review

Immunologic approaches to lipoprotein structure

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I. INTRODUCTION

The lipoproteins in plasma represent several sets of discrete particles whose structures are constantly changing because of their interactions in plasma with each other, with any of several enzymes and lipid exchange proteins, and with cell surface receptors. In addition, the entrance into plasma of nascent particles and the removal from plasma of "old" particles gives rise to heterogeneities of structure within the major classes of lipoproteins. Some of these heterogeneities consist of subtle modulations of the conformations of functionally important domains of apolipoproteins on the surfaces of lipoproteins. While appropriate changes of apolipoprotein dispositions accompany "normal" or usual metabolism, abnormal dispositions of apolipoproteins can result from defective protein structures acquired during biosynthesis or post-translational modification. Environmental or disease-induced deviations from usual body metabolism such as modifications of dietary or hormonal status or changes in physical activity also may give rise to changes in intracellular assembly or in the intravascular catabolism of lipoproteins. These deviations, too, can be expected to produce alterations in lipoprotein structure, which in turn could affect lipoprotein metabolism. Much of the appreciation of subtle modulations of apolipoprotein disposition on the surfaces of lipoproteins and the effect of this on lipoprotein structure and function have come from immunologic studies of lipoproteins and apolipoproteins. First we shall briefly discuss some of the strategies and tactics used in immunologic studies and then summarize results obtained for several apolipoproteins.

II. TERMINOLOGY

A multitude of terms has evolved to describe simple immunological concepts (1). Since controversy still exists among immunochemists as to the precise definition of several terms (2) and in view of the more general readership of this review, a brief definition of the relevant terminology as it pertains to apolipoproteins will be presented here. Detailed descriptions of terminology and immunological concepts can be found in a classic review

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Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

of Jerne (1) and in a more recent review by Benjamin et al. (2).

A *monoclonal antibody* population is one secreted by a single clone of B-lymphocytes from an animal. To obtain an "immortalized" cell line of these B-lymphocytes, the cells are fused with a malignant form of B-lymphocyte (myeloma) to form a *hybridoma* which has the ability to reproduce itself indefinitely. The production of monoclonal antibodies is now commonplace. It should be emphasized that monoclonal antibodies do not differ structurally from other antibodies elicited under natural conditions. In this review the abbreviation for monoclonal antibody is *MAb.*

Immunogenicity is the degree to which a substance (*immunogen*) can elicit an immune response and depends on the host animal's genetic makeup and previous history of the immune system. The ability to be recognized by the products of an immune response (e.g., antibodies) indicates a substance's *antigenicity.*

An *antigenic determinant* is operationally defined as that region of a protein molecule surface bound by a particular antibody molecule. In this review, an *epitope* is a single antigenic determinant. It should be pointed out that it still remains to be resolved whether or not antigenic determinants are discrete, non-overlapping portions of a protein's surface structure or whether the protein surface consists of a continuum of overlapping antigenic determinants (2).

Antigenic determinants have operationally been divided into two subsets. *Conformational determinants* are defined as being dependent on the native spatial conformation of a protein, while *sequential determinants* are defined as depending only on the primary amino acid sequence of a molecule (e.g., determinants expressed on peptide fragments of the intact molecule). Since even peptide fragments can assume a variety of conformations in solution, it is felt that *all* determinants or epitopes are conformational in the sense that the combining site on an antibody molecule will only bind to an antigen which presents a complementary spatial array of interacting side chains. As a result, epitopes may be contained within a single *segment* of the primary sequence of a protein (perhaps involving only several of a series of contiguous residues) or *assembled* from residues distant in primary sequence but brought together by the folding of the protein in its native conformation.

The authors' terms *disposition* and *configuration* are used interchangeably in this review and are alternative terms for conformation. The term *disposition* reflects the authors' intent to include the most subtle protein conformational changes that may only be detectable by immunochemical means.

III. STRATEGIES FOR IMMUNOCHEMICAL ANALYSIS OF APOLIPOPROTEIN STRUCTURE

As it became clear that different apolipoproteins performed unique roles in lipid metabolism and more specifically in the pathogenesis of atherosclerosis, polyclonal antisera directed towards several lipoproteins and apolipoproteins were raised successfully and used to develop immunoassays for the quantitation of apolipoproteins in plasma and other biological fluids. In the 1970s when much of this work began in earnest, it became obvious that polyclonal antibodies failed to recognize some epitopes on apolipoproteins when the latter were associated with heterogeneous lipoprotein species. Thus began the studies of lipoprotein structure with these antisera. By utilizing specific immunization and/or absorption protocols to narrow the range of antigenic specificities of these polyclonal antisera, much information as to the relative exposure of apolipoproteins on lipoproteins, immunogenic regions of apolipoprotein molecules, etc. has been obtained (discussed later in text). This strategy potentially enables the quantitation of a specific apolipoprotein epitope that is related to a particular function or that is expressed equally on *all* lipoprotein particles (for measuring total levels). Monoclonal antibodies, because of their inherent monospecificities, are much more useful in structural studies (3). This has led to their being used almost exclusively for such studies in recent years (3).

A. Immunogens

The use of purified apolipoproteins as immunogens has resulted in the production of monoclonal antibodies that recognize a number of "native" epitopes on hololipoproteins. Native epitopes may be defined as those ordinarily present on hololipoproteins isolated from plasmas of normal individuals. Native epitopes may be "expressed," i.e., detectable by immunologic means, or "masked" either by lipids or other apolipoproteins or by alterations in the disposition of the apolipoproteins. "Artifactual" epitopes are structures formed by nonphysiologic perturbations of the structures of apolipoproteins, e.g., by ultracentrifugal isolation or by delipidation of lipoproteins, or by chemical modification of amino acid residues. Since the physical state of the immunogen may be important in determining the range of specificities of the antibodies produced by the hybridomas, when isolated apolipoproteins are used as immunogens, antibodies may be produced not only against native expressed epitopes but also against native masked epitopes or even artifactual epitopes. Therefore, to obtain antibodies directed against native expressed epitopes, hololipoproteins may need to be used as immunogens. It is important to collect lipoproteins (or apolipoproteins) in the presence of antimicrobial (4) and antiproteolytic agents (5, 6) to reduce the potential of generating antibodies to degraded apolipoproteins or artifactual epitopes. Alternative methods of lipoprotein isolation such as affinity chromatography can be used to prevent any ultracentrifugally derived alterations in lipoprotein structure. Ideally one should try to obtain antibodies directed towards epitopes expressed on lipoproteins in vivo. Until more is known about factors influencing apolipo-

protein conformation on lipoproteins, we cannot evaluate how significantly the laboratory handling of lipoproteins alters the in vivo state of the lipoprotein.

It would appear that lipoprotein conformation is not destroyed by emulsifying in immunizing adjuvants (7). Antibodies that recognize "native" determinants on LDL (or HDL) particles have been produced using intact LDL (or HDL) as immunogens (8–11).

However, there are also potential disadvantages in using intact lipoproteins as immunogens. First, antibodies are likely to be produced against the most immunogenic apolipoprotein determinants (or immunodominant sites) on the lipoprotein particles. This is not useful when antibodies against other epitopes are desired. Second, antibodies against native but masked epitopes may not be produced.

