Generation of monoclonal antibodies specific for mouse apolipoprotein B-100 in apolipoprotein B-48-only mice

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Abstract Over the past 10 years, many laboratories have investigated lipid metabolism and atherogenesis with a variety of transgenic and gene knockout mouse models. Although many of these studies have yielded valuable insights, some have been hampered by a paucity of useful antibodies against mouse proteins. For example, many laboratories have analyzed genetic and dietary interventions affecting lipoprotein metabolism without useful antibodies against mouse apolipoprotein (apo) B. In this study, we sought to develop highly specific monoclonal antibodies against mouse apoB-100. To achieve this goal, gene-targeted mice that synthesize exclusively apoB-48 (apoB-48-only mice) were immunized with mouse apoB-100. The immune response against apoB-100 was robust, as judged by high titers of antibodies against mouse apoB-100. After fusing the splenic lymphocytes of the apoB-48-only mice with a myeloma cell line, we identified and cloned hybridomas that produced mouse apoB-100-specific monoclonal antibodies. Those antibodies were useful for developing sensitive and specific immunoassays for mouse apoB-100. This study illustrates the feasibility and utility of using gene-targeted mice to develop monoclonal antibodies against mouse proteins.—Zlot, C. H., L. M. Flynn, M. M. Véniant, E. Kim, M. Raabe, S. P. A. McCormick, P. Ambroziak, L. M. McEvoy, and S. G. Young. **Generation of monoclonal antibodies specific for mouse apolipoprotein B-100 in apolipoprotein B-48-only mice. *J. Lipid Res.* 1999.** 40: **76–84.**

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Monoclonal antibodies are important research tools for many fields of biomedical research, and the field of lipoprotein metabolism is no exception. Monoclonal antibodies have been particularly useful for investigating apolipoprotein B (apoB). These reagents were crucial for demonstrating that apoB-48 and apoB-100 are products of a single gene (1), for defining the structural relationship between apoB-48 and apoB-100 (2–4), for defining the region of the apoB-100 molecule that binds to the LDL receptor (5, 6), for defining the structure of mutant apoB proteins associated with familial hypobetalipoproteinemia and familial ligand-defective apoB-100 (7–10), and for defining the role of oxidized low density lipoprotein (LDL) in atherogenesis (11, 12). In addition, monoclonal antibodies have been valuable for establishing standardized immunoassays for human apoB (13).

Nearly all of the available apoB-specific monoclonal antibodies have been generated against human apoB or human apoB-containing lipoproteins (2, 3, 6, 14–16). A few of those antibodies, such as antibody MB47 (6, 17), bind to apoB proteins from other mammalian species (e.g., rabbit, pig, dog, and guinea pig) and therefore have been useful for experimental studies involving those species (11, 18). However, none of the available monoclonal antibodies binds to mouse apoB. The latter finding is not unexpected as nearly all of the apoB-specific monoclonal antibodies have been produced in mice, and the mouse immune system typically generates antibodies only against foreign antigenic determinants.

Although the inability of human apoB-specific monoclonal antibodies to bind to mouse apoB is not surprising, it is definitely unfortunate. During the past 5 years, a large fraction of experimental animal research in the lipoprotein metabolism/atherosclerosis field has shifted to the mouse. For experiments involving mice, antibodies against mouse apoB would be quite useful.

The goal of this study was to develop monoclonal antibodies against mouse apoB. We reasoned that it might be

Abbreviations: LDL, low density lipoproteins; SDS, sodium dodecyl sulfate; apo, apolipoprotein; RIA, radioimmunoassay: DMEM, Dulbecco's modified Eagle's medium; PBS–T/BSA, phosphate-buffered saline with 0.1% Tween 20 and 3% bovine serum albumin; SPRIA, solid-phase radioimmunoassay buffer.

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possible to produce mouse monoclonal antibodies against mouse apoB if we generated the antibodies in mice that were genetically incapable of synthesizing mouse apoB. Ideally, one would want to generate monoclonal antibodies against mouse apoB in apoB knockout mice. Unfortunately, this is not possible as apoB knockout mice die early during embryonic development (19, 20). However, we recently used gene targeting to insert a nonsense mutation into codon 2153 of the mouse apoB gene, thereby creating mice that synthesize exclusively apoB-48 (apoB-48-only mice) (21, 22). We hypothesized that the apoB-48-only mice would recognize mouse apoB-100 as a foreign antigen and thus would mount a strong immune response if immunized with that protein. In this study, we sought to test that hypothesis and to assess the feasibility of using the apoB-48-only mice to generate highly specific monoclonal antibodies against mouse apoB-100. We also sought to use the monoclonal antibodies to generate immunoassays for mouse apoB-100.

