Epitope mapping of apolipoprotein A-I using endoproteinase cleavage and monoclonal antibodies in an enzyme-linked immunosorbent assay

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Abstract The epitopes for two monoclonal antibodies (MAbs) directed towards human apolipoprotein A-I (apoA-I), designated AI-1 and AI-3, have been more precisely defined. Previous work in our laboratory demonstrated that AI-1 and AI-3 recognize antigenic determinants located within cyanogen bromide (CNBr) fragments 1 (CF1) and 3 (CF3), respectively. Using peptides generated from endoproteinase cleavage of CF1 and CF3, we now report that both MAbs are specific for two previously unreported epitopes along the apoA-I molecule. The ability of whole endoproteinase digest mixtures to bind the MAbs, as determined by means of a competitive enzyme-linked immunosorbent assay (ELISA), indicated regions of CF1 and CF3 that were likely to form the epitopes. Purified peptides derived from the digests were then used to localize the epitopes recognized by MAbs AI-1 and AI-3 to within residues 28–47 and 140–147 of apoA-I, respectively. We have previously reported that the epitopes for both MAbs are exposed on HDL_2, HDL_3, and free apoA-I. Thus, the precise mapping of the binding sites recognized by AI-1 and AI-3 has enabled the identification of regions along apoA-I that are exposed on the surface of lipoprotein particles. —**Allan, C. M., T. Tetaz, and N. H. Fidge.** Epitope mapping of apolipoprotein A-I using endoproteinase cleavage and monoclonal antibodies in an enzyme-linked immunosorbent assay. *J. Lipid Res.* 1991. **32:** 595–601.

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Apolipoprotein A-I (apoA-I), 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 (LCAT) (1) and several studies also suggest a role as a lipoprotein 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 for numerous immunochemical studies involving both polyclonal (3–6, 8, 9) and monoclonal antibodies (10–20). The applications of apoA-I specific antibodies, particularly monoclonal antibodies (MAbs), include immunoassays for quantification of serum apoA-I or HDL levels (9, 19, 20), epitope expression within genetic variants of apoA-I (13, 14) or within the different lipoprotein subclasses (8–18), and the potential use in characterizing functional domains required for LCAT activation or binding to cellular receptors.

Several panels of MAbs directed against apoA-I have been described (10–17) and most have been characterized with respect to their specificity towards the four cyanogen bromide (CNBr) fragments of apoA-I. Synthetic peptides corresponding to limited regions of the apoA-I sequence have also been used to more precisely locate the antigenic determinants within the apoA-I molecule (14–16). This procedure involves the synthesis of a set of overlapping peptides that cover the whole primary structure of the antigen, and, if possible, peptides of sufficient lengths to assume the conformation expected to exist in intact antigen molecules. In this report, we describe an alternative approach, using endoproteinase cleavage products of apoA-I in an enzyme-linked immunosorbent assay (ELISA), to obtain more precise immunochemical details of the epitopes recognized by two previously described anti-apoA-I MAbs (17).

MATERIALS AND METHODS

Isolation of apoA-I

Human HDL (d 1.063–1.210 g/ml) was obtained from human plasma (Red Cross) by ultracentrifugation (21).

Abbreviations: apoA-I, apolipoprotein A-I; CF, cyanogen bromide fragment; CNBr, cyanogen bromide; ELISA, enzyme-linked immunosorbent assay; HDL, high density lipoprotein; IgG, immunoglobulin G; MAb, monoclonal antibody; RP-HPLC, reversed phase high performance liquid chromatography; TFA, trifluoroacetic acid.

