Antibodies to the Carboxyl Terminus of Human Apolipoprotein A-I

THE PUTATIVE CELLULAR BINDING DOMAIN OF HIGH DENSITY LIPOPROTEIN 3 AND CARBOXYL-TERMINAL STRUCTURAL HOMOLOGY BETWEEN APOLIPOPROTEINS A-I AND A-II*  

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We have studied the binding of 125I-labeled high density lipoproteins (HDL3) to liver plasma membranes, which are thought to contain specific HDL receptor sites, using anti-peptide antibodies directed against two sites in the carboxyl-terminal region of human apoA-I. Two distinct antibody populations raised to peptides corresponding to amino acid residues 205-220 and 230-243, respectively, recognized the human apoA-I that are exposed in the lipid environment of HDL3. However, anti-AI[230-243] IgG, but not anti-AI[205-220] IgG, recognized HDL2, suggesting that residues 205-220 of apoA-I are expressed differently in the two HDL populations. In addition, anti-AI[230-243] IgG showed strong cross-reactivity toward apoA-II. Epitope mapping studies showed that anti-AI[230-243] IgG binds to an epitope located in the carboxyl-terminus of apoA-II, demonstrating significant structural homology between the carboxyl-terminal region of apoA-I and apoA-II, demonstrating significant structural homology between the carboxyl-terminal regions of apoA-I and apoA-II, two candidate proteins for mediating the specific cellular interaction of HDL3. Fab fragments from anti-AI[205-220] and anti-AI[230-243] inhibited the binding of 125I-HDL3 to liver plasma membranes by approximately 80% and 60%, respectively. These findings are in agreement with our recent work using isolated CNBr fragments of apoA-I (Morrison, J., Fidge, N. H., and Tozuka, M. (1991) J. Biol. Chem. 266, 18750-18755), which suggest that the carboxyl-terminal region of apoA-I contains a binding domain which mediates the specific interaction of HDL3 with liver plasma membranes, possibly through the involvement of specific HDL receptors.

Apolipoprotein A-I (apoA-I),1 the major protein constituent of human plasma high density lipoprotein (HDL), plays an important role in lipid transport and metabolism. It is an activator of lecithin:cholesterol acyltransferase (1), and evidence from several studies have also implicated a role for apoA-I as a ligand capable of recognizing specific HDL cellular binding sites (2-6). Following the recognition of an inverse correlation between plasma HDL, as well as apoA-I levels (7), and the incidence of atherosclerosis, apoA-I has become the subject of numerous immunochemical investigations. The application of apoA-I-specific antibodies include immunocytochemistry for quantification of serum apoA-I or HDL levels (8-11), studies of genetic variants (12, 13), and epitope expression of apoA-I within different lipoprotein subclasses (8, 14-20). They have also been used to probe functional domains involved in binding of HDL to cellular receptors (3-6).

Synthetic peptides representing selected regions of a protein sequence can elicit antibodies capable of reacting with the whole protein (21, 22). We have generated two populations of anti-peptide antibodies recognizing residues 205-220 and 230-243 of human apoA-I, respectively. This report describes the ability of these site-specific antibodies to inhibit the binding of HDL3 to liver plasma membranes, which are thought to contain specific receptors for HDL3 (2, 23). These studies extend our recent findings using purified cyanogen bromide digest fragments of apoA-I (24, 25) and suggest that residues 205-243 contain, or form part of, the binding domain of HDL3. We propose that the interaction of HDL3 with liver plasma membranes is mediated by HDL receptor sites which are specific for a region in the carboxyl-terminal portion of apoA-I.