METHODS

Generation of apoB-100-specific monoclonal antibodies

To isolate mouse apoB, the LDL fraction (d 1.019–1.040 g/ml) was prepared from wild-type mouse plasma by ultracentrifugation (23). The lipoprotein fractions were dialyzed against phosphate-buffered saline (PBS) containing 1.0 mm EDTA. After addition of 2% sodium deoxycholate, the lipoproteins were fractionated on a 10–40% sucrose gradient, as previously described (24). The apolipoprotein content of each fraction was assessed by electrophoresis on a 4–15% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS), followed by silver staining. Fractions containing only apoB-100 and apoB-48 were used to immunize eight apoB-48-only mice ($Apob^{48/48}$) (22) and two human apoB transgenic mice that were homozygous for a knockout mutation in the mouse apoB gene ($HuBTg^{+/o}Apob^{-/-}$) (25). ApoB-48-only mice are incapable of synthesizing mouse apoB-100; the $HuBTg^{+/o}Apob^{-/-}$ mice synthesize human apoB-100 in the liver, but do not make any mouse apoB. As controls, two wild-type mice were also immunized with mouse apoB. For the initial immunization, each mouse was injected intraperitoneally with ~2 μg of mouse apoB in complete Freund's adjuvant. The mice were boosted intraperitoneally 14 and 35 days later with ~2 μg of mouse apoB mixed with incomplete Freund's adjuvant. The response to these immunizations was assessed on day 24 and day 42 with an enzyme-linked immunoassay assay (as described below). As an additional control, two apoB-48-only mice were injected with both Freund's complete and incomplete adjuvant, but without mouse apoB. Approximately 45 days after the first immunization and 3 days before the fusion, mice were injected intravenously, via a tail vein, with ~2 μg of mouse apoB.

Fusions of splenocytes with mouse myeloma cells

Mice were killed by cervical dislocation. Splenocytes from two $Apob^{48/48}$ and two $HuBTg^{+/o}Apob^{-/-}$ mice were harvested and fused with mouse myeloma cells (Sp2/0-Ag14, ATTC catalogue no. CRL-1581) according to a standard polyethylene glycol fusion protocol (26). Fusions were resuspended and plated at different dilutions in 96-well plates in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and HAT (hypoxanthine, aminopterin, thymidine) medium (26). The plates were incubated at 37°C in a 7% CO_2 incubator for 10–14 days. At that time, the supernatant from wells containing visible hybridoma colonies was screened for the presence of antibodies against mouse LDL.

Screening and cloning of hybridomas

To screen hybridoma colonies for the production of mouse apoB-100-specific monoclonal antibodies and to assess the titer of polyclonal antibodies against mouse apoB-100 in the plasma of immunized mice, a solid-phase enzyme-linked immunoassay was used. Polypropylene 96-well plates were coated with 50 μl of PBS containing 2 μg/ml mouse LDL (d < 1.04 g/ml), incubated at 37°C for 2 h, and washed four times with PBS. To block remaining protein-binding sites, the plates were incubated with 200 μl of PBS containing 3% bovine serum albumin and 0.1% Tween 20) for 1 h at room temperature (PBS–T/BSA). The plates were again washed four times with PBS. A total of 50 μl of the cell culture supernatant fluid (or a dilution of mouse plasma) was added to each well and incubated for 2 h at 37°C. The plate was washed four times with PBS. Next, 50 μl of sheep anti-mouse IgG (diluted 1:4000 in PBS–T/BSA) (Amersham Corp., Arlington Heights, IL) conjugated to horseradish peroxidase (EC 1.11.1.7) was added to each well and incubated for 1.5 h at 37°C. The plate was again washed four times with PBS. To detect antibody binding, 50 μl of freshly prepared substrate solution [100 ml of phosphate-citrate buffer with sodium perborate (Sigma, P-4922) containing 30 mg of *o*-phenylenediamine dihydrochloride (Sigma, P-8412)] was added to the wells. The plates were then incubated at room temperature for 30 min. The color reaction was stopped by adding 50 μl of 2.5 m H_2SO_4, and the plates were read at 490 nm with a 96-well plate reader (Spectra Max 250, Molecular Devices, Sunnyvale, CA).

To confirm the specificity of the monoclonal antibodies for mouse apoB-100, we performed Western blots of mouse plasma. For these studies, 1.0 μl of mouse plasma was size-fractionated by electrophoresis on 4% polyacrylamide/SDS gels. The separated proteins were then electrophoretically transferred to a nitrocellulose membrane. The membranes were incubated with hybridoma supernatant fluid, followed by a goat anti-mouse IgG conjugated to horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA). Antibody binding was detected with an enhanced chemiluminescence kit (Amersham Corp.). In some experiments, Western blots of 4–15% polyacrylamide/SDS gels were performed with ^{125}I-labeled monoclonal antibodies, and antibody binding was detected by autoradiography.