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Plasma was adjusted to d 1.063 g/ml with solid KBr followed by centrifugation at 60,000 rpm for 20 h at 4°C. The infranate was collected and adjusted to d 1.210 g/ml with KBr and centrifuged as above. Centrifugation of the top fraction was repeated to obtain pure HDL (21). ApoA-I was dissociated from HDL by treatment with 6 M guanidine-HCl (22) and collected in the lipoprotein-free fraction after ultracentrifugation at d 1.210 g/ml. The infranate was then dialyzed against 5 mM ammonium acetate, pH 6.8, lyophilized, dissolved in urea buffer (6 M urea, 0.02 M Tris, pH 8.0), and applied to a DEAE-Sephacel column (Pharmacia, Uppsala, Sweden) previously equilibrated in the same buffer. Protein fractions were eluted using a 0–125 mM NaCl gradient, and the apoA-I containing fractions, identified by SDS 10–15% polyacrylamide gradient gels (Phastgel System, Pharmacia), were pooled, dialyzed against 5 mM ammonium acetate, pH 6.8, and lyophilized. The purified protein was stored dry, under N_2 at −80°C until required.

Cleavage and purification of CNBr fragments of apoA-I

CNBr fragments were prepared using modifications (23) of the method described by Baker, Jackson, and Gotto (24). Briefly, apoA-I was dissolved in 70% trifluoroacetic acid (TFA) containing a 500-fold molar excess of CNBr, and incubated for 24 h at 25°C, in the dark, under N_2. CNBr-treated samples were then dried under a vacuum in a Speed-Vac (Savant Inc., New York), redissolved in 6 M urea, 0.05 sodium citrate, pH 3.8, and subjected to gel filtration through a Sephadex G-50 (Pharmacia) column (1.5 × 200 cm) equilibrated in the urea buffer. The appropriate fractions were then pooled and desalted by reversed phase high performance liquid chromatography (RP-HPLC) as previously described (23). CNBr fragments 2 (CF2) and 3 (CF3) required no further purification, whereas CNBr fragments 1 (CF1) and 4 (CF4) required additional purification by DEAE-chromatography, as described elsewhere (24). The identities of the purified fragments were assessed by amino acid analysis and N-terminal sequencing, as described below.

Endoproteinase cleavage of CF1 and CF3

The endoproteinases Asp-N (Cat No. 1054-589), Arg-C (Cat No. 269-590), Glu-C (Cat No. 791-156), and Lys-C (Cat No. 476-986) were purchased from Boehringer Mannheim (Australia) and used according to the recommendations of the manufacturer. Briefly, purified CF1 was resuspended to 0.5 mg/ml in 0.1 M NH_4HCO_3 and digested with Asp-N, Arg-C, Glu-C, or Lys-C at enzyme: protein ratios of 1:20, 1:200, 1:20, and 1:20 (w/w), respectively. Similarly, purified CF3 at 0.5 mg/ml in 0.1 M NH_4HCO_3 was digested using Arg-C, Asp-N, Glu-C, and Lys-C at enzyme:protein ratios of 1:20, 1:100, 1:50, and 1:50 (w/w), respectively. The endoproteinase digest mixtures were incubated at 37°C for 24 h, then analyzed or purified by RP-HPLC as described below. The amounts of endoproteinase used in the initial digests resulted in the complete digestion of CF1 and CF3, with all concentrations falling within the ranges recommended by the manufacturer.

Purification of peptides from endoproteinase digestion of CF1 and CF3

The peptide mixture generated by endoproteinase Asp-N digestion of CF1 was analyzed, and the peptides were subsequently purified, by RP-HPLC on an RP300 column (2.1 mm i.d. × 30.0 mm, Activon). Separations were controlled by a Hewlett-Packard HP1090 HPLC equipped with a diode-array detector (DAD), and column temperature was maintained at 55°C. Analytical or preparative samples (10–30 and 100–200 μg protein, respectively) were diluted $^1/_{25}$ in 0.1% TFA and loaded via a 2-ml Rheodyne injection loop at 0.5 ml/min, to a column pre-equilibrated with 0.1% TFA. After the nonretained material had passed through the DAD, the loop was switched out of line and the flow rate was lowered to 0.3 ml/min. Peptides were then eluted with a 15-min linear gradient from 0% to 60% CH_3CN and were detected by continuous monitoring of the absorbance at 215 nm. Peaks were manually collected and the respective peptides were identified by amino acid analysis (see below). For certain peptides, N-terminal sequencing was also performed to further confirm the sequence. Purified peptides were then dried using a Speed-vac and stored at −20°C until needed. Similar procedures were used to purify the peptides generated by Glu-C digestion of CF3, except that RP-HPLC was performed on a 1.0 mm i.d. × 50.0 mm RP3000 column (using a flow rate of 0.1 ml/min), and 0.1% TFA was replaced by 50 mM NH_4HCO_3. The concentrations of peptides used in the competitive ELISA, described below, were determined by amino acid analysis.

