Eleven hybridoma antibodies specific for apoprotein B were used to examine the expression of apoprotein B epitopes on native plasma lipoproteins using fluid phase radioimmunoassays. Heterogeneity of apoprotein B in low density lipoprotein (LDL) of density 1.019-1.063 g/ml was indicated by maximum binding of radiiodinated LDL in the presence of excess antibody that ranged from 45 to 100%. Affinity constants for LDL ranged from 0.29-3.0 x 10^8 M^-1. Each of the 11 antibodies recognized apoprotein B epitopes that were expressed by very low, intermediate, and low density lipoproteins, VLDL, IDL, and LDL, respectively, since at high concentrations, each could fully displace the binding of radiiodinated LDL. In contrast, these same apoprotein B epitopes were not present on high density lipoproteins. Logit transformation analysis of competitive inhibition titrations demonstrated at least three patterns of epitope expression. The first pattern, identified by five of the antibodies, was characterized by indistinguishable expression of each of the apoprotein B epitopes on VLDL, IDL, and LDL. The second pattern, identified by three antibodies, was characterized by identical expression of the apoprotein B epitopes on VLDL and IDL, but of differing affinities than the related epitope on LDL. Three antibodies identified a third pattern of epitope expression which was characterized by identical expression on IDL and LDL, but with differing affinities for the antibodies than the same epitopes on VLDL. These observations suggest that the organization of apoprotein B in VLDL, IDL, and LDL is similar, but not identical.

Apoprotein B of rat lymph chylomicrons and VLDL differs immunologically and has a different mobility on SDS-polyacrylamide gels than apoprotein B of rat plasma LDL, suggesting that apoprotein B of intestinal origin is different from that of hepatic origin (2-4). A patient with normotriglyceridemic abetalipoproteinemia was reported to be selectively deficient in the hepatic form of apoprotein B (5). More recently, physicochemical characterization of LDL from normal humans has identified the existence of multiple distinct subpopulations of LDL that can be discriminated on the basis of characteristic hydrated densities and particle sizes (6, 7). These findings suggest that apoprotein B represents a heterogeneous set of closely related apoproteins, some of which may be under separate genetic control.

The remarkable insolubility of apoprotein B in aqueous buffers in the absence of high concentrations of detergents or denaturants (8) has hindered our understanding of the structure and the molecular organization of apoprotein B in different lipoprotein classes. Monoclonal antibodies can be used to identify the expression and to quantitate the number of single intramolecular epitopes on the hydrated surface of proteins. Thus, a panel of 11 hybridoma antibodies specific for apoprotein B were developed to facilitate the understanding of both the physical structure and the organization of apoprotein B in lipoproteins. Two of the antibodies react only with apoprotein B when it is integrated into lipoprotein particles, whereas nine others also bind denatured apoprotein B (9). An investigation of the immunochemical relationships among the denatured apoprotein B species of chylomicrons, VLDL, and LDL has established that all apoprotein B species share in common some, but not all, epitopes (9). Here we characterize these same epitopes as they are expressed by apoprotein B on native plasma lipoproteins.

**MATERIALS AND METHODS**

Lipoproteins—Lipoproteins were isolated as described by Havel et al. (10). Fresh human plasma was obtained from healthy donors by plasmapheresis and adjusted to 0.1% EDTA (w/v). Plasma pools made up of three or more donors were used. Lipoprotein classes were separated by sequential ultracentrifugation using solid KBr for density adjustment of the plasma and are VLDL (density less than 1.006 g/ml); IDL (density = 1.006-1.019 g/ml); LDL (density = 1.019-1.063 g/ml); and HDL (density = 1.063-1.21 g/ml). Chylomicrons were isolated from the VLDL fraction of nonfasting plasma by flotation through lipoprotein buffer (LLB) containing 150 mM NaCl, 0.3 mM EDTA, 0.0005% α-tocopherol, pH 7.4, by ultracentrifugation at 120,000 x g for 1 h. The lipoprotein fractions were dialyzed thoroughly against LLB, analyzed for protein content by a modified Lowry procedure (11) using bovine serum albumin as standard, and characterized as described (9). During the course of these studies, lipoproteins were isolated from six different plasma pools. Lipoprotein concentrations were expressed on the basis of protein. Molar concentrations of lipoproteins were based on the protein mass of chylomicron (6 x 10^11), VLDL (1 x 10^11), IDL (6 x 10^10), LDL (5.5 x 10^10), and HDL (2.5 x 10^10).