Hybridomas producing mouse apoB-100-specific antibodies were grown in 5% CO_2 in DMEM containing 20% fetal serum and supplemented with 2 mm l-glutamine, 50 mm 2-mercaptoethanol, 1.0 mm sodium pyruvate, 50 μg/ml penicillin, 2% essential amino acids, 1% nonessential amino acids, and 10% cloning factor (Origen, Gaithersburg, MD). Hybridomas were cloned by limiting dilution, as previously described (26). Hybridoma clones producing antibodies against mouse apoB-100 were identified by the enzyme-linked immunoassay described above. Three hybridomas (LF2, LF3, and LF5) were cloned three times and used to develop immunoassays for mouse apoB-100, as described below. Monoclonal antibodies were isotyped with IsoStrips (Boehringer Mannheim, catalogue no. 1493-027).

Ascites production and antibody purification

Balb/c female mice, age >6 weeks, were primed with an intraperitoneal injection of 300 μl of pristane (Sigma, catalogue no. P-1403). Ten days later, 5×10^6 log-phase hybridoma cells were injected intraperitoneally. After 9–14 days, the ascites fluid was recovered, and the mice were exsanguinated. The blood and as-

cites fluid were pooled, incubated at 37°C for 1 h, and centrifuged at 3,000 *g* for 10 min. The titer of antibodies in the ascites/serum pool was assessed by Western blot analysis and/or the enzyme-linked immunoassay. Monoclonal antibodies were purified from the ascites/serum pool with the HiTrap Protein G column (Pharmacia, catalogue no. 17-0404-03). The protein concentration of the IgG fraction was determined with the Bio-Rad Protein Assay (Bio-Rad, catalogue no. 500-0006).

Genetically modified mice

To characterize the monoclonal antibodies against mouse apoB-100, we used plasma samples from a variety of genetically modified mice, including apoB-48-only mice ($Apob^{48/48}$) (22), apoB-100-only mice ($Apob^{100/100}$) (22), compound heterozygous mice with one apoB-83-only allele and one apoB-100-only allele ($Apob^{83/100}$) (27), compound heterozygous mice with one apoB-39-only allele (unpublished observations, E. Kim and S. G. Young) and one apoB-83-only allele ($Apob^{39/83}$), apoE-deficient mice ($Apoe^{-/-}$) (28), apoE-deficient apoB-100-only mice ($Apoe^{-/-}$ $Apob^{100/100}$) (21, 22), apoE-deficient apoB-48-only mice ($Apoe^{-/-}Apob^{48/48}$) (21, 22), LDL receptor–deficient mice ($Ldlr^{-/-}$) (29), LDL receptor–deficient apoB-100-only mice ($Ldlr^{-/-}$ $Apob^{100/100}$) (unpublished observations, M. M. Véniant and S. G. Young), transgenic mice that express a mouse apoB transgene ($MuBTg^{+/o}$) (24), transgenic mice that overexpress human apoB ($HuBTg^{+/o}$) (30), and human apoB transgenic mice that were homozygous for a knockout mutation in the mouse apoB gene ($HuBTg^{+/o}Apob^{-/-}$) (25). Finally, we used transgenic mice expressing a mutant human apoB-100 in which human apoB-100 amino acids 4279–4536 were replaced with the corresponding sequences from mouse apoB (unpublished observations, S. P. A. McCormick and S. G. Young).

Immunoassays for mouse apoB-100

An indirect 'sandwich' immunoenzymometric assay for mouse apoB-100. Flat-bottom, 96-well polypropylene plates were coated with 50 μl of PBS containing the mouse apoB-100-specific monoclonal antibody LF3 (5 μg/ml) for 2 h at 37°C. The plates were washed five times with PBS. The remaining protein-binding sites were blocked by incubating the plates with 200 μl of PBS–T/BSA for 1 h at room temperature. The plates were then washed again five times with PBS. Mouse plasma samples (0–2.5 μl) were diluted in 50 μl of PBS–T/BSA, added to the plates in triplicate, and incubated for 2 h at 37°C. The plates were again washed five times with PBS. Next, 50 μl of PBS–T/BSA containing a 1:3000 dilution of a rabbit antiserum against mouse apoB (24) was added to the wells and incubated for 1.5 h at 37°C. The plates were again washed five times with PBS. Finally, 50 μl of a donkey anti-rabbit IgG conjugated to horseradish peroxidase was added and incubated for 1.5 h at 37°C. After the plate was washed with PBS, antibody binding was detected with the colorimetric assay described earlier. Results were plotted as absorbance units versus the volume of mouse plasma added to each well.