Another alternative is to immunize with human apolipoproteins reconstituted with lipid vesicles or microemulsions or, when immunizing mice, with mouse lipoproteins. Apolipoproteins in apolipoprotein–lipid recombinants are present in configurations that more closely resemble the native molecules than are lipid-free apolipoproteins (12, 13), therefore antibodies against some native configurations could be elicited. Human apolipoproteins complexed with appropriate mouse lipoproteins may be even more desirable immunogens because the configurations of the human apolipoproteins on these "hybrid" particles may be even closer to the native. To obtain antibodies that recognize only selected regions of apolipoproteins, it may be necessary to immunize with either lipid-free peptide fragments (14) or fragments reconstituted with lipids.

Certain apolipoproteins (e.g., apoB) tend to be immunodominant. Therefore, when lipoproteins such as VLDL that contain other apolipoproteins in addition to apoB are used as immunogen, there is a strong likelihood of obtaining hybridomas producing antiapoB antibodies. For less immunogenic apolipoproteins (e.g., apoC-II), it may be necessary to isolate the apolipoprotein and couple it to a carrier molecule such as keyhole limpet hemocyanin, autologous LDL or to immunize with a glutaraldehyde polymerized form of the molecule (2).

B. Immunizations

Polyclonal antisera against various apolipoproteins most commonly have been raised in rabbits and goats. The reader is referred to the articles referenced in the sections on the individual apolipoproteins for specific methods of immunization for polyclonal antisera. This section will focus on immunizing mice for the production of monoclonal antibodies.

Immunization of mice requires 50–100 μg of protein per injection. The antigen is injected subcutaneously, initially with complete Freund's adjuvant followed 2–3 weeks later by antigen in incomplete Freund's adjuvant. Antihuman lipoprotein monoclonal antibodies have been produced also after intraperitoneal (15, 16) and intravenous (11) immunizations. In most cases, a final booster dose of antigen (approx. 10 μg in PBS) is injected intraperitoneally or intravenously 3 days before fusion of splenic and myeloma cells.

As lipoprotein surface probes, antibodies of the IgG class are preferable because of their smaller sizes and greater stabilities. To ensure that a population of hybridomas secreting this immunoglobulin class is obtained, it may be necessary to carry out immunizations over longer periods of time (several months) using multiple injections and multiple sites. When immunized mice have high antibody titers, one can test before fusion whether antibody activity is present in the 19S or 7S fractions of the immunized mouse's serum.

C. Production and characterization of monoclonal antibodies

1) *Cell fusion and expansion of clones.* Standard methods are used for producing murine hybridomas and for expanding the clones as ascitic tumors (17).

2) *Assays for detection of antibody activity.* Several procedures for detection of antibody activity in spent hybridoma culture media or mouse ascitic fluids have been described (17). Three methods that have proved particularly useful in our laboratory are the solid phase antigen assay (17), dot immunobinding (18), and "capture" antibody assay (19). In the latter method, MAbs are immobilized by means of goat antimouse immunoglobulin and are tested for their abilities to react with radiolabeled antigen. The presence of antibody activity should be sought in assays using both purified apolipoproteins and native lipoproteins to ensure detection of antibodies directed against the maximum number of possible sequential or conformational determinants.

Once antibody activity has been detected, it is important to know the immunoglobulin isotype of the secreted antibody. The interpretation of various immunoassays (e.g., antibody competition) as well as antibody purification is reliant on the antibody isotype. Isotyping can be carried out by immunoassays employing specific antiisotype antibody preparations (20).

3) *Purification of antibodies.* Due to the presence of irrelevant proteins in ascites fluids, it is often desirable to purify the monoclonal antibodies. Several methods are employed including, *a*) ammonium sulfate precipitation (21, 22); *b*) chromatography on various column media including DEAE Affi-Gel Blue (23), Protein-A Sepharose (24, 25), insolubilized apolipoproteins (or lipoproteins), sizing gels (26), hydroxyapatite (27); and *c*) sucrose density ultracentrifugation (28). The latter method and gel permeation chromatography are particularly useful for the purification of IgM monoclonal antibodies, because alternative precipitation techniques often result in irreversibly de-

naturing some IgM antibodies.

4) *Purification of antibody Fab fragments.* The smaller size of Fab fragments makes them potentially more useful as probes of epitope expression than the parent IgG molecules. Fab fragments would be expected to have fewer steric restrictions on lipoprotein binding (8, 29). The standard methods for preparation of Fab fragments (30, 31) may need to be slightly modified for each monoclonal antibody.

5) *Identification and enumeration of distinct epitopes on apolipoproteins using monoclonal antibodies.* When a series of monoclonal antibodies known to react with a given apolipoprotein are available, it becomes important to know against how many different epitopes the antibodies are directed. The tests used for identifying or enumerating epitopes include antibody competition or antibody cotitration.

a) *Antibody competition (8).* Purified radiolabeled and unlabeled antibodies are made to compete against each other for occupancy of the antigenic binding sites. The assumption is that if two antibodies bind to the same or closely spaced epitopes, competition will occur. But if binding is to distant epitopes, the unlabeled antibody will fail to reduce the binding of the labeled antibody. When antibodies are observed to compete for binding to an apolipoprotein or lipoprotein, several explanations are possible.

i) The antibodies may be recognizing identical or overlapping epitopes.

ii) Antibodies may be directed against non-overlapping epitopes, but mutual binding of the two antibodies may be prevented merely by their physical bulk (steric hindrance). To minimize this "artifact," monovalent Fab fragments can be used.

iii) Conformational changes induced by the binding of one antibody may alter the epitope recognized by another antibody.

Failure to reduce binding of one antibody by another usually is due to their binding to distinct epitopes, but it may be observed also in cases where the labeled and competitor antibodies bind to overlapping, non-identical epitopes but the labeled antibody binds the antigen with much higher avidity than does the competitor. One way to distinguish between these possibilities is to conduct reciprocal assays in which the radiolabeled and unlabeled antibodies are reversed.

b) *Antibody cotitration (32).* For this assay, the antigen is immobilized in plastic microtiter wells, and the maximum binding of two antibodies, directed against the same protein, is determined separately. Then, saturating amounts of both antibodies are added to the antigen together and the binding of both antibodies is quantified. The amount of monoclonal antibody binding is quantified with an antimouse immunoglobulin antibody labeled either with ^{125}I or an enzyme (ELISA). If the two antibodies are directed against distinct epitopes on the insolubilized antigen, the amount of the antibodies bound will be the sum of the amounts of the antibodies bound when the antibodies were added separately. If the two antibodies recognize closely linked epitopes, then the sum of antibodies bound will be lower than that bound when either of the antibodies was added separately. Partially overlapping epitopes yield sums that are intermediate. Interpretation of the results obtained with cotitration are subject to the same pitfalls as the antibody competition assays, however cotitration offers some technical advantages. Only a single antibody (the antimouse Ig) needs to be labeled, crude antibody preparations can be used, and usually, less antibody is required than for antibody competition assays.

