The Receptor-binding Domain of Human Apolipoprotein E

MONOClonAL ANTIBODY INHIBITION OF BINDING

(Received for publication, May 9, 1983)

Karl H. Weisgraber, Thomas L. Innerarity, Karen J. Harder, Robert W. Mahley, Ross W. Milne, Yves L. Marcel, and James T. Sparrow

From the Gladstone Foundation Laboratories for Cardiovascular Disease, Cardiovascular Research Institute, Departments of Pathology and Medicine, University of California, San Francisco, San Francisco, California 94140, the §Laboratory of Lipoprotein Metabolism, Clinical Research Institute of Montreal, Montreal H3W 1R7, Quebec, Canada, and the ³Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

To investigate the potential of monoclonal antibodies as probes to determine the receptor-binding domain of apolipoprotein E (apo-E), five apo-E antibodies were tested to see if any of them inhibited 125I-apo-E3-dimyristoylphosphatidylcholine binding to apo-B,E receptors on cultured fibroblasts. Only one of the five antibodies, referred to as 1D7, was found to inhibit binding, blocking greater than 90% of the receptor-binding activity of apo-E3-dimyristoylphosphatidylcholine. The 1D7 Fab fragments were also effective inhibitors. The 1D7 bound to a Mr = 22,000 NH₂-terminal thrombolytic fragment of apo-E (residues 1-191) and to a 93-residue cyanogen bromide fragment of apo-E (residues 126-218). The four noninhibitory antibodies bound only to the NH₂-terminal thrombolytic fragment. These results suggested that the 1D7 epitope is contained between residues 126 and 191, and that the epitopes of the other antibodies are not contained in this region. The use of synthetic apo-E fragments, which cover various lengths of the sequence from residues 129-169, and human apo-E variants with substitutions at residues 145, 146, or 158, narrowed the location of the 1D7 epitope to residues 139-169 and, most likely, to the immediate vicinity of residues 140-150. It is of interest that 1D7 was found to bind to the same region of apo-E that has been implicated as the receptor-binding domain in receptor-binding studies using human apo-E variants and apo-E3 fragments.

Human apo-E is a 299-residue apoprotein that is a component of several classes of plasma lipoproteins (1). Apolipoprotein E, like apo-B, binds to the LDL or apo-B,E receptors of several lines of cultured cells. The lipoproteins containing these apoproteins are then capable of delivering cholesterol to the cells (2). In addition, a specific receptor for apo-E has been demonstrated in the liver (3, 4). It is thought that hepatocytes recognize and take up triglyceride-depleted chylomicron remnants by way of this receptor system (5-7). Because of its capability to bind to both hepatic and extrahepatic cells (see Ref. 8 for review), apo-E plays a central role in lipid metabolism. As a consequence of this important role, there is much interest in determining the region of the apo-E molecule that interacts with lipoprotein receptors.

Recently, it has been demonstrated that several structurally distinct forms of human apo-E exist (1, 9, 10). The variant forms differ from the parent form, apo-E3, by amino acid substitutions at four locations in the apo-E molecule. These variants are designated E2(Arg145→Cys), E2(Arg146→Cys), E2(Lys112→Gln), and E2(Cys112→Arg). Significantly, early studies using chemical modifications of specific amino acid residues demonstrated that a limited number of arginyl and lysyl residues of apo-E and apo-B are involved in mediating receptor-binding activity (11, 12). Recent studies aimed at determining the functional consequences of these structural variations have demonstrated that the amino acid substitution at residue 122 has no effect on the ability of apo-E to interact with apo-B,E receptors (13). In contrast, the substitution of cysteine for arginine at residue 145 or 158, and of glutamine for lysine at 146, results in a decrease in receptor activity (10, 13). This suggests that the area of the apo-E molecule in the vicinity of residues 145, 146, and 158 is important in receptor interaction.