Monoclonal antibody production and characterization

Production and initial characterization of the two anti-apoA-I MAbs have been described elsewhere (17). For simplicity, the two MAbs previously denoted by 1→5 AI^B and 6→10 AI^B will now be referred to as AI-1 and AI-3, respectively. Both MAbs were obtained after immunization and murine hybridoma production using purified human apoA-I, and both belong to the IgG1κ subclass. In the present study, assays were performed using monoclonal IgG purified from ascites fluid by affinity chromatography on Protein G-Sepharose 4B as described by Pharmacia. Protein concentrations of purified IgG were measured according to Lowry et al. (25), using bovine serum albumin as a standard.

Competitive ELISA

Ninety six-well plates (Immulon II, USA) were coated overnight at 37°C with 2–10 μg/ml purified apoA-I in 0.1 M carbonate buffer, pH 9.6 (100 μl/well). After coating, the wells were washed 3 times with phosphate-buffered saline (PBS), pH 7.4, containing 0.05% Tween 20. Duplicate wells then received 50 μl of serially diluted (1:2) digest mixture or purified peptide, followed by 50 μl of either AI-1 or AI-3. The MAbs were added at concentrations required to achieve absorbance readings of 50–60% maximal absorbance (in the absence of competing antigen), as predetermined by ELISA. AI-1 and AI-3 (at 1 mg/ml IgG) were diluted in PBS/0.5% Tween 20 to $^1/_{1000}$ and $^1/_{10000}$, respectively. After a 1 h incubation at room temperature, the wells were washed as before, then incubated with 100 μl of horseradish-peroxidase conjugated to sheep anti-mouse immunoglobulin (Silenus, Australia) diluted 1/2000 in PBS containing 0.05% Tween 20. After 1 h at room temperature, the wells were washed and the color, which developed after adding 100 μl/well of an ABTS (2', 2-azinodi [3-ethyl-benzthiazoline sulphonic acid]) substrate solution (0.1% ABTS, 0.02% H_2O_2, 0.1 M citrate, pH 4.0) for 30 min, was then quantitated using a Titertek Multiscan (Flow Laboratories Inc.) with a filter setting of 414 nm. Results were expressed as $\%(B/B_0)$ where B = absorbance of bound antibody in the presence of competing antigen, and B_0 = maximal antibody bound (absorbance in the absence of competing antigen).

Analytical methods

Samples for amino acid analysis were transferred to WISP vials (Waters Associates) containing 4 nmol of amino-guanidino propionic acid hydrochloride as an internal standard. Gas phase hydrolysis was performed for 24 h at 110°C by the method described by Meltzer et al. (26), except that thioglycolic acid was omitted. Separation and quantitation of amino acids were performed using a Beckman model 6300 amino acid analyzer equipped with a Hewlett-Packard Model 3390 integrator.

Amino acid sequences were determined using an Applied Biosystems Model 470A protein sequencer equipped with an on-line Model 120A PTH analyzer. Polybrene was used as carrier.

RESULTS

The anti-apoA-I MAbs AI-1 and AI-3 have been previously characterized in terms of their specificities towards the CNBr fragments of apoA-I (17). CNBr cleavage of apoA-I produces four fragments (24, 27) corresponding to CF1 (residues 1–86), CF2 (residues 87–112), CF3 (residues 113–148), and the C-terminal region, CF4 (residues 148–243). In previous studies, competitive ELISAs and immunoblotting techniques using purified CNBr fragments have shown that the epitopes recognized by AI-1 and AI-3 lie within the peptides CF1 and CF3, respectively (17).