D. Applications of monoclonal antibodies in antigenic analyses of apolipoproteins

The strategies described below can be applied with polyclonal antibodies as well, although this review will focus on the use of monoclonal antibodies.

1) *Assignment of epitopes to individual apolipoproteins.* When hololipoproteins containing several apolipoproteins are used as immunogens and screening of hybridomas also is performed with hololipoproteins, the detected antibody activity may be directed against any of several individual apolipoproteins. Therefore, it becomes important to identify the apolipoprotein chain specificities of the antibodies. Dot immunobinding using a "panel" of all the purified apolipoproteins against which the antibodies are likely to be directed provides a facile means of assigning chain specificities. Alternatively, the apolipoproteins are separated from each other by single dimension gel electrophoresis or isoelectric focusing or two-dimensional gel electrophoresis. The separated apolipoproteins in the gel are then transferred to nitrocellulose paper which is subsequently incubated with the antibodies to be tested. The binding of antibodies to apolipoproteins is then determined by use of an antimouse IgG antibody, labeled with ^{125}I or an enzyme (33–35). Alternatively, the antibody can be coupled to biotin and subsequently incubated with enzyme-labeled avidin (36).

2) *Assignment of epitopes to apolipoprotein domains.* The general approach in assigning epitopes to specific regions of an apolipoprotein is to fragment the protein, isolate the fragments, and assess their immunologic reactivities with the available panel of antibodies in a variety of assays. As the structures of epitopes frequently depend on protein conformation (37) as well as amino acid sequence, it may not be possible by this method unequivocally to assign every epitope. However, assignment should be easier for those epitopes that form parts of larger fragments where more of the native structure is likely to be retained. Detection of conformation-dependent epitopes may be aided if assays are carried out in the presence of lipids or detergents.

a) *Cyanogen bromide fragmentation of proteins.* Cyanogen bromide fragmentation of proteins, because it is easy to perform and reproducible, generally serves as a starting point. Fragments have been prepared of apoB (38), apoE (39), apoA-I (40), apoA-II (41), apoC-I (42), and apoC-II (43). Epitopes of apoA-I on COOH-terminal versus NH_2-terminal CNBr fragments have been distinguished using polyclonal antisera (44).

Certain apolipoprotein fragments, notably those of apoB (38) and apoA-I (44), exhibit a tendency to aggregate in solution. This may be overcome by the use of different buffer–detergent systems (44). Aggregation may result in decreased immunoreactivity and/or increased nonspecific interactions, depending on the nature of the immunoassay employed.

b) *Enzymatic cleavage of apolipoproteins.* Plasmin has been used to digest apoA-I (45) and apoB (46, 47). Digestion of apoA-I (48, 49), apoA-II (50), apoB (15, 51, 52), apoC-I (53), apoC-II (43), apoC-III (49, 54) and apoE (49, 55) has been accomplished with trypsin. ApoB of LDL has been digested with *Staphylococcus aureus* V8 protease (53, 56), chymotrypsin (56), and pronase (53). Thrombin also has been used to cleave apoB (47), apoC-III (57), and apoE (6, 58) into fragments with distinct physiologic and immunologic characteristics. Kallikrein digestion of LDL apoB-100 generates the same sized apoB-74 and apoB-26 fragments found in isolated native human LDL (47, 59).

The choice of a particular protease depends on amino acid sequence of the protein and the region of the protein under study. The conditions of incubation are determined for each protease and protein to be fragmented.

c) *Synthetic peptides.* The laboratory synthesis of apolipoprotein fragments is useful in identifying the lipid binding regions of several apolipoproteins and in assessing the effects of the addition or substitution of amino acid residues (60). Solid phase radioimmunoassays with synthetic peptidyl-residues have identified a major antigenic determinant of apoA-II (61).

d) *Chemical modification of amino acid residues.* Chemical modifications may alter the amino acids that form the epitopes themselves, or they may disrupt secondary or tertiary structure and thus affect epitopes indirectly. Therefore, any chemical modification-induced changes in immunoreactivity could be subject to more than one interpretation. However, chemical modification-induced changes in immunoreactivity would confirm the presence of an epitope on a protein or its fragment and, if none of a number of modifications produce changes in immunoreactivity, the involvement of the altered residues in the formation of an epitope can be ruled out.

It should be pointed out that not all chemical modifications are specific for a single amino acid. Appropriate conditions for protein chemical modification can be chosen to limit the degree and specificity of the modification (62). Also, as some unreacted parent molecule may exist, interpretation of the immunochemical data must rely on the ability to isolate and chemically characterize the modified protein molecule (62).

Particular attention has been paid by lipoprotein researchers to lysine and arginine residues on apolipoproteins B and E, because chemical modification of these amino acids leads to reduction of lipoprotein receptor interactions (63). Alteration of $<20\%$ of the ϵ-amino groups of lysine by acetylation (64), acetoacetylation (65), malondialdehyde treatment (66), or carbamylation (67) suffices to reduce receptor recognition (68). Chemical modification of the single arginyl residue of apoC-II with 1,2-cyclohexanedione or 2,3-butanedione diminishes the ability of apoC-II to activate lipoprotein lipase (69). Immunoreactivities of lipoproteins are altered following specific chemical modification of amino acid residues (10, 70, 71), enabling distinctions to be made between different monoclonal antibodies on the basis of their characteristic reactions with the modified lipoproteins.

The reversibility of certain chemical modifications such as cyclohexanedione treatment of arginyl residues (72, 73), citraconylation (48, 74) and acetoacetylation (75) of lysyl residues, and cysteamine charge modification of cysteine residues (39) allows one to assess whether changes in antibody reactivity are due to irreversible alterations (e.g., protein unfolding induced by manipulation) or due to an alteration of the epitope associated with the specific amino acid residue. Reversible modifications also are useful in epitope mapping because they can narrow the specificities of some proteases by blocking their access to certain cleavage sites, thereby reducing the numbers of protein fragments produced and increasing their sizes.

Protein fragments, whether obtained by cleavage or synthesis and containing all native or some chemically modified amino acid residues, can be used in immunoblotting experiments or in competitive or direct binding immunoassays to assign epitopes to fragments.

3) *Immunoassay methods.*

a) *Competitive binding assays.* When using radioiodinated proteins as tracers, the choice of iodination method is important so as not to destroy the epitope of interest (76). Displacement curves generated by the competitor species can be plotted as B/Bo (bound over total counts) versus log competitor dose. Where displacement curves are widely separated for individual competitor apolipoproteins or lipoproteins, this purely graphical analysis can provide much information as to relative potencies of epitope binding to any given antibody. Linear LOG-LOGIT transformation (77) simplifies interpretation of the data and allows estimations of the relative binding affinities of various competitors by slope comparisons (78). Curve fitting by computer-aided four-parameter logistic modelling (79, 80) generates theoretical binding curves by best fit analysis on families of competition curves which can be compared statistically on the basis of slope (affinity) or potency.

b) *Antibody affinity chromatography.* The coupling of monoclonal antibodies of known specificity to chromatographic matrices permits the isolation of subpopulations of molecules that express specific epitopes. As a result, complex mixtures of lipoproteins can be separated into subpopulations on the basis of subtle epitope determinants. Optimal conditions must be found for binding and elution of the antigen for each immunoadsorbent column. The capacity of the immunoadsorbent for various sized particles must be ascertained. This is particularly important for holo-lipoproteins of various sizes, because binding of particles to immobilized antibody may differ by size rather than by the number of epitopes expressed per particle (81). To assess the capacities of columns, it is necessary to load increasing amounts of antigen until the amount retained on the column is constant. If labeled and unlabeled antigens behave similarly on a column, determination of columnar capacity is greatly simplified. Trace amounts of radiolabeled antigen applied to an immunoadsorbent should be bound to the same degree in the absence or presence of "cold" antigen, provided the column capacity has not been exceeded. Purification of antigens from crude mixtures also is aided by labeled antigens (82).