EXPERIMENTAL PROCEDURES

Lipoprotein and Apolipoprotein Isolation—Human HDL (d 1.063-1.210 g/ml), HDLC (d 1.063-1.215 g/ml), and HDLD (d 1.125-1.210 g/ml) were obtained from human plasma (Red Cross) by ultracentrifugation as previously described (26). apoA-I and apoA-II were isolated from total HDL treated with 8 M guanidine HCl (8), followed by ultracentrifugation at 2.120 g/ml for 20 h at 60,000 rpm and 4 °C. The infranatant containing apoA-I dissociated from HDL, was dialyzed against 5 mM ammonium acetate, pH 6.8, lyophilized, dissolved in urea buffer (8 M urea, 0.02 M Tris, pH 8.0), and subjected to chromatography on DEAE-Sephacel (Pharmacia LKB Biotechnology Inc.) as described previously (27). ApoA-II was isolated from the supernatant following ethanol/ether (3:1, v/v) delipidation and chromatography on DEAE-Sephacel in 6 M urea buffer, as described (28). The purity of the isolated apolipoproteins was verified by SDS, 10-15% polyacrylamide gradient gels (Phastgel system, Pharmacia) stained with Coomassie Brilliant Blue R-250. Purified apolipoproteins were stored lyophilized at -20 °C. apoA-I-phospholipid complexes were prepared by incubation of apoA-I with dimeristoylphosphatidylcholine (DMPC; Calbiochem) liposomes (29) at a ratio of 1:4 (w/w, protein to lipid), for 20-24 h at 23 °C (30). apoA-I concentrations were determined by immunoturbidimetric analysis using human apoA-I antisera (Boehringer Mannheim) and apoA-I calibration serum (Boehringer Mannheim) on a COBAS-BIO (Roche), according to the recommendations of the manufacturer.

Peptide Synthesis and Conjugation—Two peptides, denoted AI[205-220] and AI[230-243], were synthesized using the human apoA-I sequence reported by Brewer et al. (31) and correspond to the amino acid residues 205-220 and 230-243, respectively. Both peptides represent regions of apoA-I that are predicted to be exposed in a lipid environment (32). The peptides were synthesized using Fmoc chem-
Carboxyl-terminal-specific Antibodies to Human apoA-I

ISTRY ON AN APPLIED BIOSYSTEMS MODEL 430A ANTIPEPTIDE SYNTHESIS. TO FACILITATE CONJUGATION OF THE PEPTIDES TO CARRIERS, CYSS-GLY SPACERS WERE ADDED TO THE AMINO-TERMINAL ENDS. CLEAVAGE AND DEPROTECTION WERE PERFORMED USING HYDROGEN FLUORIDE. THE CRUDE PEPTIDES WERE THEN PURIFIED BY REVERSED-PHASE (RP)-HPLC, AND THE AMINO ACID SEQUENCES WERE CONFIRMED USING AN APPLIED BIOSYSTEMS MODEL 470A PROTEIN SEQUENCER, EQUIPPED WITH AN ON-LINE MODEL 120A PTH ANALYZER. FOR THE IMMUNIZATION PROCEDURES DESCRIBED BELOW, PEPTIDES WERE COUPLED TO OVALBUMIN (OVA) WITH MALEIMIDOZENYL-N-HYDROXYSUCINIMIDINI ESTER (PIERCE). THE SPECIFICITY OF EACH ANTISERA FOR AL[205-220] AND AL[230-243] WERE DETERMINED BY ELISA (DETAILED BELOW) USING THE PEPTIDES COUPLED TO KEYHOLE LIMPET HEMOCYANIN (KLH) AS ANTIGENS. TO AVOID CROSS-REACTION WITH MALEIMIDOZENYL-N-HYDROXYSUCINIMIDINI ESTER, THE COUPLING AGENT SUCINIMIDYL 3-(2-PYRIDIDILTHIO)PROPIONATE (PIERCE) WAS USED TO PREPARE THE KLH CONJUGATES.