To test this hypothesis, two approaches have been pursued: (1) the receptor-binding activities of cyanogen bromide and thrombolytic fragments of apo-E3 have been determined (see companion paper, Ref. 14); and (2) monoclonal antibodies against apo-E have been tested to see if they interfere with the receptor-binding activity of apo-E3 and, if so, the position of the antigenic determinant(s) (epitope) in the molecule has been located. In this report, the results of the second approach have been presented. It has been determined that one of five apo-E monoclonal antibodies tested in this study inhibits apo-E3 receptor binding activity and that the epitope for this antibody is located in the region of the apo-E molecule in which the amino acid substitutions that affect receptor binding are located.

MATERIALS AND METHODS

Apolipoprotein E Monoclonal Antibodies and Apolipoprotein E Isolation—Production and characterization of the five anti-apo-E monoclonal antibodies (1D7, 3B7, 6C5, 6H7, and 7C9) have been described previously (15). The IgG subclass containing the anti-apo-E antibody

[Adjoining text not shown]
was isolated from the asctic fluid of hybridoma-bearing mice by elution from Protein A-Sepharose 4B (Pharmacia) (16). The 1D7 and 3B7 Fab fragments were prepared by papain digestion of the purified IgG (17). Undigested IgG and Fc fragments were removed by Protein A-Sepharose 4B chromatography as previously described for apo-B monoclonal antibodies (8). The various types of apo-E were obtained by elution from Protein A-Sepharose 4B (Pharmacia) (16). Undigested IgG and Fc fragments were removed by Protein A-Sepharose 4B chromatography as previously described for apo-B monoclonal antibodies (8).

The dissociation constants for the monoclonal antibodies were determined by radioimmunoassay. The relative abilities of the five antibodies to bind apo-E3 and the synthetic fragments were each labeled with 1 mCi of $^{125}$I (Amersham) by a method adapted from Hunter and Greenwood (19). The reaction was initiated with 50 $\mu$g of cholinergic-T (10 $\mu$L, 5 $\mu$g/ $\mu$L; Baker), allowed to proceed for 20 h (11). The reaction mixture was added to the 200 $\mu$L of chloramine-T (10 $\mu$L, 5 $\mu$g/ $\mu$L; Baker). Unreacted '*'I was allowed to stand 15 min. Dilutions of 1:10 were used for 20 h at room temperature, 4 $\mu$g of '*'I-apo-E3. After incubation, the wells were washed 4 to 5 times with saline containing 1% BSA. $^{125}$I-labeled rabbit anti-mouse IgG was diluted with saline containing 0.2% BSA such that the 50 $\mu$L added to each well contained 50,000 cpm. Incubation was for 2 h at room temperature, after which the wells were washed 10 to 12 times with saline containing 1% BSA, and the bound radioactivity was determined.

Competitor Studies with 1D7—Apolipoprotein E3 was diluted in 5 mM glycine, pH 9.2 and 200 $\mu$L containing 100 ng of apo-E3 were added to each well and incubated overnight at room temperature. The wells were washed with saline-Tween. Competing proteins, diluted in PBS containing 1% BSA and 0.01% thimerosal, were preincubated with the diluted 1D7 (1:150) ascites fluid for 2.5 h prior to incubation. Undigested IgG and Fc fragments were removed by Protein A-Sepharose 4B chromatography as previously described for apo-B monoclonal antibodies (8). The recombinants were isolated by density gradient centrifugation (20).

The dissociation constants for the monoclonal antibodies were determined by radioimmunoassay. The relative abilities of the five antibodies to bind apo-E3 and the synthetic fragments were each labeled with 1 mCi of $^{125}$I (Amersham) by a method adapted from Hunter and Greenwood (19). The reaction was initiated with 50 $\mu$g of cholinergic-T (10 $\mu$L, 5 $\mu$g/ $\mu$L; Baker), allowed to proceed for 20 h (11). The reaction mixture was added to the 200 $\mu$L of chloramine-T (10 $\mu$L, 5 $\mu$g/ $\mu$L; Baker). Unreacted '*'I was allowed to stand 15 min. Dilutions of 1:10 were used for 20 h at room temperature, 4 $\mu$g of '*'I-apo-E3. After incubation, the wells were washed 4 to 5 times with saline containing 1% BSA. $^{125}$I-labeled rabbit anti-mouse IgG was diluted with saline containing 0.2% BSA such that the 50 $\mu$L added to each well contained 50,000 cpm. Incubation was for 2 h at room temperature, after which the wells were washed 10 to 12 times with saline containing 1% BSA, and the bound radioactivity was determined.

