat and mouse, indicating that the MB47 epitope has been conserved throughout mammalian evolution (1).

Antibody MB47 has been widely utilized in lipoprotein and atherosclerosis research. Because the MB47 epitope is exposed on virtually all apoB-100-containing lipoproteins, it has been used in immunoassays to measure apoB-100 concentrations in plasma (5, 6). It has been suggested that antibody MB47 is an ideal antibody for a reference apoB assay (7, 8). The antibody was used to study the interaction of lipoproteins with the LDL receptor (1, 9–12) and to detect an apoB-100 aa substitution that is associated with defective binding of LDL to the LDL receptor (13–15). Antibody MB47 proved to be very useful in mapping the topography of apoB-100 on LDL particles by electron microscopy (16). It also was used in immunohistochemical studies to detect the presence of apoB-100 in human atherosclerotic lesions (17). Because antibody MB47 binds to the apoB of other mammalian species, it was used for immunohistochemical studies of rabbit atherosclerosis (17–21). Furthermore, the absence of antibody MB47 binding to mouse apoB has made it ideal for detection of human apoB in transgenic mice (22, 23).

Abbreviations: apo, apolipoprotein; LDL, low density lipoprotein; aa, amino acid(s).

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The bacterial fusion protein studies of Knott et al. (24) indicated that antibody MB47 binds weakly to apoB residues 3133–3494 but strongly to apoB residues 3350–3506. Subsequently, Pease et al. (25) reported that antibody MB47 bound strongly to fusion proteins containing amino acids (aa) 3214–3611 and 3214–3728. In addition, they found that antibody MB47 bound to a 14-kDa cyanogen bromide (CNBr) fragment of these fusion proteins, a size predicted for the CNBr fragment spanning Met_{3441} to Met_{3569}. Pease et al. (25) also found that antibody MB47 bound to fusion proteins containing two adjacent nonoverlapping segments of apoB (the 156-aa region between residues 3351 and 3506 and the 182-aa region between residues 3506 and 3687) although the binding to these fusion proteins was not as strong as it was to the fusion protein containing the 515-aa region between residues 3214 and 3728.

In this study, we used additional fusion proteins and synthetic peptides for more precise mapping of the epitope for antibody MB47. In our initial experiments, we confirmed the observation of Pease et al. (25) that antibody MB47 bound very strongly to apoB-100 aa 3214–3728. However, in contrast to their results, we were unable to detect binding of antibody MB47 to fusion proteins containing either aa 3351–3506 or aa 3506–3728. In subsequent experiments, we demonstrated that two discontinuous apoB sequences, the 25 aa from residues 3429 to 3453 and the 17 aa from residues 3507 to 3523, were important in forming the MB47 epitope. The precise localization of the MB47 epitope may prove to be important for understanding the conformation of the LDL receptor binding region on apoB-100. Also, the precise localization of the epitope for antibody MB47 should facilitate the development of stable protein fragments of apoB-100 that can be used for standardizing apoB immunoassays. Stable peptide standards would represent a significant advance over the currently available lipoprotein and plasma standards.

MATERIALS AND METHODS

β-Galactosidase-apoB fusion proteins

We obtained an *E. coli* clone from Drs. James Scott and Richard Pease that contained a plasmid coding for a β-galactosidase (B-gal)-apoB fusion protein. This plasmid contained a *Sac*I-*Bgl*II apoB cDNA fragment (apoB cDNA nucleotides 9854–11391, corresponding to apoB-100 aa 3214–3728), cloned into the internal *Sac*I site in the *lac*Z gene and polylinker *Bam*HI site in the 3' end of the *lac*Z gene of pUR278 (26); this clone yielded a β-gal-apoB fusion protein that was bound strongly by antibody MB47. The apoB cDNA insert from this plasmid was subsequently cloned into pUR291 (26). All subsequent β-gal-apoB fusion proteins were prepared by enzymatically amplifying various apoB cDNA fragments from the plasmid containing the *Sac*I-*Bgl*II insert and then cloning the amplified DNA fragments into the polylinker sites of pUR291. For the amplification reaction, we used 50 pmol of oligonucleotide primers (typically containing 30–43 nucleotides) and *Taq* Polymerase (Cetus, Emeryville, CA), with denaturation, annealing, and extension temperatures of 95°C (1 min), 52°C (2 min), and 72°C (3 min), respectively (27). The 5' (forward) primers contained nucleotide mismatches to create a *Bam*HI site and the 3' (reverse) primer contained mismatches to incorporate a TGA stop codon and a *Pst*I site. (The insertion of the stop codon into the reverse primer ensured that translation of the fusion protein would terminate at the end of the apoB cDNA sequences.) The amplified apoB cDNA fragments were then digested with restriction endonucleases, gel-purified, and ligated into *Bam*HI/*Pst*I-cleaved pUR291; the ligation reactions were transformed into SURE competent cells (Stratagene, La Jolla, CA). The size and orientation of the apoB cDNA inserts were assessed by restriction digestion of plasmid DNA (28).