Competitive ELISA using whole endoproteinase digests of CF1 and CF3

Purified CF1 and CF3 were both cleaved with four endoproteinases (Arg-C, Asp-N, Glu-C, and Lys-C), providing a series of peptides for more precise epitope mapping studies. Digest mixtures of CF1 and CF3 were analyzed by RP-HPLC to ensure complete cleavage of the parent proteins (data not shown). The ability of the whole (unfractionated) digests to compete for the binding of the MAbs to apoA-I was then assessed by means of competitive ELISA. Cleavage of CF1 by Asp-N and Arg-C resulted in digests that could inhibit the binding of MAb AI-1 to apoA-I in a manner comparable to that of undigested CF1 (**Table 1**). In contrast, CF1 digested with Glu-C or Lys-C lost the ability to inhibit the binding of AI-1.

Purified CF3 digested with Asp-N, Arg-C, or Glu-C retained the ability to compete for the binding of MAb AI-3 to immobilized apoA-I, whereas the Lys-C digest failed to inhibit AI-3 binding, as summarized in Table 1. ELISA plate wells containing the endoproteinases alone were included as controls; the binding of MAbs AI-1 or AI-3 to apoA-I during the course of the ELISA was unaffected by the presence of the enzymes.

Purification of peptides generated from endoproteinase cleavage of CF1 and CF3

Using the theoretical cleavage sites for each endoproteinase, together with the data presented in Table 1, it was possible to predict regions within CF1 and CF3 that were most likely to contain the epitopes. Asp-N and Glu-C cleavage of CF1 and CF3 respectively were selected because *1*) the peptides expected from the theoretical digests included the predicted epitope regions; and *2*) the

TABLE 1. The ability of MAbs AI-1 and AI-3 to recognize whole endoproteinase digests of CF1 and CF3, respectively

Competing Antigen[a]	Inhibition[b] of AI-1	Competing Antigen	Inhibition of AI-3
CF1	+	CF1	–
CF3	–	CF3	+
CF1 (Arg-C)	+	CF3 (Arg-C)	+
CF1 (Asp-N)	+	CF3 (Asp-N)	+
CF1 (Glu-C)	–	CF3 (Glu-C)	+
CF1 (Lys-C)	–	CF3 (Lys-C)	–

[a]Endoproteinase digests are indicated in the parentheses.
[b]Inhibition, denoted by +, refers to samples that inhibited MAbs binding to apoA-I, as determined by competitive ELISA.

peptides generated could be clearly identified and purified by means of RP-HPLC. The RP-HPLC chromatographs of the peptides generated from digestion of CF1 and CF3 are shown in **Fig. 1**. The identities of the main cleavage products from both digests were determined by amino acid analysis and, for certain peptides, confirmed by N-terminal sequencing (**Table 2**). The major products also included several unexpected peptides. Peptides AI_{51-61} and AI_{62-72} were generated by Asp-N cleavage at residue Glu^{62} of CF1; nonspecific Asp-N digestion at Glu residues has been reported by the manufacturer. Peptide $AI_{118-125}$ resulted from Glu-C digestion at residue 117 of CF3. Deamidation of Gln_{117}→Glu could account for this unexpected cleavage. Such a modification is likely since the plasma apoA-I isoforms are thought to represent sequential deamidation products (28). It should be noted that during CNBr fragmentation of apoA-I, the methionine residues are converted into homoserine lactone which is in equilibrium with homoserine (29). Therefore, the C-terminal residues of CF1 and CF3 (refer to Table 2) are in the form of homoserine or homoserine lactone, not methionine.