PRODUCTION AND PURIFICATION OF ANTI-PEPTIDE ANTIBODIES—ANTISERA AGAINST THE PEPTIDES AL[205-220] AND AL[230-243] (CONJUGATED TO OVA) WERE PRODUCED IN 5-6-MONTH-OLD RABBITS WHICH RECEIVED 0.5 mg OF PEPTIDE EMULSIFIED IN 1 ml OF PHOSPHATE-BUFFERED SALINE (PBS), pH 7.4, AND FREUND'S COMPLETE ADJUVANT, BY SUBCUTANEOUS INJECTION AT DIFFERENT SITES. THE RABBITS WERE IMMUNIZED 3 TIMES AT 1-MONTH INTERVALS, AND BLEED 2 WEEKS THEREAFTER. EACH ALANTISERUM WAS SUBJECTED TO AFFINITY CHROMATOGRAPHY ON PROTEIN A-SEPHAROSE (PHARMACB) (33). ANTIBODY ELUTED FROM PROTEIN A WAS DISSOLVED BY pH 2.8, NEUTRALIZED IMMEDIATELY WITH 1 M TRIS BUFFER, pH 8.0. PURIFIED HUMAN apoA-I AND OVA (SIGMA) WERE COUPLED TO CNBr-ACtivated SEPHAROSE 4B AS RECOMMENDED BY PHARMACIA. ANTI-PEPTIDE SPECIFIC FOR apoA-I (DESIGNATED ANTI-APOA-I) WAS ALSO OBTAINED FROM RABBITS IMMUNIZED WITH PURIFIED HUMAN apoA-I, AS PREVIOUSLY DESCRIBED (3). FOR THE PRODUCTION OF FAB FRAGMENTS, AFFINITY-PURIFIED ANTIBODY WAS SUBJETED TO PAPAIN DIGESTION ACCORDING TO THE PROCEDURES OF GORINI ET AL. (34). FAB FRAGMENTS WERE REMOVED FROM UNDIGESTED IGG AND FC FRAGMENTS BY PASSAGE THROUGH PROTEIN A-SEPHAROSE. THE ACTIVITY AND PURITY OF ISOLATED FABS WERE ASSESSED BY ELISA (DETAILED BELOW). 125I POLYCARBAMIDE PRECURSOR GEL ELECTROPHORESIS (PHASTGEL SYSTEM, PHARMACIA), RESPECTIVELY. FAB FRAGMENTS WERE ALSO PREPARED FROM TWO apoA-I-SPECIFIC MONOCLONAL ANTIBODIES, DENOTED AI-1 AND AI-3, WHICH RECOGNIZE EPITOPES EXPOSED ON THE SURFACE OF HDL2 PARTICLES (35) THAT HAVE RECENTLY BEEN LOCALIZED TO RESIDUES 28-47 AND 140-147 OF apoA-I, RESPECTIVELY (36).

ENZYME-LABELED IMMUNOASSAYS—TITRATION CURVES OF THE ANTI-PEPTIDE ANTIBODIES TOWARD DIFFERENT ANTIGENS WERE DETERMINED BY ELISA. BRIEFLY, 96-TEST WELLS (IMMULON II) WERE COATED WITH 10 pg/ml ANTIGEN IN 0.05 M SODIUM CARBOUF BUFFER, pH 9.6, FOR 1 h, AT ROOM TEMPERATURE (100 ml/well). THE WELLS WERE THEN WASHED THREE TIMES WITH PBS buffer, AND 100 ml OF 100 ml/ml DILUTED 1:2, FROM 50 ml/ml IGg, AFFINITY-PURIFIED ANTIBODY. AFTER 1 h AT ROOM TEMPERATURE, THE WELLS WERE WASHED AS BEFORE, THEN INCUBATED FOR ANOTHER 1 h WITH 100 ml OF GOAT ANTI-RABBIT IgG (H+L) HORSEARASHID PEROXIDASE CONJUGATE (BIO-RAD) DILUTED 1:200 IN PBS/0.05% TWEEN. AFTER THREE WASHES WITH PBS-TWEEN, 100 mL OF 0.1% ABTS (2',2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), 0.02% H2O2 IN 0.1 M CITRATE BUFFER, pH 4.0, WAS ADDED FOR 30 MIN, AND THE COLOR WHICH DEVELOPED WAS QUANTITATED USING A TITERTEK MULTISCAN (FLOW LABORATORIES) WITH A FILTER SETTING OF 414 nm. TO COMBINE ANTI-PEPTIDE REACTIVITY TOWARD ISOLATED apoA-I, HDL2, OR A COMBINATION OF SEPARATE ELISA SYSTEMS EMPLOYED THE SAME COATING AND WASHING PROCEDURES AS DESCRIBED ABOVE; HOWEVER, THE INCUBATION BUFFER INCLUDED 1% SKIM MILK RATHER THAN TWEEN 20, WHICH HAS BEEN SHOWN TO ALTER THE IMMUNOREACTIVITY OF apoA-I IN HDL (18, 19, 35). THESE DUPLICATE WELLS OF PLATES COATED WITH apoA-I, ABDIGESTED BIOSYSTEMS MODEL 470A PROTEIN SEQUENCER, EQUIPPED WITH AN ON-LINE MODEL 120A PTH ANALYZER. FOR THE IMMUNIZATION PROCEDURES DESCRIBED BELOW, PEPTIDES WERE COUPLED TO Keyhole Limpet Hemocyanin (KLH) AS ANTIGENS. TO AVOID CROSS-REACTION WITH MALEIMIDOZENYL-N-HYDROXYSUCINIMIDINI ESTER, THE COUPLING AGENT SUCINIMIDYL 3-(2-PYRIDIDILTHIO)PROPIONATE (PIERCE) WERE USED TO PREPARE THE KLH CONJUGATES.