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Human Apolipoprotein B-specific Hybridoma Antibodies

Immunoblotted lacroperoxidase and glucose oxidase (Enzymobeads, Bio-Rad). To 100 µg of LDL in 100 mM sodium phosphate, pH 8.5 (approximately 20 µl), 25 µl of Enzymobead suspension and 1 mM of NaCl was added and the reaction mixture was incubated at room temperature for 10 min. The Enzymobeads were pelleted at 11,000 × g for 2 min and free I1 was removed either by chromatography on Sephadex G-25, or by extensive dialysis against LLB. The specific activity of 125I-LDL ranged from 0.8-1.5 µCi/µg. Radioiodinated LDL was characterized by incubating a portion of 125I-LDL in 5% (w/v) trichloroacetic acid, 10% (w/v) phosphotungstic acid for 1 h at 4 °C. The soluble phase was extracted with chloroform as described by Bierman et al. (13), and the acid precipitate was extracted with a 10-volume excess of cold ether/ethanol (3:1, v/v) (14). Native and radioiodinated LDL was further analyzed by 1% SDS, 3-20% polyacrylamide gradient slab gel electrophoresis as described (9).

Antibodies—The anti-LDL antiserum was obtained from a rabbit that had been repeatedly immunized subcutaneously with 100 µg of human LDL in Freund's adjuvant, and was a pool of multiple late bleedings. The anti-murine Ig was obtained from a goat that had been hyperimmunized with murine IgG, IgM, and IgG (500, 125, and 125 µg, respectively) in Freund's adjuvant monthly for 2 years. It was a hyperimmunized with murine IgA, IgM, and IgG antibodies that had been repeatedly immunized subcutaneously with 100 µg of human LDL in Freund's adjuvant, and was a pool of multiple late bleedings.

Immunologically Purified Antibodies—The hybridoma antibodies were purified from ascites fluid by affinity chromatography. All antibodies except B17 and B18 were purified on insoluble apoprotein B. The insoluble apoprotein B was obtained from LDL by delipidization with ether/ethanol (3:1, v/v) followed by extensive washing of the insoluble protein with 50% acetic acid and then 3 M KI. Antibodies B17 and B18 were purified on LDL, covalently coupled to CNBr-activated Sepharose 6MB macrobeads (Pharmacia). Two ml of ascites fluid were incubated with either 15 ml of packed insoluble apoprotein B or 55 ml of LDL-Sepharose (86 mg of LDL protein) for 2 h at room temperature. The columns were washed with 1.15 M NaCl, 10 mM sodium phosphate, pH 7.2. The antibodies were rapidly eluted in 0.5 M KI and dialyzed against phosphate-buffered saline. The Ig content of these immunoclonally purified antibodies was directly estimated from the protein values obtained by the modified Lowry method (11).

Fluid Phase Radioimmunoassay—The radioimmunoassay was carried out under equilibrium conditions using the principle of double antibody precipitation. Assays were performed in glass tubes (12 × 75 mm) in duplicate in 55 mM Barbitral buffer, pH 8.0, containing 150 mM NaCl, 0.02% (w/v) sodium azide, 3% (w/v) bovine serum albumin, 1.5 mM sodium-EDTA. To 0.1 ml of 125I-LDL (3.6 nM unless otherwise indicated) were added 0.1 ml of buffer or competing antigen and 0.1 ml of 1:40 normal mouse serum. After 18 h at 4 °C, 0.1 ml of precipitating second antibody (goat anti-murine IgG) were added, followed by 1 ml of precipitating antibody for LDL 90% of the binding control (B) was removed by aspiration and 1.0 ml of precipitating second antibody for LDL was used.