Tissue Culture Assay—Cultured human fibroblasts were established from a preputial specimen of a normal human infant and grown in 75-mm flasks as described (20). The cells used for the experiments were dissociated from flasks with a 0.05% trypsin, 0.02% EDTA solution and plated at a density of 3.5 x 10$^4$ cells/35-mm Petri dish. After 5 days, the medium was changed to one containing 10% lipoprotein-deficient serum, and 2 days later the cells were used in 4°C binding experiments. The indicated concentrations of apo-E monoclonal antibodies were added to 2 ml of medium containing 10% human lipoprotein-deficient serum and 0.1 $\mu$g of human $^{125}$I-apo-E3-DMPC (2790 cpm/ng). The mixtures were incubated for 2 h at room temperature and 15 min at 4°C, and then 0.95 ml was added to 35-mm Petri dishes of cultured human fibroblasts. After 2 h at 4°C, the cells were extensively washed, dissolved with 0.1 N NaOH, and the $^{125}$I-apo-E3 bound was determined. $^{125}$I-apo-E3-DMPC incubated with nonimmune mouse IgG was added to the control plates. The amounts of the antibodies added were based on their titers against apo-E3 or apo-E3-DMPC used in the tissue culture experiments (Table I). The maximum concentrations of IgG used for 1D7, 6C5, 3B7, 7C9, and 6H7 were 272, 56, 95, 38, and 21 $\mu$g/ml, respectively. In additional experiments, the maximum concentrations of 6C5, 7C9, 246 $\mu$g/ml of the antibody failed to inhibit $^{125}$I-apo-E3-DMPC binding.

Peptide Synthesis and Purification—The peptides were synthesized by solid phase method using a previously reported program (22) on a Schwartz/Mann Bioresearch Synthesizer modified for computer control (23, 24). The amount of BOC glycine loaded on the resin was 0.15 mm/g. Resin was removed after the attachment of residues 148, 144, 139, and 129. The peptide was deprotected and cleaved from the resin using anhydrous HF. The crude peptide was purified by ion exchange chromatography on SP-Sepharose. Amino acid analysis indicated that the primary sequence matched the expected sequence of the natural peptide. Amino acid analysis of the peptide isolated from the resin was performed with a Beckman 121 automatic amino acid analyzer. The yield of peptide was approximately 70%.

RESULTS

The five apo-E monoclonal antibodies used in this study were of the IgG1 or IgG2 class and have been characterized previously (15). They are referred to as 1D7, 3B7, 6C5, 6H7, and 7C9. The relative abilities of these antibodies to bind to $^{125}$I-apo-E3-DMPC recombinants or free apo-E3 were determined by radioimmunoassay. The relative titers for free apo-E3 and apo-E3-DMPC are presented in Table I, along with the dissociation constants for the monoclonal antibodies.
with equilibrium dissociation constants for binding to apo-E.

To determine the effect of the antibodies on fibroblast binding activity, the 125I-apo-E3-DMPC recombinants were preincubated with each of the antibodies prior to their addition to the cells. The differences in antibody titers were adjusted for in the incubation mixtures so that at each point equal immunoreactive amounts were added. As shown in Fig. 1, only 1D7 had a significant effect on binding activity; binding of 125I-apo-E3-DMPC recombinants to fibroblasts was reduced to less than 10% of the control value at higher concentrations of this antibody. The four other antibodies had little or no effect on binding. The Fab fragments of 1D7 were also effective inhibitors, blocking greater than 90% of 125I-apo-E3-DMPC binding (data not shown). When added at equal immunoreactive amounts, the addition of both 1D7 Fab fragments and IgG resulted in identical levels of binding inhibition.