The use of monoclonal antibodies to isolate subpopulations of lipoproteins assumes that binding has occurred via the epitope in question. Any nonspecific binding produces artifacts. To circumvent this problem, one can remove substances likely to be nonspecifically bound to column matrices by passing the antigen-containing mixture over a column containing no antibodies (22, 82, 83) or a column containing nonspecific mouse IgG (22, 84) before it is passed over the monoclonal affinity column.

IV. EXPRESSION OF EPITOPES ON HOLOLIPOPROTEINS

Panels of monoclonal antibodies directed against identified regions of apolipoproteins can be used to assess the uniformity of epitope expression in a given population of lipoproteins, the number of epitopes expressed per particle, and the expression of the epitopes of a given apolipoprotein on different populations of related particles (e.g., epitopes of apoB or apoE on VLDL, IDL, LDL, and epitopes of apoA-I or apoA-II on subclasses of HDL). With the antibodies it is also possible to determine the effects of genetic, physiologic, and pathologic perturbations on epitope expression and to relate epitope expression to metabolic functions and interactions of lipoproteins.

Information on the degree of homogeneity of epitope expression (11, 70, 78, 85, 86) in a given lipoprotein population can be obtained in direct binding or competition assays. Such assays also are useful in comparing epitope expression of a given apolipoprotein on related lipoproteins.

Calculation of binding of antibodies or their Fab fragments to lipoproteins by conventional Scatchard or other similar analyses can provide information on the uniformity of binding and the number of times per particle an epitope is expressed (29). By affinity chromatography of lipoproteins on antibody-containing columns or by immunoprecipitation, it is possible to identify and isolate subpopulations of particles on which given epitopes are expressed.

A. Immunochemistry of apoA-I, apoA-II and apoA-IV

1) *ApoA-I.* ApoA-I (**Table 1**) is the major structural component of HDL and its main physiologic function is the activation of the enzyme LCAT. It is the most abundant apolipoprotein in human plasma. (For a review of the functions of apoA-I, apoA-II, and apoA-IV, see ref. 87.) Most apoA-I molecules are not detected in immunoassays meant to quantify the apoA-I contents of holoHDL, but disruption of HDL structure renders all apoA-I detectable (88–93). Recently, Curtiss and Edgington (94), using monoclonal antiapoA-I antibodies, reported that none of their antibodies was able to bind to more than 60% of either HDL or apoA-I. These data suggest that there is heterogeneity of expression of apoA-I epitopes in HDL. The immunochemical data are compatible with physicochemical evidence of HDL heterogeneity (95–97).

However, all apoA-I in plasma can be detected in some polyclonal antibody-based immunoassays (98–102). Some assays employ antisera from which subpopulations of antibodies were isolated on affinity columns containing HDL or apoA-I coupled to solid phase matrices (98, 102, 103). These isolations tend to select antibodies to exposed "surface determinants" of HDL apoA-I. Assays using the resulting antibodies were able to detect apoA-I accurately in plasma, holoHDL, and in preparations of isolated apoA-I, suggesting that some epitopes of apoA-I may be expressed on all HDL particles.

The reduced immunoreactivity of some epitopes of apoA-I in holoHDL may be a result of apoA-I interactions with lipid and other apoproteins or both. Schonfeld, Pfleger, and Roy (104) demonstrated progressive reduction of apoA-I immunoreactivity in recombinant lipoprotein particles as the recombinants became more and more HDL-like in lipid and apoprotein composition. The two terminal regions of the apoA-I molecule are immunogenically distinct (44), and the development of specific radioimmunoassays for the COOH-terminal and the NH_2-terminal cyanogen bromide fragments of apoA-I has made it possible to quantify the "exposure" of these two regions in HDL. The COOH-terminal region of apoA-I appears to be more "exposed" than the NH_2-terminal region. This finding was substantiated by demonstrating that anti-holoHDL antisera contained more antiCOOH-terminal than antiNH_2-terminal antibodies, i.e., the COOH-termi-

TABLE 1. Monoclonal antibodies directed towards apolipoprotein A-I

Immunogen	Techniques	Main Observations	Reference
ApoA-I	Competitive radioimmunoassay	Five stable clones directed against at least three antigenic determinants on apoA-I; differing reactivities with CNBr fragments of apoA-I indicating specificities vs. the mid-region of the molecule; apoA-I epitope expression on HDL heterogeneous.	9
VLDL	Western blotting	Antibody not characterized in this study.	15
Soluble apoVLDL, HDL	Competitive radioimmunoassay; Western blotting	Three clones specific for three separate epitopes on apoA-I; differences in the expression of the three epitopes in HDL subpopulations separated by density gradient ultracentrifugation or chromatofocusing.	94
ApoA-I	Competitive radioimmunoassay	Four clones specific for three separate antigenic determinants on apoA-I; reactivities against CNBr fragments of apoA-I indicate epitope localization between amino acid residues 1–148.	106

nal region of apoA-I in holoHDL was more immunogenic than the NH_2-terminal region (105). Monoclonal antibodies to apoA-I have been generated using apoA-I (9, 106) or HDL (9, 94) as immunogens. These studies indicate that there are at least three distinct antigenic determinants on apoA-I. Of the monoclonal antibodies reported thus far, most are directed against the midportion of the molecule.

Recently, a novel prebeta-migrating apoA-I-containing lipoprotein was isolated from plasma by immunoaffinity chromatography (107). The molecular weight of the particle is ~80,000. It is composed of ~90% protein and ~10% phospholipid, and very small amounts of free and esterified cholesterol. These low molecular weight lipoproteins may be the same as the ~50,000 molecular weight apoA-I-containing species observed earlier by Schonfeld, Bailey, and Steelman (108) on molecular sieve chromatography of plasma.

2) *ApoA-II.* HDL apolipoproteins other than apoA-I are more "exposed" on the surfaces of the particles than is apoA-I. For example, apoA-II, C-II, and C-III on HDL lose virtually all of their immunoreactivities after pronase treatment, whereas 80% of apoA-I immunoreactivity is retained (109). HDL-associated apoA-II is also more reactive in immunoassays than is apoA-I, and delipidation does not significantly enhance "exposure" of apoA-II on HDL (50, 110–116). The antiapoA-II antisera generated by Mao, Gotto, and Jackson (50) were found to be directed towards several regions of apoA-II. A major antigenic determinant of apoA-II was assigned between amino acid residues 60–77, and two other less reactive antigenic determinants were localized between amino acid residues 4–23 and 31–46 (117).