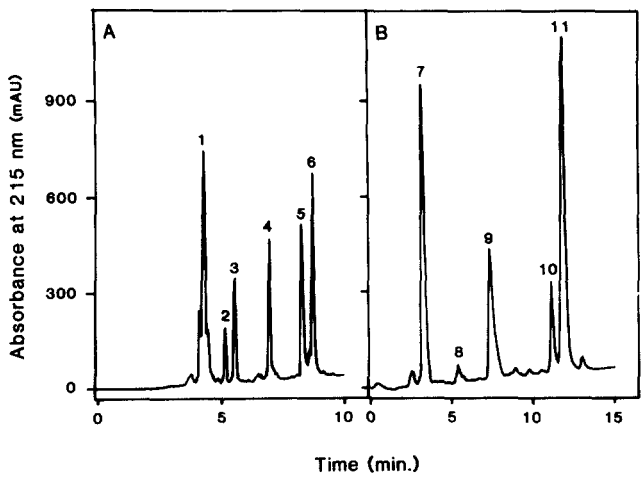

Fig. 1. RP-HPLC chromatographs showing the separation of peptides generated by endoproteinase Asp-N digestion of CF1 (panel A), and Glu-C digestion of CF3 (panel B). The numbered peaks were identified by amino acid analysis, as summarized in Table 2.

Competitive ELISA using the purified peptides from endoproteinase cleavage of CF1 and CF3

Purified peptides from the Asp-N digestion of CF1 and from the Glu-C digestion of CF3 were compared for their abilities to inhibit the binding of the MAbs to apoA-I, as described in Materials and Methods. Of the five major peptides selected from the CF1 cleavage (Table 2), only one (AI_{28-47}) could reduce the binding of MAb AI-1 to apoA-I (**Fig. 2**). The inhibition produced by peptide AI_{28-47} was similar to that produced by CF1. Using the same competitive ELISA procedure, three peptides obtained from Glu-C digestion of CF3 were tested against the MAb AI-3. Peptide $AI_{140-148}$ produced a competition curve almost identical to that shown by whole CF-3 (**Fig. 3**). Peptide $AI_{113-125}$ also produced inhibition, but only at higher concentrations (100-fold greater) than those used for $AI_{140-148}$ or CF3. It is possible that trace amounts of partially digested or undigested CF3 were present within the sample, or alternatively, there may be low level cross-reactivity against the peptide itself.

DISCUSSION

In comparison with the other apoA-I MAbs identified in the literature, AI-1 and AI-3 produced in this labora-

TABLE 2. Peptides obtained from RP-HPLC after Asp-digestion of CF1 and Glu-C digestion of CF3

Peptide[a]	Sequence	Chromatograph Peak[b]
CF1 (AI_{1-86})	**DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLN-LKLLDNWDSVTSTFSKLREQLGPVTQEFWDNLEKETEGLRQEM**	
AI_{1-8}	DEPPQSPW	1
AI_{13-19}	DLATVYV	3
AI_{28-47}	DYVSQFEGSALGKQLNKLL	5
AI_{51-72}	DSVTSTFSKLREQLGPVTQEFW	6
AI_{51-61}	DSVTSTFSKLR	2
AI_{62-72}	EQLGPVTQEFW	4
AI_{73-86}	DNLEKETEGLRQEM	1
CF3 ($AI_{113-148}$)	**ELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEEM**	
$AI_{113-125}$	ELYRQKVEPLRAE	10
$AI_{114-125}$	LYRQKVEPLRAE	11
$AI_{118-125}$	KVEPLRAE	8
$AI_{129-136}$	GARQKLHE	7
$AI_{140-148}$	KLSPLGEEM	9

[a]Peptides identified by amino acid analysis. Peptides from peaks 1-6 and 9 were also characterized by N-terminal sequencing.
[b]Refer to numbered peaks on the chromatographs in Figs. 1A and 1B, for CF1 and CF3, respectively.