To establish the location of the epitope for each antibody, the ability of the antibodies to bind to various fragments of apo-E3 was tested. It has been demonstrated that thrombin has a limited ability to cleave apo-E3. Two major cleavage sites are at residues 191 and 215, producing a M, = 22,800 NH2-terminal fragment (residues 1–191) and a M, = 10,000 COOH-terminal fragment (residues 216–299) (see companion paper, Ref. 14). Similar fragments of apo-E produced by thrombin digestion have been reported by Gianturco et al. (25). Cyanogen bromide digestion of apo-E3 yields a 93-residue peptide, CB5, also referred to as CNBr II, which contains residues 126–218 (1). Because the amino acid substitutions at residues 145, 146, and 158 are known to affect receptor binding, four synthetic fragments that included these residues were tested to determine if 1D7 bound to apo-E3 in the area of the substitutions. Table II summarizes the relationship of the synthetic fragments to intact apo-E3.

Apolipoprotein E3 and the synthetic fragments were bound to microtiter plates that were then incubated with the antibodies. Antibody binding was detected by the addition of 125I-labeled rabbit anti-mouse IgG. All five antibodies bound to the NH2-terminal thrombolytic fragment preferentially (Table III), suggesting that their respective epitopes are contained in the NH2-terminal region of apo-E. When CNBr II (CB5) was tested, only 1D7 demonstrated a high level of binding to this fragment. The other antibodies exhibited low levels of binding for CNBr II, similar to those exhibited for the COOH-terminal thrombolytic fragment (Table III). These results suggest that the epitope for 1D7 is contained within CNBr II, and that the epitope for the other antibodies are not contained in this fragment.

The binding of 1D7 to the two shortest synthetic fragments, residues 148–169 and 144–169, was approximately equal to the nonspecific binding observed for apo-A-I and the COOH-terminal thrombolytic fragment (Table III). The 1D7 binding to residues 139–169 was slightly higher than the nonspecific binding observed in experiment A and significantly higher than the nonspecific binding observed in experiments B and C. The fragment spanning residues 129–169 showed a consistently high level of 1D7 binding. These results support the hypothesis that the 1D7 epitope is located between residues 129–169 and, as suggested by experiments B and C (Table III), that it may actually be contained in the 139–169 fragment. As expected, 3B7, 6C5, 6H7, and 7C9 did not bind to the synthetic fragments. Binding of the synthetic fragments to the plastic wells was demonstrated with 125I-labeled fragments. With each fragment, approximately 20% of the 200 ng applied bound to the wells. In the case of intact apo-E3, 50% was bound to the wells. As 3B7, 6C5, 6H7, and 7C9 did not affect receptor activity, the characterization of their epitopes was not pursued further.

The conditions of the binding assay were then optimized to determine if a higher specific binding of 1D7 to the 139–169 fragment could be demonstrated. The changes in the assay included adding the fragments to the wells at a higher concentration and washing the wells after fragment incubation with a BSA buffer without detergent. When 1D7 binding was examined in this manner at a series of antibody dilutions, 1D7 was shown to bind similarly and effectively to both the 129–169 and 139–169 fragments, but not to the 144–169 and

| Antibody | Titer* | Kd (M) |
|----------|--------|--------|
| 1D7      | 480    | 2.0 × 10^-2 |
| 3B7      | 50     | 2.2 × 10^-10 |
| 6C5      | 84     | 2.2 × 10^-10 |
| 6H7      | 34     | 7.1 × 10^-10 |
| 7C9      | 38     | 5.3 × 10^-10 |

* The titers were determined by solid phase radioimmunoassay for apo-E3 and by liquid phase assay for apo-E3-DMPC as described under "Materials and Methods," and they are expressed as the ng of IgG required to achieve 50% maximum binding to 1 ng of apo-E3.
Receptor-binding Domain of Apo-E

Relative binding of monoclonal antibodies to apo-E3 and various apo-E3 fragments

The results are expressed as a percentage of apo-E3 binding for each antibody. Values represent triplicate determinations. Typical ranges were less than ±8% of the average value.