A 'sandwich' radioimmunoassay (RIA) for mouse apoB-100. Flat-bottom, 96-well polypropylene plates were coated with 50 μl per well of PBS containing the mouse apoB-100-specific monoclonal antibody LF5 (25 μg/ml) for 4 h at room temperature. The plates were then washed four times with PBS containing 0.1% BSA, 0.05% Tween-20, and 0.08% sodium azide [solid-phase radioimmunoassay buffer (SPRIA)], and the remaining binding sites were blocked by adding 200 μl of PBS containing 2% BSA to each well for 3 h at room temperature. The plates were again washed with SPRIA. SPRIA (50 μl) containing 0–10 μl of mouse plasma was pipetted in triplicate into the wells, and the plates were incubated at 4°C for 16 h. The plates were then washed five times with SPRIA. A total of 50 μl of SPRIA containing ^{125}I-labeled LF3 (300,000 cpm/well) was added to each well. Antibody LF3 was radioiodinated to a specific activity of ~10,000 cpm/ng with Iodogen (Pierce, Rockford, IL) according to the manufacturer's instructions. The plates were incubated at 4°C for 16 h and then washed five times with SPRIA. Individual wells were counted in a gamma counter. Results were plotted as ^{125}I cpm versus the volume of mouse plasma added to each well. The background binding ^{125}I-labeled LF3 in the absence of antibody LF5 (or in the absence of mouse plasma samples) was measured in each experiment and invariably was very low (<250 cpm).

A competitive RIA for mouse apoB-100. Flat-bottom, 96-well polypropylene plates were coated with PBS containing mouse LDL (d 1.02–1.04 g/ml) (50 μl/well) at room temperature for 4 h. After the plates were washed with SPRIA, the remaining binding sites were blocked by adding 200 μl of PBS containing 2% BSA to each well and incubating the plates for 3 h at room temperature. The plates were then washed five times with SPRIA. SPRIA (25 μl) containing 0–10 μl of mouse plasma was then added to the wells in triplicate, followed by 25 μl of SPRIA containing 300,000 cpm of ^{125}I-labeled LF3. The plates were incubated overnight at 4°C. The plates were washed five times with SPRIA, and the individual wells were counted in a gamma counter. The results were plotted as B/B_o versus log of the amount of plasma added to the assay, where B and B_o are specific cpm bound in the presence and absence of a competitor, respectively. The binding of ^{125}I-labeled LF3 in the absence of immobilized mouse LDL was extremely low (<250 cpm). The concentration of mouse apoB-100 in individual mouse plasma samples was determined from a secondary standard of wild-type mouse plasma, as previously described (31–33). The amount of apoB in the secondary standard was determined by chemical techniques (34).

RESULTS

$Apob^{48/48}$ mice, which are genetically incapable of synthesizing apoB-100, developed a strong immune response against mouse apoB, as demonstrated by a high titer of

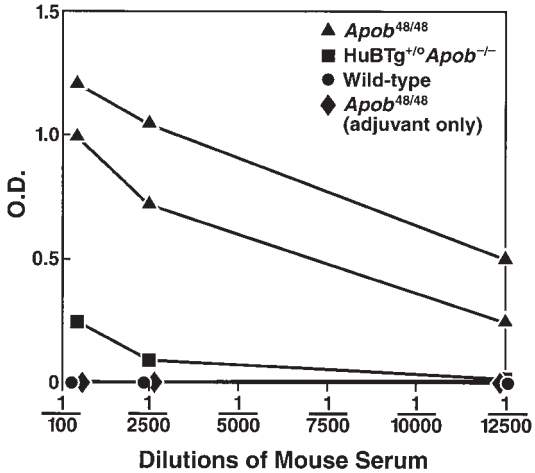

Fig. 1. Solid-phase enzyme-linked immunoassay documenting the development of antibodies against mouse LDL in mice that had been immunized with mouse apoB. Aliquots of serum from immunized mice were incubated on 96-well polypropylene plates that had been coated with mouse LDL, and binding of the polyclonal antibodies was detected with a second antibody against mouse IgG (see Methods). This figure shows the immune responses for two different $Apob^{48/48}$ mice.

antibodies against mouse LDL (**Fig. 1**). No specific antibody response was observed in wild-type mice or in *Apob*$^{48/48}$ mice injected with adjuvant alone (Fig. 1). The HuBTg$^{+/o}$ *Apob*$^{-/-}$ mouse also developed antibodies against mouse LDL, but the titers were lower than those in *Apob*$^{48/48}$ mice.