β-Galactosidase-apoA-I/apoB fusion proteins

In the course of these studies, we generated several β-gal-apoA-I/apoB fusion proteins that contained both the epitope for the apoA-I specific monoclonal antibody, AI-11, and the epitope for antibody MB47. Antibodies AI-11 and MB47 have proven to be useful for apoA-I and apoB immunoassays, respectively (5, 6, 8). To generate a single fusion protein expressing the epitopes for both antibodies, a fragment of the apoA-I cDNA (cDNA nucleotides 142–807, corresponding to apoA-I aa 1–222) was enzymatically amplified from an apoA-I cDNA clone (generously provided by Dr. Jan Breslow) using *Taq* polymerase and oligonucleotide primers containing a *Pst*I site. The amplified fragment was cleaved with *Pst*I and ligated into the *Pst*I site of pUR291. The plasmid containing the apoA-I cDNA was then linearized at the polylinker *Hin*dIII site (located 3' to the *Pst*I site) and the cohesive ends were filled in using T4 DNA polymerase (28); also, the plasmid was digested with *Xho*I, which cleaves the apoA-I cDNA at nucleotide 791 (corresponding to aa 203). Then, four different apoB cDNA fragments containing the MB47 epitope were cloned in-frame to the 3' end of the apoA-I coding sequence. The four apoB cDNA fragments coded for apoB aa 3429–3590, 3429–3565, 3529–3544, and 3529–3523, respectively, and were generated by PCR using a 5' oligonucleotide containing an *Xho*I site. The four enzymatically amplified apoB cDNA fragments were cleaved with *Xho*I and cloned into the *Xho*I-cleaved plasmid described above. The resultant clones coded for β-gal-apoA-I/apoB fusion proteins.

Preparation of fusion proteins

To prepare fusion proteins, 40 μl of SURE competent cells (Stratagene, La Jolla, CA) was transformed with 0.5

μg of plasmid DNA, and the transformed cells were grown for 6 h in 10 ml of LB medium (28) containing 50 μg of ampicillin per ml. A volume of 2.5 ml of this culture was then inoculated into 250 ml of LB containing 50 μg of ampicillin per ml and grown at 37°C in a shaking water bath to an absorbance of 0.5 (600 nm). Then, isopropyl β-thiogalactoside (IPTG) was added (final concentration 250 μg/ml), and the bacteria were grown for an additional 3 h. Bacteria were pelleted by centrifugation at 4000 *g*; the supernatant fluid was decanted and the bacteria were resuspended in 2 ml of phosphate-buffered saline (PBS) (0.154 M NaCl/21 mM Na_2HPO_4/15 mM NaH_2PO_4/0.3 mM EDTA, pH 7.35) containing 5% sodium dodecyl sulfate (SDS). The cells were disrupted by sonication using the procedure of Chang et al. (29). The bacterial debris was pelleted by centrifugation at 4000 *g* and the protein concentration of the supernatant fluid (containing the solubilized β-gal-apoB fusion protein) was determined by a modified Lowry assay (30).

Antibodies and Western blots

Antibodies MB47 (1) and AI-11 (31, 32) were purified from mouse ascites fluid on a Pharmacia FPLC Mono-Q column (Pharmacia Inc., Uppsala, Sweden) as previously described (5). A rabbit polyclonal antiserum generated to a synthetic apoB peptide containing apoB-100 aa 3490–3511 (33) was used in the Western blot studies to confirm that the fusion proteins were appropriately folded and stable to the extent that they could react with the polyclonal antibody. A polyclonal rabbit antibody specific for β-galactosidase (Cappel, Durham, NC) was used in the Western blot studies to control for differences in the expression or loading of the various fusion proteins.

To perform the Western blot studies, from 20 to 40 μg of the solubilized bacterial extracts were electrophoresed on 3–20% gradient SDS-polyacrylamide gels under reducing conditions. The separated proteins were then electrophoretically transferred to Immun-Lite (Bio-Rad, Richmond, CA) membranes. Western blots were then visualized as previously described (2, 34) using an Immun-Lite chemiluminescent Assay Kit (Bio-Rad, Richmond, CA) for detection of either mouse or rabbit antibody.