The combined use of antiapoA-I or antiapoA-II antisera has provided important insights into the nature of HDL subpopulations. Virtually all apoA-II in plasma is associated with HDL particles that also contain apoA-I, whereas apoA-I-containing lipoproteins without apoA-II can be found (118). Both of these major HDL populations are heterogeneous with respect to size and hydrated density (118). Since apoA-II can form molecular associations with apoA-I, whereas apoC-II and C-III cannot (119), it is possible that molecular interactions of apoA-I with apoA-II in HDL may "mask" the expression of some apoA-I epitopes on the surfaces (104) of some HDL particles. This may account for part of the immunochemical heterogeneity of HDL.

3) *ApoA-IV.* ApoA-IV synthesized primarily in intestine, occurs in several isoforms. Ohta, Fidge, and Nestel (120) using immunoaffinity chromatography and immunoblotting techniques detected that the apoA-IV isoforms were unequally distributed between the various lipoproteins and the d > 1.21 g/ml fractions of plasma. These results suggest that the different forms of apoA-IV may have different physiological roles (120). Monospecific antisera to human apo-IV and a sensitive radioimmunoassay are available (121, 122). ApoA-IV is found primarily in the d > 1.21 g/ml fraction of centrifuged plasma, but is distributed throughout all HDL fractions in noncentrifuged plasma. A particle 8 nm in diameter also has been identified (122). This particle is the only apoA-IV-containing lipoprotein present in abetalipoproteinemic plasma.

B. Immunochemistry of apolipoprotein B (Table 2)

1) *Forms of apoB.* Two secretory forms of apoB are present in mammals (123). The apoB synthesized in human liver is referred to as apoB-100 (approximate molecular weight ~550,000), the apoB of intestinal origin is apoB-48 (approximate molecular weight ~260,000) (124–126). (For reviews of apoB structure and metabolism see references 87 and 123.) Thus, intestinal lymph chylomicrons contain almost exclusively apoB-48, and VLDL from normolipidemic individuals contains apoB-100 (123). In normal subjects, much of the VLDL is converted to LDL before being

taken up by cells, while chylomicron remnants are removed by the liver before being converted to smaller particles. Therefore, LDL contains virtually only apoB-100 and in some cases also apoB-74 and apoB-26, which by amino acid composition appear to be complementary cleavage fragments of apoB-100 (123), probably generated by proteolysis of LDL apoB-100 by tissue and/or plasma kallikreins (5). The d < 1.006 g/ml lipoproteins, or VLDL, of hyperlipidemic individuals frequently contain both intestinal and hepatic-derived triglyceride-rich lipoproteins, i.e., the VLDL are comprised of subpopulations of particles, some of which contain apoB-100 and other apoB-48 (127, 128). ApoB-100- and apoB-48-containing VLDL particles have been separately isolated from plasmas of hyperlipoproteinemic and normal subjects by immunoaffinity chromatography using monoclonal anti-LDL antibodies (84, 128).

Many laboratories have produced antibodies against human apoB. Immunization with intact "narrow density cut" human LDL (1.030–1.050 g/ml) which contain >98% of protein as apoB-100, yields high affinity monospecific antisera (129–137) and monoclonal antibodies. Several libraries of antiLDL monoclonal antibodies produced in mice are available (8, 10, 11, 15, 16).

2) *Enumeration and localization of apoB epitopes.* Between five and eight distinct epitopes have been identified on LDL (8, 10, 11, 15). Some epitopes are near the cellular LDL receptor recognition site of LDL (8, 10, 83). Some appear to manifest genetic heterogeneity (85, 138–140), some are partially "masked" by lipids (78) or dependent on lipids for structural integrity (141), while others are not (86). With respect to localization of epitopes, polyclonal antisera raised either against apoB-74 or apoB-26 react with apoB-100, but antiapoB-74 does not bind to apoB-26 and vice versa (123). Compatible findings have been reported with some sets of monoclonal antibodies (142). However Curtiss and Edgington (15) and Maynard et al. (143) each have reported on monoclonal antibodies that bind to both apoB-74 and apoB-26. Thus, some epitopes are unique to apoB-74 or apoB-26 while others are shared by both fragments, suggesting that some epitopes may be present more than once on apoB-100 (15). Studies with monoclonal antibodies also indicate that hepatic apoB-100 and intestinal apoB-48 share some epitopes (78, 142). The sharing of epitopes between the various forms of apoB is the strongest evidence available to date for their structural relatedness. The lack, to date, of a MAb specific for apoB-48 is intriguing. Perhaps epitopes unique to apoB-48 are masked in the "native" lipoprotein-associated state. This would be consistent with a lack of receptor recognition of apoB-48 by cells (discussed later).

Although carbohydrate comprises about 8–10% of apoB-100 mass (144) and has been shown to participate in the antigenic sites of LDL (145), no direct involvement of carbohydrate in any epitope recognized by a monoclonal antibody has yet been shown (16, 146).

3) *ApoB epitope expression.* A role of lipids in maintaining the structural integrity of apoB was demonstrated first by Scanu, Pollard, and Reader (71) who observed that the conformation of apoB is grossly altered by delipidation. The lipids of LDL must "anchor" apoB, as considerable immunoreactivity (51, 52, 147) and cellular reactivity (51, 147) remain after proteolysis of up to 20% of LDL apoB protein. Theolis et al. (146) noted that all seven of their antiLDL monoclonal antibodies reacted with a single tryptic fragment of apoB of approximately 125 KDa, indicating that perhaps 25% or less of the apoB protein molecule is immunogenic. The rest may be hidden or "masked" by lipid in holoLDL.

Even larger portions of the apoB molecular may be "masked" by lipid in the more triglyceride-rich VLDL particles. Some laboratories claim that their assays accurately detect apoB both in LDL and VLDL (129–133); others find VLDL apoB to have reduced immunoreactivity compared with LDL apoB (134–137). These apparently disparate observations could reflect differing specificities of polyclonal antisera as demonstrated by Schonfeld et al. (148). They reported that lipolysis of VLDL particles enhanced their immunoreactivity with some antisera but not with others, suggesting that their antisera were directed against at least two different sets of epitopes. One set of epitopes was not affected by lipolysis of the VLDL particles, whereas the other set probably became more "exposed" on the lipid-depleted VLDL particles (148).

Monoclonal antibodies, depending on their specificities, can be very sensitive to subtle conformational alterations of apoB. One group (16, 141) noted higher affinities of their monoclonal antibodies for LDL at 4°C versus 37°C, probably resulting from the changeable conformation of apoB at different temperatures. Organic extraction of LDL lipids or lipolysis of VLDL resulted in a substantial loss of apoB immunoreactivity (141, 149). Loss of immunoreactivity of LDL apoB by ionic detergent treatment was also demonstrated for certain monoclonal antibodies by Watt and Watt (11) and Curtiss and Edgington (15).