RESULTS

Characterization of 125I-LDL—Greater than 90% of the 125I in 125I-LDL was acid precipitable. Half of the nonprecipitable radioactivity was free iodide by chloroform extraction, and only 7-10% of the acid-precipitable radioactivity was extractable into organic solvent. Thus, in all cases, greater than 85% of 125I in the 125I-LDL appeared to be protein-associated. The distribution of the 125I label in the apoproteins of LDL was assessed by electrophoresis of 125I-LDL on 3-20% polyacrylamide gradient slab gels containing 1% SDS. Densitometric analysis of a 24-h autoradiograph of the polyacrylamide gel (Fig. 1) revealed that greater than 94% of the protein-associated 125I was present in apoprotein B.

To establish that radioiodination did not immunologically alter the LDL ligand, varying proportions of 125I-LDL and LDL were incubated with either a directly precipitating hyperimmune rabbit antiserum specific for human LDL or a

\[
\text{where } X = \text{mean radioactivity precipitated in the presence of a given amount of specific antibody.}
\]

The molar concentration of LDL (competitor) required for 50% inhibition of specific binding (C) at a given molar concentration of 125I-LDL (L) and the maximum amount of 125I-LDL bound in the absence of competitors (B) were obtained from competitive radioimmunoassays and used to calculate the mean affinity constant (Ks) of antibody for LDL as described (15).

\[
K_s = \frac{1}{(C - L) (1 - 1.5b + 0.5b^2)}
\]

LDL 125I-LDL

FIG. 1. Electrophoresis of LDL in a 1% SDS, 3-20% polyacrylamide gradient slab gel. Left lane, 50 µg of LDL stained for protein with Coomassie brilliant blue, R-250. Right lane, 24-h autoradiograph of the polyacrylamide gel (Fig. 1) revealed that greater than 94% of the protein-associated 125I was present in apoprotein B.

1. Electrophoresis of LDL in a 1% SDS, 3-20% polyacrylamide gradient slab gel. Left lane, 50 µg of LDL stained for protein with Coomassie brilliant blue, R-250. Right lane, 24-h autoradiograph of the polyacrylamide gel.
pool of mouse ascites fluids containing equal volumes of each of the 11 apoprotein B-specific antibodies, which were then precipitated by the goat anti-murine Ig. A concentration of 3 nM LDL (\textsuperscript{125}I-LDL plus LDL) was maintained. The plots of \textsuperscript{125}I-LDL bound versus per cent \textsuperscript{125}I-LDL added were both linear with correlation coefficients (r) of 0.990 and 0.994 for the two methods, respectively.

The maximum amount of \textsuperscript{125}I-LDL (1.2 nM) that could be bound by each of the individual hybridoma antibody ascites fluids varied from 43 to 97% (Fig. 2), although a pool made up of all 11 hybridoma antibodies was capable of binding 100% of the \textsuperscript{125}I-LDL. Saturating conditions of antibody excess were approached with some, but not all, of the antibodies (Fig. 2). To increase the quantity of specific antibody that could be added to the radioimmunoassay, each of the hybridoma antibodies was immunochemically purified and used in a modified fluid phase radioimmunoassay. Under these conditions, saturation of binding by use of antibody in clear excess was achieved (Fig. 3). Notably, complete binding of all \textsuperscript{125}I-LDL was not observed for all of the hybridoma antibodies. From 45 to 100% of the \textsuperscript{125}I-LDL was bound by different antibodies (Table I) and suggests that epitopes defined by some of the hybridoma antibodies were not expressed by certain subsets of \textsuperscript{125}I-LDL.

Differences in the amount of maximum binding between the ascites fluids and the purified antibodies were less than 10% for all antibodies, except B3 and B17. The \textsuperscript{125}I-LDL binding of these antibodies increased following purification and use in antibody excess from 77 to 92% for B3 and from 43 to 100% for B17. These data established that all \textsuperscript{125}I-LDL contained apoprotein B epitopes that were recognized by three of the hybridoma antibodies. More importantly, they demonstrated heterogeneity of LDL, since the other antibodies recognized only 45-94% of the \textsuperscript{125}I-LDL.