Generation of apoB synthetic peptides and competitive solid phase immunoassays

To test whether a synthetic peptide containing from 20 to 40 aa residues could specify the epitope for antibody

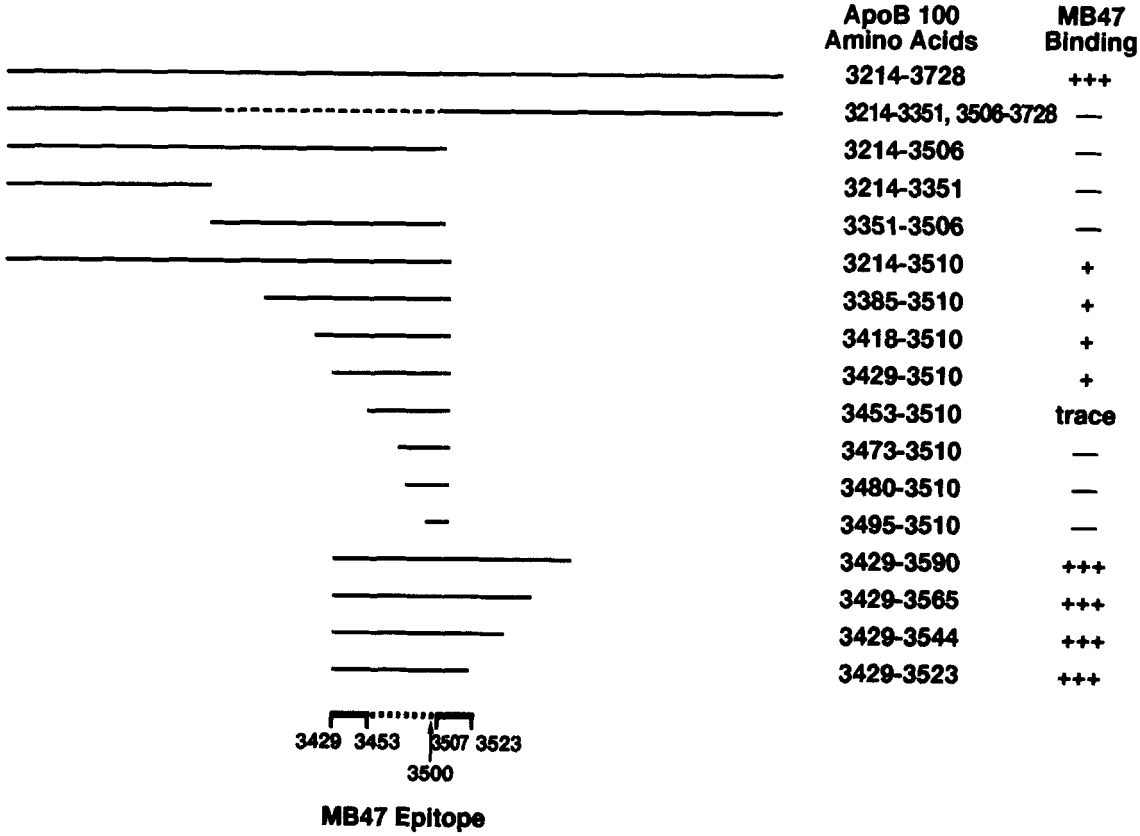

Fig. 1. Summary of antibody binding to β-gal-apoB and β-gal apoA-I/apoB fusion proteins. The data illustrate the spatial relationship between the various apoB-100 fusion proteins used in this study. Shown are the results described in the text of the binding of monoclonal antibody MB47 to the various fusion proteins (−), no antibody binding; (+), weak but detectable binding; (+ + +), strong binding.

MB47, we used an Applied Biosystems 430-A peptide synthesizer to synthesize peptides containing apoB aa 3426–3456, 3417–3446, 3415–3456, 3441–3460, 3446–3465, 3451–3470, 3461–3480, 3466–3485, 3471–3490, 3471–3500, .3476–3495, 3479–3505, 3481–3500, 3486–3505, and 3491–3510. The peptides were purified by HPLC. In all cases, the peptides were analyzed by aa analysis, and in some cases the peptides were sequenced (31).