Ten hybridomas producing monoclonal antibodies against mouse apoB-100 were selected in the initial screening immunoassays, nine from *Apob*$^{48/48}$ mice and one from HuBTg$^{+/o}$*Apob*$^{-/-}$ mice. Three of the hybridomas from one of the *Apob*$^{48/48}$ fusions (LF2, LF3, and LF5) were cloned and are the subject of this report. Hybridomas LF2 and LF3 produced IgG1κ antibodies, whereas clone LF5 produced an IgG2aκ antibody.

As expected, each of the three monoclonal antibodies bound specifically to apoB-100 and did not bind to apoB-48 (**Fig. 2**). None of the antibodies bound to human apoB, but antibody LF5 bound to rat apoB (data not shown). We localized the epitopes for each of the antibodies to the carboxyl terminus of apoB-100 (amino acids 3797–4536) by demonstrating that none bound to mouse apoB-83 (which terminates at mouse apoB amino acid residue 3797) (data not shown). The epitope for antibody LF5 was further localized to mouse apoB-100 amino acids 4279–4536 by demonstrating that it bound strongly to a mutant human apoB in which human amino acids 4279–4536 were replaced with the corresponding sequences from mouse apoB (**Fig. 3**).

When coated onto 96-well plates, each of the three antibodies was effective in capturing apoB-100 in mouse plasma samples. In addition, each of the antibodies bound strongly to mouse LDL that had been coated on 96-well plates. As a result, it was possible to devise many different immunoassays for mouse apoB-100. We used antibody LF3 and a rabbit antiserum against mouse apoB (24) to develop an indirect sandwich immunoenzymometric assay for mouse apoB-100. This immunoassay was very sensitive and specific. It detected mouse apoB-100 in as little as 0.05 μl of plasma from wild-type mice or transgenic mice that expressed a mouse apoB transgene (MuBTg$^{+/o}$) but did not detect human apoB-100 in human plasma or in the plasma of HuBTg$^{+/o}$*Apob*$^{-/-}$ mice (**Fig. 4**).