Antibody Affinities for LDL—Expression of apoprotein B epitopes on LDL were characterized by equilibrium competitive inhibition analyses in which the ability of LDL to compete with \textsuperscript{125}I-LDL for binding by each of the antibodies was assessed. Each apoprotein B-specific antibody (ascites fluid) was used at a concentration that gave 50% of its maximum binding capacity. Full displacement of \textsuperscript{125}I-LDL and LDL was observed for each antibody. Estimated average affinity constants (K_a) for LDL epitopes were calculated from the competitive inhibition assays according to Muller (15). As shown in Table I, seven antibodies had affinity constants for apoprotein B epitopes on intact LDL of 1.8-3.0 x 10^9 M^-1, whereas four others were of lower affinity, i.e. 1.9-8.1 x 10^8 M^-1. When these 11 hybridoma antibodies were considered as a group, there was no correlation between the maximum amount of LDL that was bound and the affinity constant. For example, 100% binding of \textsuperscript{125}I-LDL was observed for antibody B17 which had a relatively lower K_a of 1.9 x 10^9 M^-1, whereas 45% of \textsuperscript{125}I-LDL was bound by antibody B20, which had a higher K_a of 2.8 x 10^9 M^-1.

Apoprotein B Expression by LDL, VLDL, and IDL—Apoprotein B is present in varying abundance in plasma chylomicrons, VLDL, and IDL, as well as LDL. Plasma HDL, which does not contain apoprotein B, did not compete with \textsuperscript{125}I-LDL for binding to any of the antibodies. Complete competition was observed with each antibody at high concentrations of VLDL and IDL, indicating that each of these apoprotein B epitopes was expressed by both VLDL and IDL. When the competition curves were analyzed by logit transformation (16-18) to compare qualitative epitope expression by each class of lipoprotein, three consistent and reproducible patterns were apparent. Five antibodies (B1, B3, B11, B19, and B14) identified the Type I pattern of epitope expression, of which antibody B11 is representative (Fig 4). When these antibodies were used in competitive inhibition analyses with VLDL and IDL, the slopes of the LDL dose titration regression lines did not differ significantly by a Student’s t test (19) from the
Apoprotein B-specific hybridoma antibodies

Each of the hybridomas and their secreted apoprotein B-specific antibodies are identical with those described previously (9). Maximum binding values were obtained with immunochemically purified antibody by radioimmunoassay as described. LDL concentrations used are indicated in Fig. 3. The antibody affinity constants ($K_a$) for LDL were obtained from competitive radioimmunoassays as described. Types I, II, and III refer to the different patterns of apoprotein B epitope expression on VLDL, IDL, and LDL and are defined in the text.

| Antibody | Hybridoma | Maximum binding for LDL | Affinity constant ($K_a$) for LDL | Patterns of epitope expression |
|----------|-----------|-------------------------|---------------------------------|-------------------------------|
| B1       | V82G3-R1D6 | 78                      | 2.7                             | I                             |
| B3       | V81C6-R1B6 | 92                      | 1.0                             | I                             |
| B11      | I101C9-R1A5 | 91                      | 0.81                            | I                             |
| B14      | V82B1-R2D4 | 90                      | 1.8                             | I                             |
| B19      | V82B2-R1F6 | 100                     | 0.5                             | I                             |
| B2       | V82G12-R1G1 | 70                      | 2.5                             | II                            |
| B18      | I101F5-R1G2 | 50                      | 0.29                            | II                            |
| B20      | I101F8-R1G5 | 45                      | 2.8                             | II                            |
| B16      | I101D4-R1D8 | 94                      | 1.8                             | III                           |
| B17      | I101H12-R1G5 | 100                     | 0.19                            | III                           |
| B24      | V82A6-R1G4 | 100                     | 3.0                             | III                           |