Marcel et al. (7) defined three types of antigenic determinants using their panel of monoclonal antibodies. One antibody reacted with lipid and detergent-free solubilized LDL apoB; a second antibody reacted with soluble apoB only when it was combined with SDS micelles or cholesterol/lecithin liposomes; and a third set of antibodies recognized apoB determinants only when the apoB was reconstituted with a microemulsion containing a hydrophobic core of cholesteryl esters (7). It should be noted that the third set of antibodies recognized epitopes close to the LDL receptor recognition site (7). Tikkanen et al. (78) reported that most of their antiLDL monoclonal antibodies (including two that blocked the uptake of LDL by cells) recognized apoB epitopes on lipoprotein particles in the order LDL > $VLDL_3$(S_f 20–60) > $VLDL_2$ (S_f

60–120) > $VLDL_1$(S_f > 120). Others too, noted that VLDL apoB is less immunoreactive than LDL apoB (86, 150). The increased immunoreactivity of apoB associated with particles of decreasing size was also demonstrated using monoclonal antibodies when "VLDL remnants" were produced by in vitro lipolysis of $VLDL_1$ (78).

4) *Stoichiometric binding studies.* Conventional physical and chemical methods to assess molecular weight and subunit structure of apoB have yielded conflicting results (123). Two research groups using monoclonal antibodies in stoichiometric binding studies to address this question have reached compatible conclusions, namely that one IgG molecule or Fab fragment is bound per LDL particle (29, 151). This favors the concept that LDL apoB is composed of a single polypeptide chain of nonrepetitive subunits. Molecular weight estimates of apoB in these studies were 600,000 (29) and 520,000 (151). However, these studies cannot entirely rule out the presence of repetitive subunit structures because the binding of one antibody (or Fab) per particle could have hindered the binding of additional antibodies or certain repetitive epitopes on LDL apoB could be "masked."

5) *Heterogeneity of apoB epitopes.* Alloantisera isolated from multitransfused humans have been used to identify LDL allotypes (152, 153). These alloantibodies were generated against a polymorphic variant of LDL apoB, suggesting that this protein is encoded by allelic genes. These allelic forms offer sufficient variability as to elicit an immune response in an individual of the same species lacking that (allo)antigenic determinant. Schumaker et al. (138), using monoclonal antibodies and a data analysis method referred to as a "binding ratio profile," demonstrated apoB polymorphism at one to three very closely related epitopes on LDL isolated from 32 human subjects. Pedigree analysis favored genetic causes for the polymorphism. Heterogeneity in the immunoreactivities of LDL preparations isolated from different individuals was also noted in studies using polyclonal antisera (129) and other sets of monoclonal antibodies (84–86, 140). Other workers relate heterogeneity of epitope expression to the presence of LDL subfractions of different sizes and/or densities. Teng et al. (139) noted that certain antiapoB monoclonal antibodies reacted equally with three LDL subfractions, whereas another group of monoclonal antibodies reacted progressively less well with LDL of increasing density or decreasing cholesterol content. One explanation for the epitope heterogeneity detected in these studies is that lipids modulate apoB conformation (85); an alternative interpretation is that apoB allotypes are preferentially expressed on the various sized LDL (139). The latter is compatible with the observation that certain apoB allotypes are differentially distributed on lipoproteins in rabbits (154). Thus, there may be heterogeneity based on genetic variations of apoB primary structures and also based on nongenetic determinants of the structures of lipoproteins. It is also worth noting that some epitopes on apoB are constantly and equally expressed on apoB-containing particles (85, 150, 155). A recent intriguing observation is that LDL isolated from patients with coronary heart disease may be immunologically different from "normal" LDL (140).

6) *ApoB epitopes involved in cell recognition.* Some monoclonal antibodies inhibit the LDL (apoB,E) receptor-mediated cellular endocytosis of LDL (8, 10, 83). These monoclonal antibodies bind to apoB-100 and apoB-74 (78, 142). An antiapoB-48 monoclonal antibody did not block binding of either chylomicron remnants or β-VLDL to the B,E receptor or apoE receptor, suggesting that apoB-100 and apoB-74, but not apoB-48, contain the domain responsible for the cellular recognition of lipoproteins (127). Somewhat unexpected are the findings that antiapoB monoclonal antibodies that inhibit LDL uptake by fibroblasts do not necessarily inhibit apoE-mediated binding of VLDL particles to fibroblasts and, conversely, a monoclonal antibody directed against the receptor-binding domain of apoE does not cross-react with apoB (156). These findings are somewhat unexpected because apoB-100 and apoE share the ability to interact with the B,E receptor on cells (83).

7) *Conservation of apoB epitopes.* Several epitopes on LDL apoB are shared among animal species (132, 157). The epitopes on LDL located near the recognition site of apoB for cellular apoB,E receptors are most highly conserved (155).

8) *Antibodies to chemically modified LDL.* Steinbrecher et al. (158) have produced antisera against several modified preparations of guinea pig LDL in guinea pigs. The antisera were directed almost exclusively against the derivatized lysine residues. Monoclonal antibodies have also been generated in mice against glucosylated homologous LDL (159) (**Table 3**). Immunoassays, using these antibodies are effective in detecting glucosylated amino acids of other apoproteins as well as several other glucosylated plasma proteins in diabetics (160). The ability of LDL to elicit immune responses by such subtle alterations of its structure raises the possibility that the autoantibodies directed against LDL in diseased or elderly subjects may be directed against modified LDL particles; the nature of the modification, if any, of course is unknown (158).

C. Immunochemistry of apoE (Table 4)

Although the relative concentration of apoE plasma is low compared with apolipoproteins A-I or B, apoE plays several major roles in lipoprotein metabolism (87). First, it serves as a recognition marker for several cellular lipoprotein receptors, including hepatocyte receptors for chylomicron and VLDL remnants (156, 161–163), receptors for LDL on hepatic and extrahepatic cells (164), and receptors for VLDL on macrophages (165). Second, apoE

TABLE 2. Monoclonal antibodies directed towards LDL (apoB)