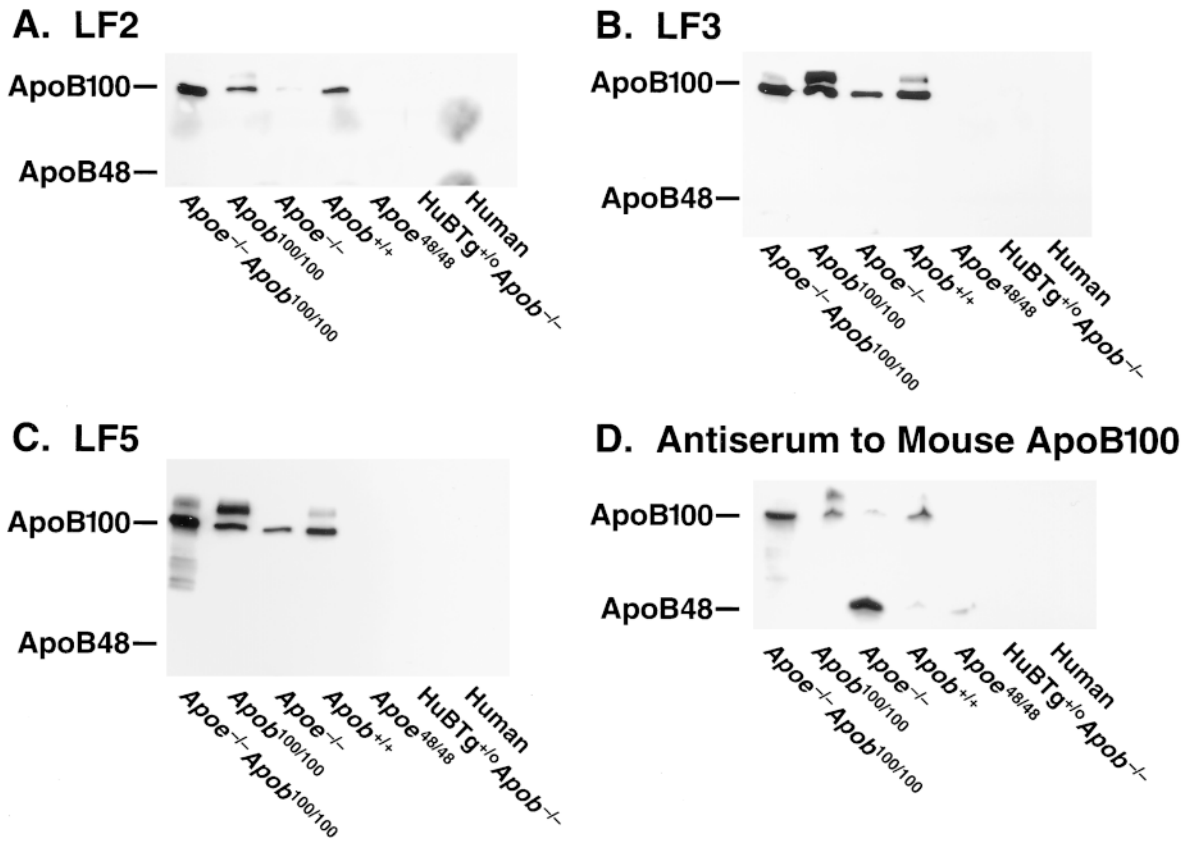

Fig. 2. Analysis of plasma from mice with several different genotypes by Western blot with three different monoclonal antibodies against mouse apoB-100. Plasma samples (1.0 μl) from *Apob*$^{100/100}$*Apoe*$^{-/-}$, *Apob*$^{100/100}$, *Apoe*$^{-/-}$, *Apob*$^{+/+}$, *Apob*$^{48/48}$, and HuBTg$^{+/o}$ *Apob*$^{-/-}$ mice and from a normolipidemic human subject were electrophoresed on a 4% polyacrylamide/SDS gel. The separated proteins were transferred to nitrocellulose and probed with antibodies LF2, LF3, and LF5 (a 1:500 dilution of ascites). Binding of the monoclonal antibodies to apoB-100 was detected with a second antibody against mouse IgG and chemiluminescence reagents. In separate experiments, we used ^{125}I-labeled monoclonal antibodies to probe plasma samples that had been size-fractionated on 4–15% polyacrylamide gels. Each of the monoclonal antibodies bound specifically to apoB-100 (data not shown). None of the monoclonal antibodies reacted with human apoB. In panel A, there are smudges in the human plasma lane, but there are no bona fide apoB-100 or apoB-48 bands. In several of the panels, there are two bands in the vicinity of apoB-100. The lower of those two bands is apoB-100. The higher band is an artifact resulting from the fact that the plasma samples were not fully delipidated and reduced before they were loaded onto the gel.

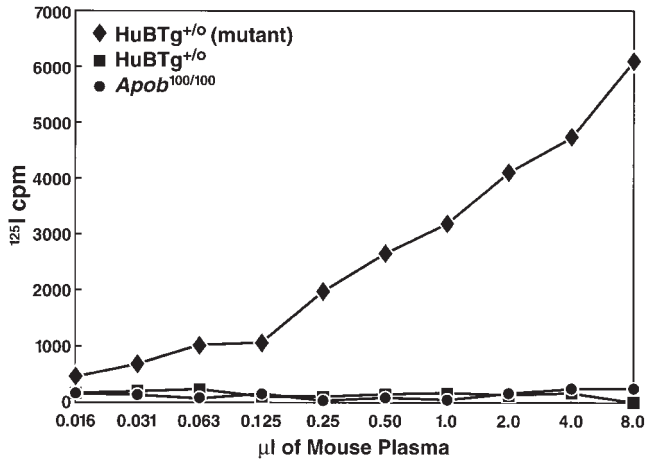

Fig. 3. A solid-phase RIA demonstrating that the epitope for antibody LF5 is located within the extreme carboxyl terminus of mouse apoB-100. Flat-bottom, 96-well polypropylene plates were coated with monoclonal antibody MB47 (6, 17) , which is specific for human apoB-100 and does not bind to mouse apoB-100. We then added dilutions of plasma samples from an *Apob*$^{100/100}$ mouse, a transgenic mouse expressing human apoB, and a transgenic mouse expressing a mutant human apoB in which human apoB amino acids 4279–4536 were replaced by the corresponding mouse apoB sequences. The plates were washed, and bound lipoproteins were detected with ^{125}I-labeled LF5. Antibody LF5 is specific for mouse apoB-100 and does not bind to human apoB-100. As expected, no ^{125}I-labeled LF5 binding was observed with plasma from the *Apob*$^{100/100}$ mouse or from the transgenic mouse expressing a wild-type human apoB. However, robust binding of ^{125}I-labeled LF5 was observed with the plasma from the transgenic mouse expressing a mutant human apoB in which human apoB amino acids 4279–4536 were replaced by the corresponding mouse apoB sequences. Neither ^{125}I-labeled LF2 nor ^{125}I-labeled LF3 bound to the mutant human apoB in this radioimmunoassay.