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**Type I**

**Type II**

**Type III**

Differences in the expression of LDL apoprotein B epitopes on VLDL and IDL were analyzed by fluid phase double antibody competitive radioimmunoassays and were performed as described in Fig. 4. The slope of the linear regression line of the homologous competitor (LDL) was compared for each antibody with the slopes of the heterologous competitors, VLDL and IDL, by a two-tailed Student's $t$ test (20). The 11 hybridoma antibodies were grouped into three patterns of epitope expression (Types I, II, and III) on the basis of the significance of the reproducible slope differences. Similar analyses performed with these same antibodies on a second set of lipoproteins isolated from a different plasma pool gave identical results. Type I antibodies showed no slope differences for LDL, VLDL, or IDL. Type II antibodies showed significant differences between LDL and VLDL, and LDL and IDL, while the Type III antibodies showed no differences between LDL and IDL.

| Apoprotein B epitope | Hybridoma antibody | Homologous competitor (LDL) | Heterologous competitor |
|----------------------|--------------------|-----------------------------|-------------------------|
| Type I               | B11                | $-2.167$                    | $-2.095^*$               |
|                      | B3                 | $-1.966^*$                  | $-2.688^*$               |
|                      | B14                | $-2.471^*$                  | $-2.749^*$               |
|                      | B19                | $-2.181^*$                  | $-2.324^*$               |
|                      | B1                 | $-1.870^*$                  | $-1.642^*$               |
| Type II              | B18                | $-2.818^*$                  | $-2.143^*$               |
|                      | B2                 | $-1.421^*$                  | $-2.134^*$               |
|                      | B3                 | $-1.886^*$                  | $-1.512^*$               |
|                      | B20                | $-1.656^*$                  | $-1.754^*$               |
| Type III             | B24                | $-2.342$                    | $-1.883^*$               |
|                      | B17                | $-2.305$                    | $-2.047^*$               |
|                      | B16                | $-2.190$                    | $-2.146^*$               |

$p > 0.1$

$p < 0.01$

$p < 0.05$
and IDL competitive inhibition slopes for each of these antibodies indicated that the VLDL and IDL epitopes were in all cases expressed in a remarkably similar manner \( (p > 0.6) \). Thus, in contrast to Type I apoprotein B epitopes which appeared to be expressed equally by VLDL, IDL, and LDL, the Type II apoprotein B epitopes were modified on VLDL and IDL relative to LDL.

The remaining three antibodies, B24, B16, and B17, appeared to define a third category of epitope expression. As shown in Fig. 6 and represented by antibody B16, these antibodies exhibited regression lines for LDL and IDL that had similar slopes, whereas the slope of the VLDL dose titration was different. Significant slope differences were observed for LDL and VLDL \( (p < 0.05) \) while the slopes of IDL

**Fig. 4.** Type I pattern of apoprotein B epitope expression. Logit transformation analysis of the ability of chylomicrons (Chylo), VLDL, IDL, LDL, and HDL to compete with \(^{125}\text{I}-\text{LDL}\) for binding to apoprotein B-specific B11 antibody (ascites fluid). The fluid phase radioimmunoassay was incubated at \( 4 \) °C for 22 h and contained 1.2 nM \(^{125}\text{I}-\text{LDL}\) and 0.24 nM B11 Ig. Lipoprotein competitors were added at the same time as the ligand and at the concentrations indicated on the X axis. Logit \((Y)\) is the \( \log, (Y/1-Y)\), where \(Y\) equals the percent binding of \(^{125}\text{I}-\text{LDL}\) in the presence of a given amount of competitor. Linear regression correlation coefficients \((r)\) for chylomicrons, VLDL, IDL, and LDL were 0.986, 0.995, 0.998, and 0.997, respectively.

**Fig. 5.** Type II pattern of apoprotein B epitope expression. Logit transformation analysis of the ability of VLDL, IDL, LDL, and HDL to compete with \(^{125}\text{I}-\text{LDL}\) for binding to antibody B20. Assay conditions are the same as in Fig. 4. Antibody B20 was used at 0.61 nM. \( r = 0.999, 0.988, \) and 0.983 for VLDL, IDL, and LDL, respectively.