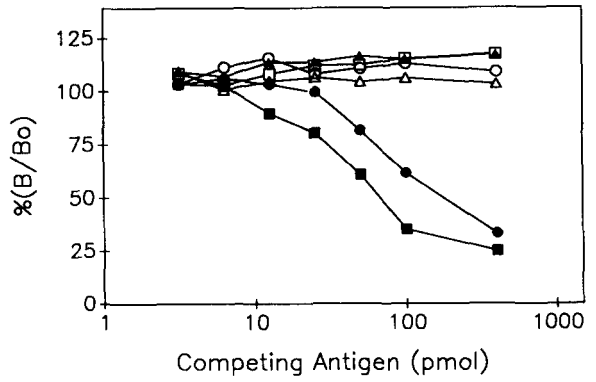

Fig. 2. Competition curves of MAb AI-1 binding to apoA-I in the presence of Asp-N digest peptides of CF1. Ninety six-well plates coated with apoA-I received increasing amounts of the purified peptides; (■), CF1; (□), AI_{1-8}; (▲), AI_{13-19}; (●), AI_{28-47}; (△), AI_{51-72} and (○), AI_{73-86}, followed by the addition of MAb AI-1. Bound MAb was detected by ELISA as described in Materials and Methods.

tory appear to recognize two previously unreported epitopes, as summarized in **Table 3**. Our earlier study demonstrated that these MAbs are directed against two distinct regions of the apoA-I sequence, between residues 1–86 (CF1) and 112–148 (CF3), respectively (17). In addition, both epitopes were equally expressed on HDL_2, HDL_3, and apoA-I alone, suggesting the involvement of lipid-independent epitopes (17). MAbs with similar properties have been described in several studies, and are particularly useful for the establishment of immunoassays to accurately determine plasma apoA-I levels (19, 20). The majority of apoA-I-specific MAbs reported in the literature have been characterized in terms of their specificity towards the four CNBr fragments of apoA-I (10–17). Several laboratories have also described the use of synthetic peptides analogous to limited regions of the apoA-I sequence (14–16), or chemical modification of particular amino acids (18), to further delineate the antigenic determinants of apoA-I (Table 3). This report describes the production and use of endoproteinase cleavage products of CF1 and CF3 to further localize the epitopes recognized by MAbs AI-1 and AI-3.

The probable locations of the epitopes within CF1 and CF3 were initially determined by the ability of whole endoproteinase digest mixtures to compete for the binding of the MAbs to apoA-I (Table 1). That is, amino acids that were included in, or essential for, the integrity of the epitope were identified when digests failed to recognize (i.e., inhibit) the MAbs. Similarly, residues lying outside the epitope regions were indicated by the digests that could still recognize the MAbs. To this end, the initial studies suggested that Asp-N and Glu-C cleavage of CF1 and CF3, respectively, generated peptides most likely to retain the predicted epitope regions. After purification by RP-HPLC and identification by amino acid analysis (Table 2), selected peptides were then tested (by competitive ELISA) for their ability to recognize the MAbs.

Of the five major peptides derived from Asp-N digestion of CF1, only AI_{28-47} could recognize the MAb AI-1 (Fig. 2). The presence of two lysines (residues 41 and 45) and one glutamic acid (residue 35) within this sequence is consistent with the initial competition data (Table 1), which suggested that Lys-C or Glu-C digestion of CF1 destroys the epitope of AI-1. Furthermore, the epitope was unaffected by Arg-C cleavage, providing more evidence for its location within peptide AI_{28-47}, which contains no arginine residues. According to the predictions of Welling et al. (30), the most antigenic region along this 28–47 amino acid sequence resides in the COOH-terminal portion, between residues 39 and 47. In addition, this region of apoA-I is thought to include a small β-structure centered around the leucine at position 46 (31). On the basis of these predictions, it is possible that MAb AI-1 recognizes a β-conformation that is exposed on apoA-I either associated or unassociated with lipid (17).