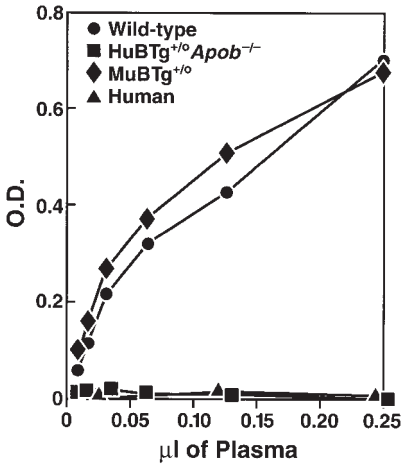

Fig. 4. An indirect sandwich immunoenzymometric assay demonstrating the binding of monoclonal antibody LF3 to the apoB in the plasma of wild-type mice and transgenic mice that overexpressed mouse apoB (MuBTg$^{+/o}$) (24). The format of this enzyme-linked immunoassay is described in Methods.

We also developed a sandwich RIA for mouse apoB-100 by using monoclonal antibodies LF3 and LF5. This RIA revealed that the concentration of apoB-100 in the plasma of *Apob*$^{100/100}$ mice was greater than in wild-type mice. It also revealed that the apoB-100 concentration was much greater in *Ldlr*$^{-/-}$*Apob*$^{100/100}$ than in *Apob*$^{100/100}$ mice (**Fig. 5**). Variability within the RIA was low; in a typical assay, the amount of ^{125}I binding to triplicate wells varied by only ~5%.

The sandwich RIA requires two steps, one in which lipoproteins are captured with an immobilized monoclonal antibody, and a second in which a ^{125}I-labeled monoclonal antibody is used to detect captured lipoproteins. To establish a single-step assay, we developed a competitive RIA with monoclonal antibody LF3. That assay measured the ability of the apoB-100 in mouse plasma samples to compete with immobilized mouse LDL for binding to ^{125}I-labeled LF3. The competitive RIA revealed that the plasma levels of apoB-100 are much lower in *Apoe*$^{-/-}$ mice than in wild-type mice (**Fig. 6A**). The reduction in plasma apoB-100 levels in the setting of apoE deficiency was also obvious in the Western blot experiments (Fig. 2). Interestingly, apoE deficiency did not reduce plasma apoB-100 levels in the setting of homozygosity for the apoB-100-only allele. The plasma apoB-100 levels were significantly higher in *Apoe*$^{-/-}$*Apob*$^{100/100}$ mice than in *Apob*$^{100/100}$ mice (Fig. 6B). Once again, this difference was corroborated by the Western blot studies (Fig. 2).[3]

We also used the competitive RIA to determine whether overexpression of a human apoB transgene affects the plasma levels of mouse apoB-100. For these studies, we analyzed mouse apoB-100 levels in a "high-expressing" line of human apoB transgenic mice (line 1102) (30). In that line, the total amount of apoB synthesis and secretion is increased about 2- to 3-fold (M. M. Véniant, J. Borén, and S. G. Young, unpublished observations). In the competitive assay for mouse apoB-100, the competition curves for a pooled plasma sample from human apoB transgenic mice and a pooled plasma sample from nontransgenic littermate controls were virtually superimposable, indicating that overexpression of human apoB has no impact on the plasma levels of mouse apoB-100 (**Fig. 7A**). When we assayed the plasma levels of mouse apoB-100 in each of 23 female human apoB transgenic mice and 23 nontransgenic female littermates, we found no differences (Fig. 7B).

[3]The reason why apoE deficiency reduces plasma apoB-100 levels in the setting of a wild-type *Apob* allele (*Apob*$^{+}$) but increases apoB-100 levels in the setting of the *Apob*^{100} allele has not been established with certainty. In a recent study (43), we speculated that this difference might relate to the fact that the apoB-100 is produced by both the intestine and liver in the setting of the *Apob*^{100} allele but is produced only by the liver in the setting of the *Apob*$^{+}$ allele. In the setting of apoE deficiency, it is possible that liver-derived apoB-100–containing lipoproteins are cleared efficiently by the LDL receptor, but that intestine-derived apoB-100–containing lipoproteins are cleared inefficiently. A difference in the intrinsic metabolic properties of apoB-100–containing lipoproteins produced by the liver and intestine conceivably could occur as a result in differences in the size or composition of lipoproteins produced by the two organs.