To assess binding of antibody MB47 to the peptides, the peptides were solubilized in PBS and tested for their ability to compete with human LDL for binding to antibody MB47. For this assay, human LDL was immobilized on 96-well Falcon microtest plates by coating the plates with LDL (10 μg/ml) for 2 h. To determine whether the synthetic peptides could compete with the immobilized LDL for binding to antibody MB47, 0.05 ml of a limiting concentration of antibody MB47 and 0.05 ml of various concentrations of the solubilized peptide (from 0.1 to 2 mg/ml) were added to the LDL-coated wells. After 18 h at 4°C, the plates were washed, and antibody MB47 binding to the immobilized LDL was assessed after a 4-h incubation with radioiodinated goat anti-mouse Ig (1, 35). Direct binding of the antibodies to the peptides (1 mM) immobilized on nitrocellulose also was assessed with the radioiodinated goat anti-mouse Ig.

RESULTS

The schematic shown in **Fig. 1** illustrates the linear relationship of the various apoB-fusion proteins used in this study. Also shown is a summary of their MB47 binding properties as discussed in detail below. Furthermore, each of these fusion proteins was screened by Western blotting for binding to an apoB-peptide-specific rabbit polyvalent antibody that was generated by immunization with a synthetic peptide representing apoB-100 residues 3490–3511. This rabbit antiserum has been described previously (33). All fusion proteins except the deleted proteins (aa 3414–3351, 3506–3728) and the aa 3214–3351 protein were bound by this antiserum. This control was included to verify that all fusion proteins containing this region of apoB-100 had a conformation that was recognized by a polyclonal apoB-100 antibody.

As shown in **Fig. 2,** antibody MB47 bound strongly to the β-gal-apoB fusion protein containing apoB aa 3214–3728, confirming the result of Pease et al. (25). In repeated experiments, however, antibody MB47 did not bind to fusion proteins containing apoB aa 3214–3506, 3214–3351, 3351–3506, or to a fusion protein containing aa 3214–3351 and 3506–3728 (Fig. 2). These results indicated that the MB47 epitope was not contained solely within aa 3214–3351, 3351–3506, or 3506–3728.

We next used enzymatically amplified apoB cDNA fragments to generate a series of β-gal-apoB fusion proteins that terminated with apoB aa 3510. We found that antibody MB47 bound to fusion proteins containing apoB-100 aa 3214–3510, 3385–3510, 3418–3510, and 3429–3510, but not to fusion proteins containing aa 3473–3510, 3480–3510, or 3495–3510 (**Fig. 3** and **Fig. 4**) even though these same fusions could bind the peptide-specific polyclonal antiserum. There was trace MB47

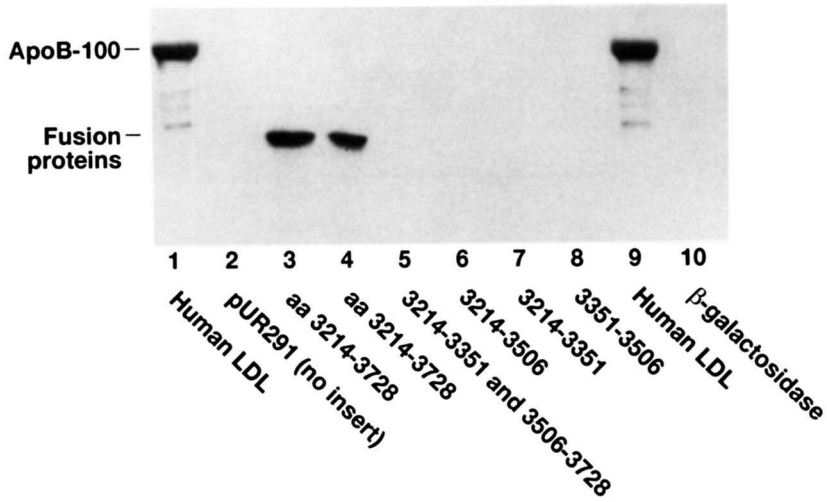

Fig. 2. Western blots demonstrating binding of MB47 to β-gal-apoB fusion proteins prepared in pUR278 or pUR291. Forty μg of each solubilized *E. coli* extract was electrophoresed on SDS-polyacrylamide gels, and Western blots were performed as previously described (2, 34). Lane 1 shows human LDL; lane 2, pUR291 with no insert; lane 3, a fusion protein containing apoB aa 3214–3728 (in pUR291); lane 4, a fusion protein containing aa 3214–3728 (in pUR278); lane 5, a single fusion protein coding for aa 3214–3351 and 3506–3728; lane 6, a fusion protein containing aa 3214–3506; lane 7, a fusion protein containing aa 3214–3349; lane 8, a fusion protein containing aa 3351–3506; lane 9, human LDL; lane 10, β-galactosidase. The β-gal-apoB fusion proteins that contained apoB-100 aa 3214–3728, aa 3214–3506, and aa 3351–3506 were also bound by a rabbit antiserum to a synthetic peptide containing aa 3490–3511 (data not shown).