RESULTS

SPECIFICITY OF THE ANTI-PEPTIDE ANTIBODIES—TO SELECT ANTIBODIES IMMUNOREACTIVE TOWARD THE PARENTERAL PROTEIN, HUMAN apoA-I, ANTI-PEPTIDE ANTIBODIES WERE PURIFIED BY AFFINITY CHROMATOGRAHY ON apoA-I-SEPHAROSE 4B AS DESCRIBED UNDER "EXPERIMENTAL PROCEDURES." THE RECOGNITION OF apoA-I-SPECIFIC IN HUMAN PLASMA MEMBRANES (23) WAS 5-FOLD GREATER THAN THE AMOUNTS RECOVERED FROM anti-AI[205-220] SERA (DATA NOT SHOWN). THE ANTIGENIC REACTIVITIES OF ANTISERA AND AFFINITY-PURIFIED ANTIBODY WERE DETERMINED BY ELISA.

THE PURIFIED ANTI-PEPTIDE ANTIBODIES BOUND ONLY TO THEIR RESPECTIVE PEPTIDES, WITH NO CROSS-REACTIVITY OBSERVED TOWARD THE OTHER PEPTIDE (FIG. 1). ALTHOUGH BOTH ANTIBODY POPULATION RECOGNIZED THE PARENT PROTEIN (apoA-I), OF PARTICULAR INTEREST WAS THE ABILITY OF ANTI-AI[230-243] IgG TO RECOGNIZE HUMAN apoA-I WITH AN UNUSUALLY HIGH CROSS-REACTIVITY (FIG. 1, PANEL B). IN CONTRAST, anti-AI[205-220] IgG WAS UNABLE TO BIND apoA-II. TO DETERMINE WHETHER THE ANTI-AI[205-220] AND ANTI-AI[230-243] ANTIBODIES RECOGNIZED THE PROMINENT apoA-I ISOFORMS FOUND IN HDL, IMMUNOBLOTTING WAS PERFORMED FOLLOWING ISOELECTRIC FOCUSING OF apoA-I (AND apoA-II) DERIVED FROM HUMAN HDL (d 1.063-1.210 g/ml). THE IMMUNOBLOT PATTERNS FOR EACH ANTIBODY WERE IDENTICAL, BUT CLEARLY DISTINGUISHABLE FROM EACH OTHER (FIG. 2). FURTHERMORE, THE RECOGNITIVITY OF ANTI-AI[230-243] TOWARD THE apoA-II ISOFORMS CONFIRMED THE CROSS-REACTIVITY IDENTIFIED BY THE ELISA METHOD.

BOTH ANTI-PEPTIDE ANTIBODY PREPARATIONS REACTED WITH ISOLATED apoA-I-DMPC AND HDL2 PARTICLES (FIG. 3), DEMONSTRATING THAT apoA-I RESIDUES 205-220 AND 230-243 CONTAIN EPITOPES THAT ARE EXPOSED IN THE LIPID ENVIRONMENT OF THESE
particles. However, different reactivities of the anti-peptide antibodies toward isolated apoA-I, HDLs, and HDL2 sug-
ggested structural differences in the expression of these two regions of apoA-I. Anti-AI[230-243] showed reactivity toward all three lipid-bound forms of apoA-I, whereas anti-AI[205-220] had little or no reactivity toward apoA-I in HDL2 (Fig. 3). Both anti-peptide populations were also compared for their ability to immunoprecipitate radiolabeled HDL2 relative to anti-apoA-I IgG. Assigning an arbitrary 100% for the maximum immunoprecipitation produced by anti-apoA-I IgG, the affinity-purified anti-AI[205-220] and anti-AI[230-243] IgG precipitated 82% and 96% of 125I-HDLs, respectively.