Glu-C digestion of CF3 enabled the identification of a region within peptide $AI_{140-148}$ as the epitope recognized by AI-3 (Fig. 3). Furthermore, AI-3 was unable to recognize CF3 digested with Lys-C, demonstrating that the N-terminal lysine of the 140–148 amino acid sequence (Table 2) forms an important part of the epitope. It should also be noted that the C-terminal residue of $AI_{140-148}$ corresponds to homoserine or homoserine lactone, after the conversion of methionine during CNBr digestion of apoA-I. This implies that the methionine is not required for the epitope, thus narrowing down the region recognized by MAb AI-3 to seven amino acids, between residues 140 and 147. This region of apoA-I contains a proline at position 143 which is thought to occupy a β-turn between two amphipathic helices (31). Thus, the present findings suggest that MAb AI-3 is directed towards a β-turn of apoA-I that assumes the same conformation in apoA-I, HDL_2, or HDL_3 (17). A similar epitope has been reported by Curtiss and Smith (16) for MAb AI-18 (Table 3), in which the

TABLE 3. The identified regions of human apoA-I corresponding to epitopes recognized by apoA-I-specific MAbs

MAb	Predicted Residues [Δ] or Peptides of ApoA-I	Reference
M-30	$AI_{107-124}$	Silberman et al., 1987 (15)
AI-16	AI_{1-15}	Curtiss and Smith, 1988 (16)
AI-18	AI_{95-107}	Curtiss and Smith, 1988 (16)
2G11	$^{\Delta}AI_{13-20}$	Ayrault-Jarrier et al., 1988 (18)
4A12	$^{\Delta}AI_{233-239}$	Ayrault-Jarrier et al., 1988 (18)
A-I-12	AI_{87-124}	Marcovina et al., 1990 (14)
A-I-57	$AI_{148-182}$	Marcovina et al., 1990 (14)
AI-1	AI_{28-47}	Present study
AI-3	$AI_{140-148}$	Present study

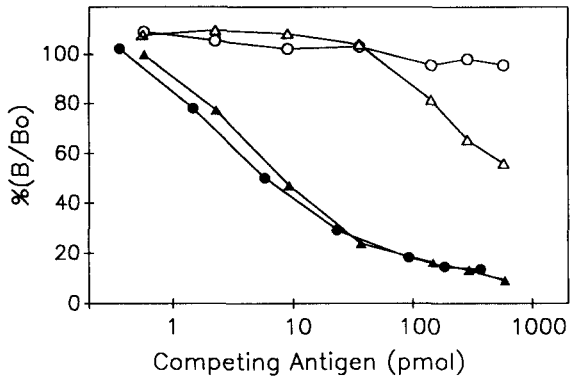

Fig. 3. Competition curves of MAb AI-3 binding to apoA-I in the presence of Glu-C digest products of CF3. Ninety six-well plates coated with apoA-I received increasing amounts of the purified peptides; (●), CF3; (△), $AI_{113-125}$; (○), $AI_{129-136}$ and (▲) $AI_{140-148}$, followed by the addition of MAb AI-3. Bound antibody was detected by ELISA as described in Methods.

antibody was shown to recognize a β-turn situated in residues 95–107. Unlike AI-3, however, AI-18 reacted poorly to purified apoA-I, while effectively recognizing apoA-I in HDL (16), presumably due to conformational changes upon lipid binding.

In conclusion, we have localized the epitopes recognized by the MAbs AI-1 and AI-3 to regions within residues 28–47 and 140–147 of apoA-I, respectively. Our earlier studies demonstrated that the epitopes for both MAbs are expressed on the surface of HDL_2 and HDL_3 particles (17). Thus, the precise mapping of the binding sites recognized by AI-1 and AI-3 has enabled the identification of regions of apoA-I that are exposed on HDL. The location of epitopes on the apoA-I molecule has been the objective of several recent investigations using chemical modifications (18) or series of overlapping, synthetic peptides representing portions of the apoA-I sequence (14–16). This report describes an alternative approach, using peptides obtained after endoproteinase cleavage of apoA-I, which has identified two previously unreported epitopes on the apoA-I molecule. Such details regarding the immunochemical properties of apoA-I are important for the development of MAbs as probes to study the structural and functional domains of apoA-I that mediate its role in lipid metabolism.

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