Immunogen	Techniques	Main Observations	Reference
As in ref. 10	ApoB-lipid recombinants; competitive radioimmunoassay	Three types of antigenic determinants defined on apoB, a) lipid independent; b) dependent on surface, polar lipid; c) dependent on core lipid.	7
LDL (d 1.025–1.050 g/ml)	Competitive radioimmunoassay; lipoprotein-cell culture binding experiments	Seven stable clones defining at least five antigenic determinants on apoB; inhibition of LDL binding to fibroblasts by antibodies 464B1B3, 464B1B6.	8
LDL (d 1.030–1.050 g/ml)	Competitive radioimmunoassay; lipoprotein-cell culture binding experiments	Seven stable clones define at least six antigenic determinants on apoB; inhibition of LDL degradation pathway in fibroblasts by antibodies 3F5, 4G3, 3A8, 3A10, 5E11.	10
LDL (d 1.020–1.050 g/ml)	Competition ELISA;radio-binding assays	Six characterized clones indicating at least two groups of antigenic determinants on apoB; a) SDS-labile; b) non-SDS-labile.	11
VLDL (d < 1.006 g/ml) IDL (d 1.006–1.019 g/ml)	Competitive radioimmunoassay; Western blotting	Eleven antiapoB clones directed against two groups of determinants on apoB, a) complex epitopes dependent on lipoprotein association; b) epitopes expressed on denatured apoB; assignment of epitopes to major apoB fragments.	15
LDL (d 1.019–1.063 g/ml)	Competitive radioimmunoassay	Temperature-dependent interaction of LDL with monoclonal antibodies (decreased binding at 37°C compared to 4°C); no apparent recognition of carbohydrate moieties in LDL.	16
As in ref. 10	Stoichiometric binding assays	One Fab fragment bound to single LDL particle favoring concept of a single, large apoB molecule per LDL.	29
As in ref. 8	Enzymatic digestion of LDL-apoB; competitive radioimmunoassay; lipoprotein-cell culture binding experiments; Western blotting	Expression of apoB epitopes differentially affected by proteolytic enzymes; some soluble peptides manifested cell reactivity and antigenicity which is compatible with idea of repeating structures on apoB.	51
As in ref. 8	Competitive radioimmunoassay; Western blotting	Immunoreactivity of apoB in VLDL subfractions decreased with increasing flotation rate; specificity of antibodies towards apoB fragments.	78
As in ref. 8	Competitive radioimmunoassay	Variation of LDL-apoB epitope expression observed on LDL from a variety of subjects; appeared to be related to percentage composition of lipids although heterogeneity in apoB primary structure cannot be ruled out.	85
As in ref. 15	Competitive radioimmunoassay	Three patterns of apoB epitope expression on native lipoproteins, a) expression of apoB epitopes on VLDL = IDL = LDL; b) VLDL = IDL but differing affinities for epitopes on LDL; c) IDL = LDL but differing affinities for epitopes on VLDL.	86
As in ref. 8	Lipoprotein-cell culture binding experiments	Inhibition of hypertriglyceridemic VLDL binding to fibroblasts by antiapoB antibody progresses in the order LDL > $VLDL_3$ > $VLDL_2$ > $VLDL_1$.	83
As in ref. 10	Affinity chromatography; competitive radioimmunoassay	Separation of type III VLDL into apoB-48 VLDL and apoB-100 VLDL; the two apoB species exist on different particles.	84
As in refs. 10 and 156	Lipoprotein-cell culture binding experiments; affinity chromatography	No inhibition of binding of chylomicron remnants or β-VLDL to cellular receptors by antiapoB-48; minor inhibition of binding of apoB-100 containing β-VLDL by antiapoB-100.	127

may play a role in lipoprotein lipase-mediated lipolysis of lipoproteins (166, 167); and third, it may facilitate the transport of sterols under certain conditions (168). Virtually all of apoE plasma is associated with lipoproteins. Approximately 50% is associated with VLDL and 50% with HDL-sized particles (169).

TABLE 2. (continued)

Immunogen	Techniques	Main Observations	Reference
As in ref. 10	Affinity chromatography	Separation of apoB-48 VLDL and apoB-100 VLDL from normal, type III, or type IV VLDL; composition of apoB-48 VLDL is most variable and CE/TG ratio in this fraction appears to be characteristic of the type of hyperlipidemia.	128
As in ref. 15	Direct binding assays	Three different phenotypes distinguishable by monoclonal antibodies; data fit a model consisting of two co-dominant apoB alleles to account for the observed genetic polymorphism.	138
As in ref. 10	Competitive radioimmunoassay	Two groups of antibodies defined, a) LDL subclasses equally immunoreactive; b) the immunoreactivity of apoB decreases as LDL particle size decreases; differential reactivity could be due to modulation of epitopes by lipoprotein structure or due to apoB genetic polymorphism.	139
As in ref. 16	Competitive radioimmunoassay	Heterogeneous immunoreactivity of LDL from different subjects; increased apparent apoB contents in patients with angiographically documented coronary artery disease.	140
As in ref. 16	Direct radio-binding; competitive radioimmunoassay, ELISA	Removal of lipids from LDL by organic extraction resulted in 50% loss of immunoreactivity; removal of triglycerides from VLDL by lipoprotein lipase also resulted in substantial loss of immunoreactivity suggesting role of lipids in maintaining apoB antigenic structure.	141
As in ref. 10	Competitive radioimmunoassay; Western blotting	Antigenic map of apoB created indicating that apoB-100 and apoB-48 share epitopes; specificities of antibodies for apoB-74 or apoB-26 suggest these are complementary fragments of apoB-100.	142
As in ref. 8	Competitive radioimmunoassay	Effectiveness of monoclonal antibodies in quantitative radioimmunoassay.	143
As in ref. 10	Enzymatic digestion of LDL-apoB; Western blotting	Peptide of 125 KDa was smallest fragment of apoB recognized by all antibodies; proximity of carbohydrate groups to certain epitopes was indicated.	146
LDL (d 1.030–1.050 g/ml)	Immunoradiometric assay	Effectiveness of monoclonal antibodies in quantitative immunoassay for LDL-apoB.	150
As in ref. 8	Stoichiometric binding assays	One antibody molecular or Fab fragment bound per LDL particle; epitope at or very near cell receptor recognition domains of apoB and uniformly expressed, once per particle.	151
As in ref. 8	Competitive radioimmunoassay	Rank order of binding of LDL from several animal species to specific antibodies correlated with the antibodies ability to interfere with LDL uptake by cells; suggests that epitopes near the cell recognition domain on apoB have a greater tendency to be evolutionarily conserved.	155
LDL (d 1.020–1.050 g/ml)	Lipoprotein-cell culture binding experiments	AntiapoB-100 effectively inhibited LDL binding to fibroblast or liver cell receptors but was less effective in inhibiting β-VLDL or HTG-VLDL binding to these cells.	156

There are several major isoforms of human apoE referred to as apo-E1, apoE2,...apoE7 (170). Differences between isoforms are either due to differing degrees of sialylation or to amino acid substitutions (170). The sialylation of apoE occurs intracellularly before secretion (171, 172), and apoE is progressively desialylated in plasma so that approximately 80% of plasma apoE exists in the desialylated form (87). Amino acid substitutions are due to point mutations. Isoform heterogeneity resulting from genetic or post-translational modification can be distinguished from each other. ApoE3 is the most frequently found isoform in free-living, unselected populations and is consid-

TABLE 3. Monoclonal antibodies generated by immunization with glucosylated LDL

Immunogen	Techniques	Main Observations	Reference
Reduced glucosylated murine LDL	Competitive radioimmunoassay	Six clones generated, each of which is capable of identifying glucitollysine epitopes on a variety of reduced glucosylated proteins including isolated lipoproteins from normal and diabetic human individuals.	159
As in ref. 159	Affinity chromatography; Western blotting	Glucitollysine-specific antibodies can be used to quantitate the extent of glucosylation of plasma proteins; apos A-I, A-II, B, C-I, and E are glucosylated in hyperglycemic diabetic subjects.	160

ered to be the "wild type." The apoE2 phenotype may be due to several different amino acid substitutions which produce dysfunctional molecules, with the severity of the dysfunction depending on the site of the amino acid substitution (173, 174). The dysfunction consists of reduced binding of apoE-containing lipoproteins to cellular receptors that recognize apoE.