Epitope Mapping of ApoA-II Using Anti-AI[230-243]—As described above, anti-AI[230-243], although raised to a peptide representing a portion of human apoA-I, displayed strong reactivity toward purified apoA-II using both ELISA and immunoblotting techniques. Close inspection of the primary structures of apoA-I (31) and apoA-II (38) reveals a 41% sequence homology between the last 17 carboxyl-terminal residues of both apolipoproteins (Fig. 4). Most of the region showing this homology is included in the peptide AI[230-243]. To confirm that the carboxyl-terminus of apoA-II contains the epitope(s) recognized by anti-AI[230-243] IgG, tryptic fragments of apoA-II were prepared and used in a competitive ELISA. The RP-HPLC chromatogram of the generated peptides is shown in Fig. 5. The identities of the peaks, determined by amino acid analysis and amino-terminal sequencing, agreed with the expected peptides previously reported by Lux et al. (38). Selected peptides were then compared for their abilities to inhibit the binding of anti-AI[230-243] to apoA-II, as described under “Experimental Procedures.” Only peptide AI[56-77] could reduce the binding of anti-AI[230-243] to apoA-II (Fig. 6). The inhibition produced by peptide AI[56-77] was identical with that produced by whole apoA-II, confirming that the AI/AI1 cross-

Fig. 3. Competition curves of anti-AI[205-220] (panel A) and anti-AI[230-243] (panel B) IgG binding to isolated apoA-I (C), apoA-I-DMPC (V), HDL2 (●), HDL3 (▲), and apoA-II (C), as determined by ELISA. 96-well plates coated with apoA-I received increasing amounts of competing antigen, followed by the addition of diluted (1/1000) antibody. Specifically bound antibody was detected as described under “Experimental Procedures.” Results are shown as the absorbance obtained in the presence of competing antigen (B), expressed as a percent of the maximum absorbance (Bo), obtained in the absence of competing antigen.

Fig. 4. Comparison between the carboxyl-terminal amino acid sequences of human apoA-I and apoA-II. Amino acid residues at identical positions within the carboxyl-terminus are indicated by the boxes.
Prepared. Fab's of anti-apoA-I, anti-AI[205-220], and anti-AI[230-243], with similar reactivities toward apoA-I, all prepared from the use of whole IgG molecules, Fab fragments were approximately 60%.

Under the same conditions, Fab fragments of human apoA-I, were found to inhibit the binding of HDL3 size from two distinct regions of the carboxyl-terminal region.

Anti-AI[205-220] and anti-AI[230-243] Fab fragments inhibited binding by approximately 60%.

Concentration of Antigen (pmol)

Absorbance (A)

1 10 100 1000

Peak Peptide
1 A[4-23] 2 A[45-54] 3 A[56-77] 4 A[60-64] 5 A[65-74] 6 A[67-77]

Concentration of Fab fragments (µg/ml)

% Total HDL bound

0 10 20 30 40 50 60

Concentration of Anti-AI[230–243] IgG bind-
ing to apoA-I at concentrations of 300 µg/ml Fab fragments reduced the binding of apoA-I.

Anti-AI[205–220] and anti-AI[230–243] Fab fragments significantly reduced the interaction between HDLz and rat liver plasma membranes.

Inhibition of 125I-HDLz binding to rat liver plasma membranes. 125I-labeled HDLz (1 µg/ml) was preincubated with increasing amounts of Fab fragments derived from the monoclonal antibodies AI-1 (■) and AI-3 (□) and from anti-apoA-I (○), unrelated anti-peptide (◆), anti-AI[205–220] (▲), and anti-AI[230–243] IgG (▼), for 4 h at 37°C, followed by the addition of liver plasma membranes for 4-h binding studies at 37°C. Each point represents the mean of triplicate (or duplicate for AI-1 and AI-3) determinations.

Fig. 5. RP-HPLC chromatograph showing the separation of peptides generated by trypsin digestion of apoA-II. The purified peptides, corresponding to the numbered peaks, were identified by amino acid and amino-terminal sequencing analysis, as summarized in the right-hand table.

Fig. 6. Competition curves of anti-AI[230–243] IgG binding to apoA-II in the presence of apoA-II tryptic peptides. 96-well plates coated with apoA-II received increasing amounts of the purified peptides AII[4–23] (●), AII[29–39] (△), AII[40–44] (□), AII[45–54] (○), AII[56–77] (△), and intact apoA-II (●), followed by the addition of anti-AI[230–243] IgG. Results are shown as the absorbance obtained in the presence of competing antigen (B), expressed as a percent of the maximum absorbance (Bo), obtained without competing antigen.