### Table III

| Antibody | Table IV |
|----------|----------|
| A | B | C | 3B7 | 6C5 | 6H7 | 7C9 |
| Apo-E3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Apo-A-I | 15.1 | 10.4 | 4.2 | 4.9 | 5.1 | 4.3 | 3.5 |
| Thrombolytic fragment, \( M_r = 22,000 \) (residues 1-191) | 181 | 94.6 | 102 | 104 | 109 | 107 |
| Thrombolytic fragment, \( M_r = 10,000 \) (residues 216-299) | 18.0 | 4.2 | 22.1 | 21.1 | 12.8 | 16.4 |
| CNBr II (CB5) (residues 126-218) | 133 | 98.7 | 103 | 26.6 | 23.4 | 12.2 | 15.5 |
| Synthetic fragment (139-169) | 39.4 | 68.9 | 29.4 | 4.2 | 3.5 | 2.8 | 3.0 |
| Synthetic fragment (144-169) | 26.5 | 11.8 | 4.6 | 4.1 | 3.3 | 2.4 | 1.9 |
| Synthetic fragment (148-169) | 35.1 | 10.4 | 4.5 | 5.5 | 4.8 | 3.8 | 4.1 |
| Synthetic fragment (149-169) | 26.5 | 11.8 | 4.6 | 4.5 | 3.3 | 2.4 | 1.9 |
| Synthetic fragment (148-169) | 25.1 | 10.4 | 4.5 | 5.5 | 4.8 | 3.8 | 4.1 |

Average total counts of \(^{125}\)I-goat anti-mouse IgG bound per well for 1D7 A, 1D7 B, 1D7 C, 3B7, 6C5, 6H7, and 7C9 were 1688, 4077, 5310, 2500, 2545, 2308, and 2317 cpm, respectively.

Fig. 2. Binding of 1D7 IgG to synthetic fragments at varying antibody dilutions. A and B are the results from two experiments: fragment 129-169 (C), fragment 139-169 (B), fragment 144-169 (A), and apo-E3 (D).

Fig. 3. Logit/log transformation of the competitive displacement of apo-E3 from 1D7 by various types of apo-E. Competitive assays with E3 (O), E2(Arg\(_{148}^{\text{Lys}}\), Gln) (O), E2(Arg\(_{148}^{\text{Lys}}\), Gln) (O), canine apo-E (O), and rat apo-E (O) were performed as described under "Materials and Methods." Each point represents the average of three determinations.

### Table IV

The ability of human apo-E variants and canine and rat apo-E to compete with immobilized apo-E3 for 1D7 binding

| Apo-E | Concentration at which 50% of 1D7 was displaced from immobilized apo-E3 | Relative competitive ability |
|-------|--------------------------------------------------------------------------------|-----------------------------|
| Human E3 | 7.5 | 100.0 |
| Human E2(Arg\(_{158}^{\text{Lys}}\), Gln) | 8.7 | 111.9 |
| Human E2(Arg\(_{158}^{\text{Lys}}\), Gln) | 15.4 | 48.7 |
| Human E2(Lys\(_{158}^{\text{Lys}}\), Gln) | 23.6 | 31.8 |
| Canine apo-E | 37.3 | 20.1 |
| Rat apo-E | 87.3 | 8.6 |

occurred with E2(Arg\(_{158}^{\text{Lys}}\), Gln). Another F mutant, E2(Lys\(_{158}^{\text{Lys}}\), Gln), also competed poorly. The rat and canine apo-E displayed a binding level of ~20% or less than the apo-E3 level. Inspection of the amino acid sequences of both rat
and canine apo-E revealed that several amino acid substitutions occur in the vicinity of the two human E2 substitutions at residues 145 and 146 (Table V).