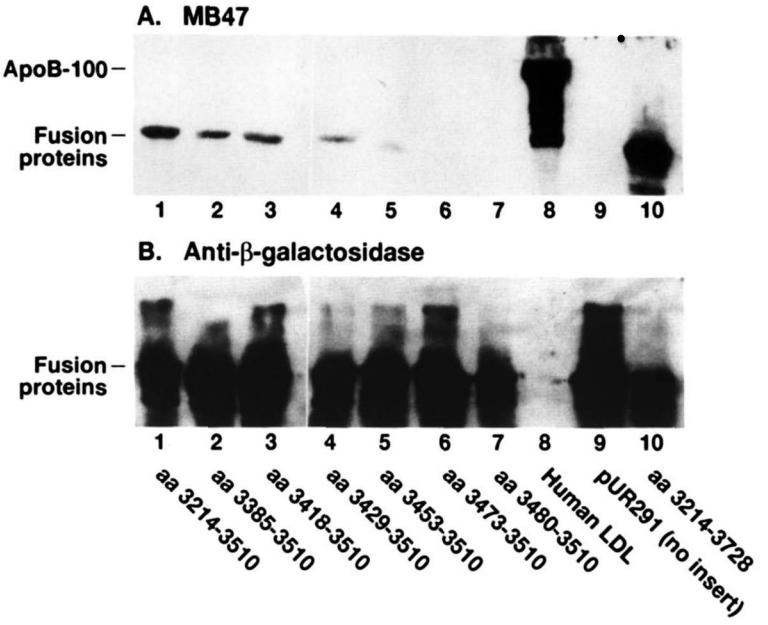

Fig. 3. Binding of antibody MB47 (panel A) and an antiserum to β-galactosidase (panel B) to a series of β-gal-apoB fusion proteins that terminate with apoB-100 aa 3510. Twenty μg of each bacterial extract was electrophoresed on a polyacrylamide gel, and Western blots were performed. For each panel, lane 1 shows a fusion protein containing apoB aa 3214–3510; lane 2, a fusion protein containing aa 3385–3510; lane 3, a fusion protein containing aa 3418–3510; lane 4, a fusion protein containing aa 3429–3510; lane 5, a fusion protein containing aa 3453–3510; lane 6, a fusion protein containing aa 3473–3510; lane 7, a fusion protein containing aa 3480–3510; lane 8, human LDL; lane 9, pUR 291 with no insert; lane 10, a fusion protein containing apoB aa 3214–3728. The fusion proteins in lanes 1–7 were also bound by the rabbit polyvalent antiserum specific for aa 3490–3511 (data not shown).