Fig. 7. Inhibition of 125I-HDLz binding to rat liver plasma membranes. 125I-labeled HDLz (1 µg/ml) was preincubated with increasing amounts of Fab fragments derived from the monoclonal antibodies AI-1 (■) and AI-3 (□) and from anti-apoA-I (○), unrelated anti-peptide (◆), anti-AI[205–220] (▲), and anti-AI[230–243] IgG (▼), for 4 h at 37°C, followed by the addition of liver plasma membranes for 4-h binding studies at 37°C. Each point represents the mean of triplicate (or duplicate for AI-1 and AI-3) determinations.

Reactivity is due to similarities which reside in the carboxyl-terminal regions.

Inhibition of 125I-HDLz Binding to Rat Liver Plasma Membranes—To determine the effects of the antibodies on the binding of HDLz to rat liver plasma membranes, 125I-labeled HDLz particles were preincubated with each antibody prior to the addition of plasma membranes. Initial studies using whole antibody molecules resulted in enhanced levels of 125I-HDLz binding to membranes, presumably due to the formation of antibody-HDLa aggregates (3). To avoid this effect, and to minimize the possibility of steric inhibition resulting from the use of whole IgG molecules, Fab fragments were prepared. Fab's of anti-apoA-I, anti-AI[205–220], and anti-AI[230–243], with similar reactivities toward apoA-I, all produced inhibition of binding, whereas Fab's from an unrelated anti-peptide antibody with no specificity toward apoA-I, had little or no effect (Fig. 7). Anti-AI[205–220] and anti-apoA-I at concentrations of 300 µg/ml Fab's reduced the binding of 125I-HDLz to less than 80% of control values. At similar concentrations, anti-AI[230–243] inhibited binding by approximately 60%.

Under the same conditions, Fab fragments from the two monoclonal antibodies, AI-1 and AI-3, had no significant effect on the binding of 125I-HDLz to the hepatic membranes (Fig. 7).

**Discussion**

Anti-peptide antibodies generated to two peptides, synthesized from two distinct regions of the carboxyl-terminal region of human apoA-I, were found to inhibit the binding of HDLz to liver plasma membranes. In addition to supporting the proposal that apoA-I can act as a specific ligand for HDLz cellular binding sites (2–6), the present studies further suggest that a specific region in the carboxyl-terminus of apoA-I is responsible for the cellular binding of HDLz. Furthermore, characterization of the specificities displayed by the anti-peptide antibodies has identified a region of structural homology between human apoA-I and apoA-II, two proteins previously implicated in mediating the binding of HDL to human (6) and rat (2) liver plasma membranes.

Peptides AI[205–220] and AI[230–243], corresponding to residues 205–220 and 230–243 of apoA-1, respectively, generated two distinct populations of anti-peptide antibodies which could recognize the major isoforms of apoA-I from HDLz (Fig. 2). Anti-AI[205–220] and anti-AI[230–243] also recognized apoA-I associated with apoA-I-DMPC and HDLz particles (Fig. 3), which is consistent with the proposed orientation of apoA-I in the lipid environment (32), in which both regions are thought to contain sites exposed on the lipoprotein surface. Such sites are potentially available for interactions with enzymes, receptors, or other blood components involved in lipid metabolism; therefore, these antibodies may provide useful tools for further probing the structural-functional properties of apoA-I. However, apparent structural differences between the expression of apoA-I in HDLz and HDLz were identified by the inability of anti-AI[205–220] to recognize HDLz particles. It is possible that residues 205–220 become hidden in the lipid environment of the larger HDLz particles due to conformational changes of apoA-I, or, alternatively, these residues may be masked by other protein moieties on the particle surfaces.

Anti-AI[205–220] and anti-AI[230–243] Fab fragments significantly reduced the interaction between HDLz and rat liver plasma membranes (Fig. 7), whereas two monoclonal antibodies, AI-1 and AI-3, recognizing epitopes positioned toward the amino-terminal (residues 28–47) and middle (residues 140–147) portions of apoA-I, respectively (37), had little or no effect. Thus, both anti-peptide antibodies may recognize epitopes which contain, or lie close to, a cellular binding domain located in the carboxyl-terminal region of apoA-I. The higher levels of inhibition observed with anti-AI[205–220] may indicate that residues within 205–220 are more specifically involved in, or lie closer to, the actual binding region. The region of apoA-I recognized by anti-AI[205–220] IgG is thought to include a β-conformation between two amphipathic α-helical regions (32). Recent studies involving chemical modification of lysine or arginine residues of HDLz (43,
for metabolism. We anticipate that these new antibodies will provide useful tools for immunochemical characterization of previously unknown functional domains of apoA-I involved in lipid metabolism.

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