**Fig. 6.** Type III pattern of apoprotein B epitope expression. Logit transformation analysis of the ability of VLDL, IDL, LDL, and HDL to compete with \(^{125}\text{I}-\text{LDL}\) for binding to antibody B16. Assay conditions are the same as in Fig. 4. Antibody B16 was used at 3.2 pm. \( r = 0.997, 0.990, \) and 0.990 for VLDL, IDL, and LDL, respectively.
and LDL were not significantly different (p > 0.1) (Table II). Therefore, the Type III epitopes recognized by these antibodies were expressed in a similar manner only on IDL and LDL.

**DISCUSSION**

Analysis of apoprotein B has presented an inordinately difficult problem in the molecular biology of the plasma lipoproteins. Whereas some investigators have assumed homogeneity of LDL and have suggested the existence of a single size form of apoprotein B (20), others have indicated that apoprotein B may represent a more heterogeneous population of protein chains (21, 22). Recent studies have provided more detailed insight into the heterogeneity of plasma apoprotein B. Not only are there mobility differences for subunits of human (23) and rat (2-4) apoprotein B on SDS-polyacrylamide gel electrophoresis, but there is genetic evidence to suggest differences between apoprotein B of hepatic and intestinal origin (5).

Evidence of apoprotein B heterogeneity has now been expanded in this and the preceding report (9), using monoclonal antibodies of hybrid cell origin that are specific for apoprotein B. Both the physical chemical behavior of apoprotein B, and the existence of different epitopes on different subsets of denatured apoprotein B suggest that there may be a variety of highly similar but nonidentical species of plasma apoprotein B. In the preceding study, we identified differences between different species of apoprotein B following the removal of lipids (9). In the present study, we have asked whether there are differences in the organization of apoprotein B on different lipoproteins. Indeed, evidence from the present study indicates considerable heterogeneity in apoprotein B as it is expressed at the hydrated surface of lipoproteins.

The binding of LDL by apoprotein B antibodies was specific based on the lack of competitive inhibition by HDL and was not due to trivial events such as modification of the LDL by radiolodination. The existence of different subsets even within as homogeneous a class as LDL was clearly indicated by the observation that not all LDL could be bound by a number of the antibodies. Maximum binding of 125I-LDL varied from 45 to 100% for different hybridoma antibodies in antibody molar excess (Fig. 3), indicating that the intramolecular loci defined by certain antibodies were not expressed at the hydrated surface of a significant proportion of LDL.

Theoretically, the interaction of a single antibody molecule with one apoprotein B epitope on a lipoprotein particle is sufficient to mediate the subsequent binding and precipitation of the lipoprotein by the second antibody. Three of the antibodies (B19, B24, and B17) bound to and permitted precipitation of 100% of LDL and can therefore be considered pan-LDL antibodies. The existence of pan-LDL antibodies indicates that all LDL particles of density 1.019-1.063 g/ml share some epitopes in common. However, eight hybridoma antibodies did not recognize all LDL. Indeed, binding of 45-94% of LDL was the maximum that could be achieved even when taken to antibody excess. The fact that there are some apoprotein B epitopes that are not expressed by all LDL particles indicates that there are either different species of apoprotein B on different LDL particles, or the same apoprotein B is organized differently on different particles (i.e., organized so as to expose the epitope in a different fashion). Since LDL contains lipids, no distinction can be made between true apoprotein B heterogeneity and heterogeneity of expression caused by the conformational effects of boundary lipids. Nevertheless, LDL does exhibit a newly recognized degree of immunological heterogeneity which is consistent with recent reports of the biochemical and physical heterogeneity of LDL (6, 7).