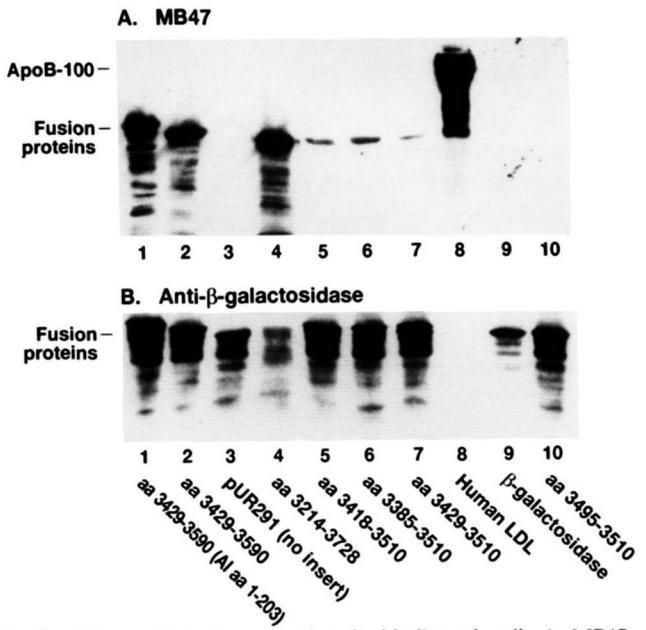

Fig. 4. Western blots demonstrating the binding of antibody MB47 (panel A) and an antiserum to β-galactosidase (panel B) to β-gal-apoB and β-gal-apoA-I/apoB fusion proteins. Lane 1 shows a fusion protein containing aa 1–203 of apoA-I and aa 3429–3590 of apoB; lane 2, a fusion protein with apoB aa 3429–3590 but no apoA-I aa; lane 3, pUR 291 with no insert; lane 4, a fusion protein with apoB aa 3214–3728; lane 5, a fusion protein with apoB aa 3418–3510; lane 6, a fusion protein with apoB aa 3385–3510; lane 7, a fusion protein with apoB aa 3429–3510; lane 8, human LDL; lane 9, β-galactosidase; lane 10, a fusion protein with apoB aa 3495–3510. The fusion proteins in lanes 1–2, 4–7, and 10 were bound by the rabbit antiserum specific for aa 3490–3511 (data not shown). In lane 4 of panel B, the binding of the anti-β-galactosidase antibody is less intense than in other lanes. This finding is due to the fact that the apoB cDNA insert in this construct is cloned into the internal *Sac*I site of the *lac*Z gene; consequently, this β-gal-apoB fusion protein contains a deletion of the carboxyl-terminal 402 amino acids of β-galactosidase and therefore contains fewer binding sites for the polyclonal antibody to β-galactosidase. The multiple bands reacting with MB47 and the β-galactosidase-specific antibody are presumably proteolytic breakdown products produced by bacterial endopeptidases.

binding to a fusion protein containing aa 3453–3510 (Fig. 3, lane 5). These data indicated that aa 3429–3453 constitute an important part of the MB47 epitope. In addition, when considered together with the fact that antibody MB47 did not bind to aa 3214–3506 (Fig. 2, lane 6), these data indicated that aa 3507–3510 were critical for MB47 binding. Although antibody MB47 bound to fusion proteins containing aa 3214–3510 and 3429–3510, it did not bind these fusion proteins nearly as strongly as the fusion protein containing aa 3214–3728 (Fig. 3, lane 10). These data indicated that aa sequences carboxyl-terminal to residue 3510 were important for the high affinity binding of antibody MB47. To test this issue, we created an apoB fusion protein that contained apoB-100 aa 3429–3590, and compared its binding to a fusion protein containing only aa 3429–3510. Antibody MB47 bound to aa 3429–3590 as strongly as it bound to aa 3214–3728. In contrast, it bound aa 3429–3510 weakly (Fig. 4, lane 7).

One reason for localizing the MB47 epitope was our desire to generate a stable polypeptide that could be used to standardize apoB immunoassays. Current reagents for standardizing apoB immunoassays (LDL or plasma) have drawbacks, including instability at 4°C or −20°C (36, 37). There are similar problems with standards for apoA-I immunoassays (37–41). Therefore, we tested the feasibility of generating a single fusion protein that contained the epitopes for both antibody MB47 and the apoA-I specific monoclonal antibody, AI-11. The initial fusion protein contained apoA-I aa 1–203 followed by apoB aa 3429–3590. Antibody MB47 bound to this β-gal-apoA-I/apoB fusion protein as strongly as to the β-gal-apoB fusion protein containing only apoB aa 3429–3590 (Fig. 4). Subsequent studies with additional β-gal-apoA-I/apoB fusion proteins revealed that antibody MB47 bound equally

strongly to apoB aa 3429–3590, 3429–3565, 3429–3544, and 3429–3523 (**Fig. 5**). The fact that antibody MB47 bound to aa 3429–3523 as strongly as apoB aa 3214–3728 implies that the carboxyl-terminal sequences responsible for high-affinity MB47 binding are located between aa 3511 and 3523. Again, antibody MB47 bound weakly to aa 3429–3510 (Fig. 5, lane 2). As expected, antibody AI-11 bound to all β-gal-apoA-I/apoB fusion proteins strongly (Fig. 5, panel C).

The apoB and apoA-I/apoB fusion proteins were soluble in buffers containing 1% SDS. When the SDS was removed by extensive dialysis against an ammonium bicarbonate buffer (25 mM), the apoA-I/apoB fusion proteins remained soluble for 1–2 days and could be used to coat 96-well Falcon microtest plates for immunoassays (data not shown). However, the apoA-I/apoB fusion protein was not soluble in the ammonium bicarbonate buffer for prolonged periods (> 1 week).