**DISCUSSION**

To gain further insight into the specific region of the apo-E molecule that interacts with the apo-B,E receptor, the ability of apo-E monoclonal antibodies to inhibit this interaction was examined. A similar inhibition study has been performed with anti-LDL monoclonal antibodies and fibroblast receptors (18, 27). A basic assumption of this approach is that to block receptor-apo-E binding a monoclonal antibody would have to bind at or near the recognition site and, thereby, sterically inhibit receptor interaction. Alternatively, by binding near the recognition site the antibody might affect the recognition site conformation and thereby inhibit binding. Implicit in this approach is that antibody binding to a non-critical region of apo-E would not affect receptor activity. This condition was satisfied when it was determined that only one of the five antibodies inhibited receptor binding, although the other four antibodies did bind to apo-E. In addition, for the approach to be successful, the binding affinity of the antibody should at least be on the same order as that of receptor-apo-E interaction. This would insure that the receptor would not displace the antibody off the apo-E molecule. In the case where the antibody was binding at or near the receptor-binding site, this would lead to a false negative result. This latter condition was met, since the binding equilibrium dissociation constants of the five antibodies ranged from $2.0 \times 10^{-9}$ to $2.2 \times 10^{-10}$ M. These values are within the range of $K_d = 1.0 \times 10^{-9}$ M determined for apo-E binding to fibroblast receptors (28).

A combination of different apo-E3 fragments and apo-E variants were used to characterize the antibody-antigenic sites on apo-E. Two useful fragments of apo-E3 that have been shown to bind to the apo-B,E receptors on cultured fibroblasts and adrenal membranes (14) include the NH$_2$-terminal two-thirds of the apo-E molecule (residues 1–191) produced by thrombin digestion and the large CNBr II fragment encompassing residues 126–218. All five antibodies specifically bound to the NH$_2$-terminal thrombolytic fragment (residues 1–191), suggesting that their respective epitopes are contained in this portion of the molecule. However, only 1D7 showed significant binding to the apo-E3 CNBr II fragment. This suggests that 3B7, 6C5, 6H7, and 7C9 all recognize epitopes contained in the sequence prior to residue 126. Alternatively, it is possible that one or more of the antibodies recognize topographic or conformational determinants in the NH$_2$-terminal thrombolytic fragment not present in CNBr II. Recent studies with monoclonal antibodies against myoglobin have indicated that topographic determinants may constitute a more important class of determinants than it was previously thought (29, 30).

Determination of 1D7 binding to four synthetic fragments spanning various lengths of apo-E3 between residues 129–169 revealed that 1D7 bound effectively to the two longest fragments. These results suggest that the 1D7 epitope is entirely contained within residues 139–169. The binding of 1D7 to this rather short segment of apo-E would suggest that the 1D7 epitope is not a topographic determinant in which the tertiary structure of the protein is important. However, as will be discussed below, this does not rule out the possibility that secondary structure plays a role in defining the epitope.

Since 1D7 does not bind to the 144–169 fragment, it might be assumed that the 1D7 epitope is contained in the 139–144 span. However, the possibility exists that the epitope actually

---

**Table V.** Partial sequence comparisons of human apo-E variants and canine and rat apo-E.

| Residue | Arg | Asp | Ala | Phe | Ser | Thr | Met | Thr |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| Human E2 | Arg | Asp | Ala | Phe | Ser | Thr | Met | Thr |
| Human E2 (Lys$_{138}$-Glu$_{139}$) | Arg | Asp | Ala | Phe | Ser | Thr | Met | Thr |
| Canine apo-E | Arg | Asp | Ala | Phe | Ser | Thr | Met | Thr |
| Rat apo-E | Arg | Asp | Ala | Phe | Ser | Thr | Met | Thr |