Quantitative immunoassays for human apoE using polyclonal antiserum yield widely differing apoE concentrations in plasma probably because different standards and antisera are used (175). Most assays require the use of denaturants or detergents to "expose" all apoE epitopes in human plasma and in isolated lipoproteins (175). This may be because most of the antisera are produced by immunization with isolated apoE, and isolated apoE tends to self-aggregate (175, 176) and also to form hetero- and homodimers via disulfide bridges (177). Therefore, it is highly likely that the antisera contain populations of antibodies directed towards "artifactual" apoE epitopes that are likely to be "masked" in lipid-rich lipoproteins. This necessitates the use of detergents or denaturants for full immunologic detection of apoE in plasma or intact lipoprotein samples.

Two antiapoE monoclonal antibodies reported to date appear to be directed against regions near the cellular receptor recognition domain(s) on apoE (80, 83, 178). Other monoclonal antibodies define several other epitopes, however, these have not been assigned to specific regions of the molecule. As yet, no MAb has been reported that can distinguish between the different apoE isoforms. Clinically, this would be of much significance, as the individual apoE isoforms may be differentially implicated in the development of atherosclerosis (179).

AntiapoE and antiapoB monoclonal antibodies have been used together to distinguish between the relative roles of apoB and apoE in mediating the uptake of tri-

TABLE 4. Monoclonal antibodies directed towards apoE

Immunogen	Techniques	Main Observations	Reference
ApoE	Lipoprotein-cell culture binding experiments; lipid-apoE recombinants	Antibody 1D7 blocked apoE · DMPC and apoE$_2$ (T-22K)-CysNH$_2$ · DMPC binding to fibroblasts (see ref. 53 for details).	58
As in ref. 58	Competitive radioimmunoassay	Five stable clones with different intramolecular specificities.	70
ApoE, HDL, apoHDL	Competitive radioimmunoassay; Western blotting; affinity chromatography	Five stable clones identify at least three antigenic determinants on apoE; different patterns of expression of these epitopes on native lipoproteins.	80
As in ref. 80	Lipoprotein-cell culture binding experiments	Inhibition of hypertriglyceridemic VLDL binding to fibroblasts by antiapoE antibodies progresses in the order VLDL$_1$ > VLDL$_2$, VLDL$_3$.	83
As in ref. 58	Lipoprotein-cell culture binding experiments	Almost total inhibition of binding of chylomicron remnants and β-VLDL to cellular receptors by antiapoE antibody 1D7.	127
As in ref. 58	Lipoprotein-cell culture binding experiments	AntiapoE antibody 1D7 capable of inhibiting binding of HDL$_c$, β-VLDL, and HTG-VLDL to fibroblasts or liver cell receptors.	156
As in ref. 58	Lipoprotein-cell culture binding experiments; apoE-lipid recombinants, competitive radioimmunoassay	Only one of five clones (1D7) able to inhibit apoE · DMPC binding to cells; use of synthetic peptide fragments localized epitope to amino acid residues 139–169 of apoE.	178

TABLE 5. Monoclonal antibodies directed towards apoC-I

Immunogen	Techniques	Main Observations	Reference
VLDL	Western blotting	Antibody not characterized in this study.	15
"ApoB" (see ref. 191 for details)	ELISA; Western blotting	Three clones identify at least two antigenic determinants on apoC-I; lipolysis resulted in 30% increase in apoC-I epitope expression with one clone, whereas non-ionic detergents inhibit the antibody–antigen reaction.	191
VLDL	Competitive radioimmunoassay	Ten clones identify at least three distinct antigenic determinants on the CNBr NH_2-terminal residue of apoC-I; delipidated apoC-I showed very little immunoreactivity compared to apoC-I complexed with Intralipid or lipoproteins.	192

glyceride-rich lipoproteins by cultured cells (83, 127, 156). ApoE appears to mediate the recognition of the larger sized hypertriglyceridemic VLDL (83). ApoB becomes more important as the particles become smaller in size and virtually all LDL are recognized via apoB (83). Anti-apoE monoclonal antibodies are also useful for studying the expression of apoE epitopes on the surfaces of major classes of lipoproteins. An epitope related to cellular recognition site of apoE appears to be expressed equally well on all lipoproteins, since monoclonal antibodies 1D7 from Milne's group (70) and A1.4 from Schonfeld's group (80) recognize an epitope equally on all lipoproteins and also block apoE-mediated uptake of lipoproteins (83, 127, 156). Other apoE epitopes are more expressed on VLDL than HDL and still others are most expressed on HDL (80). Thus, there are significant differences in the disposition of certain apoE epitopes on various lipoproteins. The immunochemical data are in accord with observations that lipoprotein lipids do modulate the conformation of apoE (as detected by circular dichroism (180) and optical rotatory dispersion (181)). The heterogeneity of apoE conformation or disposition on the surfaces of lipoproteins is also confirmed by the varying accessibility to thrombin cleavage of apoE on differently sized VLDL particles (147). It is clear that some functions of lipoproteins (e.g., their interactions with cells) can be adversely affected by some alterations in apoE disposition that are produced by amino acid substitutions at positions 158 and 146 (182).

D. Immunochemistry of the C apolipoproteins

The apolipoproteins C are designated as apoC-I, apoC-II, and apoC-III. Although these apolipoproteins tend to redistribute on the surface of lipoproteins as a group, their metabolic functions are diverse (87). All three proteins have been shown to stimulate LCAT (183, 184), however, this function appears to be most specific for apoC-I (183). ApoC-II is an activator of lipoprotein lipase (185) whereas apoC-III may exhibit the activation (186). ApoC-III also appears to modulate the uptake of triglyceride-rich remnants by liver cells (161).

Due to their low molecular weights (6,600–8,800), the development of high-titer antisera for the apoC proteins poses more of a problem than is the case with larger apolipoproteins (187). Curry et al. (188) coupled human apoC-I to albumin to increase its immunogenicity in recipient animals and was successful in obtaining antisera. Raising monospecific antisera to apoC-II is complicated, in addition, by the relative difficulty of obtaining "pure" apoC-II (189, 190).

Both of the above problems are minimized by use of monoclonal antibodies (**Table 5**). Antirat apoC-I monoclonal antibodies recently were elicited by immunization with a Sephacryl S-300 chromatographic peak corresponding to apoB (191). Antihuman apoC-I monoclonal antibodies were obtained by immunizing mice with human holoVLDL (192). Both groups of antibodies are directed against conformationally dependent epitopes. Lipolysis increased and detergent treatment decreased the binding of the monoclonal antibody to rat apoC-I (191), whereas the antihuman apoC-I antibodies bound human apoC-I only when it was complexed to lipid (unpublished results). It is expected that further studies using these antibodies will contribute to our understanding of apoC-I structure and function.

Monoclonal antibodies directed against apoC-II or apoC-III have not been reported in the literature to date. However, studies with polyclonal antisera have demonstrated that apoC-III antigenic sites are fully exposed on the surfaces of HDL or VLDL (193). By contrast, not all antigenic determinants of apoC-II are exposed on native plasma lipoproteins (194). The need for inclusion of detergents in assays in order to obtain accurate quantitation of apoC in hyperlipidemic plasmas suggests that apoCs may become increasingly "masked" in lipid-rich particles (188, 195).

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