The fusion protein data illustrated in Figs. 2–5 and summarized in Fig. 1 suggested that apoB-100 aa 3429–3453 and 3507–3523 were important for the formation of the MB47 epitope. These results implied that it might be difficult to create a short synthetic peptide that would be bound by antibody MB47. We nevertheless used solid phase competitive immunoassays and nitrocellular dot blot assays to assess the binding of MB47 to 15 synthetic apoB peptides (peptides containing aa 3426–3456, 3417–3446, 3415–3456, 3441–3460, 3446–3465, 3451–3470, 3461–3480, 3466–3485, 3471–3490, 3471–3500, 3476–3495, 3479–3505, 3481–3500, 3486–3505, and 3491–3510). None of these peptides, even in high concentrations (2 mg/ml), either bound MB47 or competed with LDL for binding to antibody MB47. These data indicate that the MB47 epitope was not specified by any of the short synthetic peptides.

DISCUSSION

In this study we used β-gal-apoB fusion proteins to localize the epitope for antibody MB47, a monoclonal antibody that has not only proved useful in apoB immunoassays but has also been widely used in lipoprotein metabolism and atherosclerosis research (5, 6, 8, 11–13, 16–23, 42). Earlier studies by Pease et al. (25) indicated that MB47 binds strongly to a fusion protein containing aa 3214–3728 and binds weakly to two non-overlapping apoB sequences, aa 3351–3506 and aa 3506–3687. We confirmed the finding that antibody MB47 binds strongly to aa 3214–3728 (Fig. 2); however, in repeated experiments, we were unable to demonstrate any binding of antibody MB47 to fusion proteins containing aa 3351–3506 or 3506–3728. The reason for this discrepancy is not known, although it is conceivable that it relates to techniques of Western blotting or to the construction of the fusion proteins (see below). In any case, by generating a number of new fusion proteins, we were able to localize the epitope for antibody MB47 more precisely. Our studies indicated there are two discontinuous sequences within apoB-100 that are topographically involved in