*See Ref. 1.
See Ref. 10.
extends beyond residue 144 and that part of the epitope is contained in the 144–169 span. An alternative possibility is that conformation in this region of the protein plays a role in defining the 1D7 epitope and that the smaller fragments (144–169 and 148–169) do not contain enough of the sequence to orient them in the proper conformation for 1D7 binding. Examination of 1D7 binding to available apo-E variants, E2(Arg149→Cys), E2(Lys144→Gln), and E2(Arg146→Cys), has provided some clarification of these points. Determination of the relative binding affinities of the human mutants has demonstrated that amino acid substitutions at residues 145 and 146 result in a significant lowering of the apparent binding affinity of 1D7, while the substitution at residue 158 does not affect 1D7 binding. These results suggest that residues 145 and 146 are part of the 1D7 epitope, while residue 158 lies outside the epitope.

This is somewhat difficult to interpret in terms of providing additional information on the 1D7 epitope because there are several differences in amino acid sequences between residues 129 and 158 in canine, rat, and human apo-E (Table V). However, when the predicted secondary structure of this region is taken into account, an interesting picture develops. Application of the Chou-Fasman rules (31, 32) predicts that residues 131–150 form an α-helix (1). When the positions of the various amino acid substitutions that occur in canine and rat apo-E and in human apo-E variants were located on a helical model of this region (33), all of the substitutions were shown to occur either in the center or at the edges of one face of the helix (Fig. 4). These results raise the speculative point that the 1D7 epitope may lie on the face of this helix rather than being part of a simple linear sequence determinant. Although this question cannot be resolved at this time, the data presented in this report indicate that the 1D7 epitope is contained within the 139–169 span, and is most likely localized in the immediate vicinity of residue 145 and 146.

It is noteworthy that 1D7, which inhibits receptor binding of apo-E, binds to apo-E in the vicinity of residues 145 and 146. Several independent lines of evidence have also indicated the importance of this region of apo-E in lipoprotein-receptor interaction. It has previously been demonstrated that a limited number of lysyl and arginyl residues appear to be involved in receptor interaction (11, 12). This is consistent with the observation that the region around residues 145 and 146 are rich in basic residues; 11 of the 30 residues shown in Table V are arginyl or lysyl residues. Also of significance in this regard is that, of the five monoclonal antibodies tested, only 1D7 binding to apo-E was inhibited by carbamylation of the apo-E lysyl groups (15). Additional evidence underscoring the importance of this region in receptor interaction comes from receptor binding studies of various apo-E mutants, which have demonstrated that residues 145, 146, and 158 are important for receptor interaction (10, 13). Finally, as demonstrated in the accompanying study (14), the CNBr II fragment, which contains this region, retains receptor-binding activity.
Receptor-binding Domain of Apo-E

28. Pitas, R. E., Innerarity, T. L., and Mahley, R. W. (1980) J. Biol. Chem. 255, 5454-5460
29. Berzofsky, J. A., Buckenmeyer, G. K., Hicks, G., Gurd, F. R. N., Feldmann, R. J., and Minna, J. (1982) J. Biol. Chem. 257, 3189-3198
30. East, I. J., Hurrell, J. G. R. Todd, P. E. E., and Leach, S. J. (1982) J. Biol. Chem. 257, 3199-3202
31. Chou, P. Y., and Fasman, G. D. (1974) Biochemistry 13, 211-222
32. Chou, P. Y., and Fasman, G. D. (1974) Biochemistry 13, 222-245
33. Schiffer, M., and Edmundson, A. B. (1967) Biophys. J. 7, 121-135
The receptor-binding domain of human apolipoprotein E. Monoclonal antibody inhibition of binding.
K H Weisgraber, T L Innerarity, K J Harder, R W Mahley, R W Milne, Y L Marcel and J T Sparrow

J. Biol. Chem. 1983, 258:12348-12354.