Competitive inhibition analyses employing each of the antibodies indicated that VLDL and IDL expressed many of these apoprotein B epitopes to nearly the same extent as LDL. Logistic transformation analysis of the competitive inhibition data was used to assess the identity, partial identity, and nonidentity of epitope expression on these apoprotein B-containing lipoproteins. One group of antibodies exhibited no significant difference in the slopes of regression profiles for VLDL, IDL, and LDL (Fig. 4 and Table II). Therefore, the single intramolecular loci responsible for these Type I epitopes were expressed in an indistinguishable fashion on VLDL, IDL, and LDL. Since one of these antibodies recognized 100% of LDL (antibody B19), it follows that this antibody is probably a pan-apoprotein B antibody, since it recognized an apoprotein B epitope that was expressed identically on all plasma VLDL, IDL, and LDL. In contrast, chylomicrons demonstrated nonequivalent competitive inhibition of antibody B11. This indicated that this B11 epitope was present on chylomicrons but was not identical.

The Type II pattern of epitope expression was exhibited by antibodies B2, B18, and B20. These apoprotein B epitopes were expressed in an indistinguishable manner on IDL and VLDL but in a significantly different manner on LDL. Therefore, these antibodies appeared to be capable of distinguishing LDL from VLDL and IDL. Finally, three hybridoma antibodies identified a third pattern of epitope expression. These antibodies were characterized by the fact that the Type III apoprotein B epitopes expressed on IDL were not significantly different from those expressed by LDL, but they were different from those expressed on VLDL. Therefore, these antibodies appeared to be capable of distinguishing IDL and LDL from VLDL. The differential affinity exhibited by the Type II and Type III apoprotein B epitopes on VLDL, IDL, and LDL indicated that the expression of these epitopes on different lipoproteins is different. The altered expression could result from either differences in primary structure or differences in the conformational organization of the epitopes. In view of the metabolism of apoprotein B, it is more likely that the primary structure was identical, but that the conformational organization of these epitopes at the hydrated surface of the lipoproteins was differentially influenced by lipid or other apoproteins that resulted in alternative patterns of folding and exposure of the epitope.

It can be hypothesized that there are at least three types of apoprotein B epitopes on the hydrated surface of a lipoprotein. One type would represent those portions of apoprotein B that interact only with solvent. Another type would represent those portions of apoprotein B that interact with or border on the regions involved in protein/protein interactions, whereas the third type would represent those portions that interact with or border on the regions of apoprotein B that are involved with protein/lipid interactions. The data are consistent with the hypothesis that the antibodies that recognize the identical Type I epitopes on VLDL, IDL, and LDL may be specific for epitopes that interact only with solvent and are not influenced by the presence of lipid and other apoproteins. The antibodies that appear to distinguish LDL from VLDL and IDL may be directed at a class of apoprotein B epitopes that participate in protein/protein interactions, since a distinguishing feature between LDL and IDL/VLDL is the additional apoprotein E and C content of IDL and VLDL. The antibodies that appear to distinguish LDL and IDL from VLDL may bind to apoprotein B epitopes that participate in protein/lipid interactions, since a distinguishing feature between LDL/IDL and VLDL is the proportion of lipid. In any case, these results are consistent with the characterization of these epitopes on denatured apoprotein B species (9), where it was observed that...
apparently complete immunochemical identity was shared among apoprotein B species B-100 and B-26 of LDL, B-100 of VLDL, and B-100 of chylomicrons. However, there are insufficient data at present to correlate for any given antibody the pattern of epitope expression observed for each of the various lipoproteins with the pattern of reactivity observed for each of the denatured species of apoprotein B (9).

None of this panel of 11 hybridoma antibodies appeared to be of the same clonal origin, since they identified a minimum of eight different patterns of reactivity for the denatured species of apoprotein B (9). However, there are insufficient data to determine whether these 11 antibodies bind to 11 spatially independent apoprotein B epitopes. A subsequent report will address the number of different LDL apoprotein B epitopes that can be identified by this hybridoma antibody panel and how many of each unique epitope are expressed per lipoprotein particle. We anticipate that this will permit the construction of initial structural and spatial maps of apoprotein B as it is organized on the various plasma lipoproteins.

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