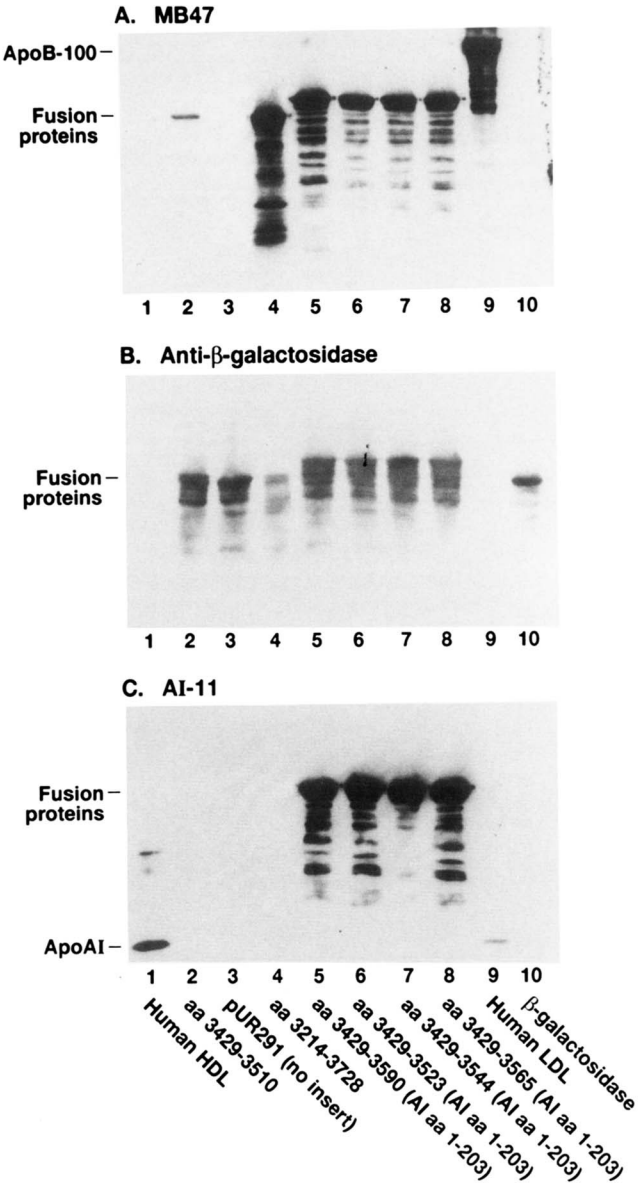

Fig. 5. Western blots illustrating the binding of antibody MB47 (panel A), an antibody to β-galactosidase (panel B), and antibody AI-11 (panel C) to β-gal-apoB and β-gal-apoA-I/apoB fusion proteins. For each blot, lane 1 shows HDL (5 μg); lane 2, a fusion protein containing apoB aa 3429–3510; lane 3, the β-galactosidase protein produced by pUR 291 with no insert; lane 4, a fusion protein containing apoB aa 3214–3728; lane 5, a fusion protein containing apoA-I aa 1–203 and apoB aa 3429–3590; lane 6, a fusion protein containing apoA-I aa 1–203 and apoB aa 3429–3523; lane 7, a fusion protein containing apoA-I aa 1–203 and apoB aa 3429–3544; lane 8, fusion protein containing apoA-I aa 1–203 and apoB aa 3429–3565; lane 9, human LDL (20 μg); lane 10, purified β-galactosidase (2 μg). The fusion proteins in lanes 2 and 4–8 were bound by the rabbit antiserum specific for aa 3490–3511 (data not shown). In panel B, lane 4, the less-intense staining is due to the fact that this fusion protein lacks the carboxyl terminal 402 aa of β-galactosidase (see legend to Fig. 4).

forming the MB47 epitope, the 25 amino acid residues from 3429 to 3453 and the 17 amino acids from 3507 to 3523 (Fig. 1). Consistent with these data implicating discontinuous sequences in the formation of the MB47 epitope, we found that antibody MB47 failed to bind to a series of 15 overlapping synthetic apoB peptides between apoB aa 3415 and 3510. Our data do not mean that the 53 intervening amino acid residues are not important for maintenance of the MB47 epitope. However, our data indicate that these 53 residues probably are not involved in direct contact with the MB47 antibody combining site.

Weisgraber et al. (13) have reported that antibody MB47 binds to the LDL of familial defective apoB-100 (FDB) heterozygotes with a slightly greater affinity than to LDL of normal subjects. FDB is caused by an Arg → Gln substitution at apoB-100 residue 3500, which is located between the two discontinuous sequences identified as being important for MB47 binding (Fig. 1). Recently, Lund-Katz et al. (43) reported NMR data indicating that the aa substitution at residue 3500 significantly alters the conformation of the receptor binding region of apoB-100 on LDL particles. Presumably the conformational change caused by the glutamine substitution at residue 3500 affects both antibody MB47 binding to apoB and the ability of apoB-100 to bind to the LDL receptor.

It is not surprising that our fusion protein data indicated that two discontinuous sequences are important for the binding of antibody MB47. The epitopes for many monoclonal antibodies contain discontinuous sequences. Barlow, Edwards, and Thornton (44) recently argued that virtually all of the antigenic determinants on the surface of globular proteins are discontinuous. Using the known crystallographic structures of myoglobin, trypsin, and lysozyme, they calculated the percentage of all possible protein surface determinants that would contain purely continuous sequences. All surface regions of 20 Å diameter (a typical recognition area for a high-affinity antibody) contained discontinuous protein sequences. Even for a low-affinity antibody with a determinant having a diameter of 16 Å, very few ($< 10\%$) of the possible determinants involved strictly continuous sequences. Crystallographic analyses of lysozyme bound to Fab fragments of monoclonal antibodies reveal that large surface areas are involved in the antibody-protein interaction, and indicate the importance of discontinuous sequences in forming the antibody epitopes (45, 46).

To develop the fusion proteins used in this study, we used the β-galactosidase fusion protein vector pUR291 (26). The various apoB cDNA inserts that were cloned into this plasmid were obtained by enzymatically amplifying cDNA segments using the polymerase chain reaction. This approach, which was used to a limited extent by Pease et al. (25), eliminates reliance on naturally occurring restriction sites and permits creating a wide range of fusion proteins for accurate mapping of sequences involved in the epitope. The use of enzymatically amplified cDNA inserts rather than naturally occurring restriction fragments also has the advantage of avoiding irrelevant aa sequences at the carboxyl terminus of the fusion protein. Because the 3' oligonucleotides used in our amplification reactions encoded a TGA stop codon, each of the fusion proteins that we generated terminated with the apoB protein sequence rather than extending into irrelevant aa sequences specified by plasmid DNA. We found that cloning of the constructs described in this report, as well as the expression of the fusion proteins, was greatly facilitated by the use of the SURE *E. coli* strain (Stratagene, La Jolla, CA). The reason that we obtained better results with this *E. coli* strain than other commonly used strains (such as DH5a) is unknown.

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