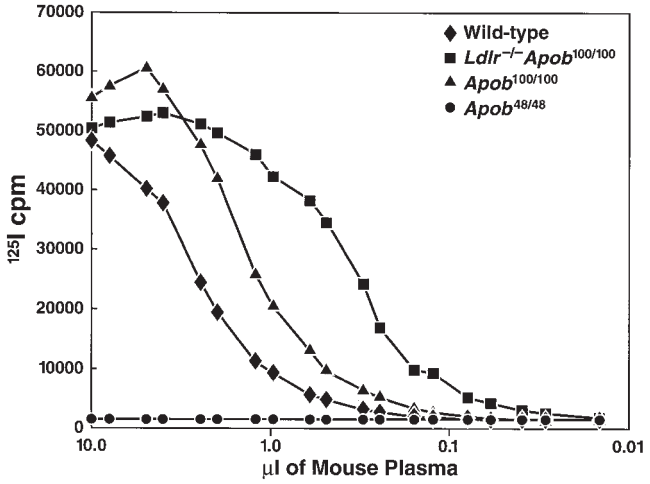

Fig. 5. A 'sandwich' radioimmunoassay illustrating differences in the plasma concentrations of mouse apoB-100 in wild-type mice (*Apob*$^{+/+}$), apoB-100-only mice (*Apob*$^{100/100}$), and LDL receptor–deficient apoB-100-only mice (*Ldlr*$^{-/-}$*Apob*$^{100/100}$). In this RIA, apoB-100 in mouse plasma samples is captured with immobilized antibody LF5. The captured apoB-100 is then detected with ^{125}I-labeled LF3 (see Methods for a complete description of this RIA).

DISCUSSION

In this study, we developed and characterized three murine monoclonal antibodies against mouse apoB-100. The development of monoclonal antibodies against mouse apoB-100 was made possible by using apoB-48-only mice, which recognize mouse apoB-100 as a foreign protein. Each of the three monoclonal antibodies was very specific for mouse apoB-100, and Western blots using these monoclonal antibodies were useful in identifying differences in plasma apoB-100 levels in different groups of genetically modified mice. Perhaps more importantly, the monoclonal antibodies were effective in establishing sensitive solid-phase immunoassays for mouse apoB-100. These immunoassays will undoubtedly be useful for any study designed to assess the impact of an environmental or genetic intervention on plasma apoB-100 levels. In addition, several laboratories are using genetic techniques to define the chromosomal loci affecting lipoprotein and apolipoprotein levels in different inbred mouse strains. The immunoassays described here could be extremely important for the success of those studies.

Generating monoclonal antibodies against mouse antigens in gene-targeted mice is new for the lipid and lipoprotein field, but we suspect that this approach will prove to be generally useful. For example, it should be possible to generate monoclonal antibodies against mouse apoE in *Apoe*$^{-/-}$ mice (28, 35) or monoclonal antibodies against SR-B1 in *Srb1* knockout mice (36). Although monoclonal antibodies against mouse proteins can easily be generated by using rats and rat myeloma cells, developing antibodies in gene-knockout mice could have distinct advantages. For example, rat monoclonal antibodies against mouse SR-B1 would likely be directed against domains within the protein that are structurally distinct from rat SR-B1 (i.e., foreign domains) and not necessarily the functionally important and highly conserved regions of the molecule. In contrast, a panel of monoclonal antibodies developed in *Srb1* knockout mice might prove to have far more diverse specificities, as the entire SR-B1 protein, including all of its most highly conserved domains, would be recognized as foreign. A recent study by Declerck and co-workers (37) lends support to this view. They developed mouse mono-

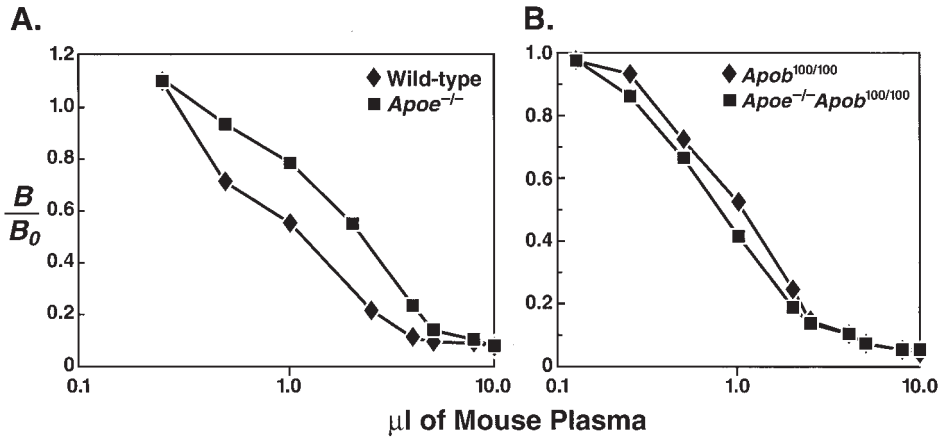

Fig. 6. (A) A competitive RIA demonstrating differences in the plasma concentrations of mouse apoB-100 in the plasma of wild-type mice (*Apob*$^{+/+}$) and apoE-deficient mice (*Apoe*$^{-/-}$). Each plasma sample represents a pooled plasma sample prepared from 4–6 female mice of the same genotype. (B) A competitive RIA demonstrating differences in the plasma concentrations of mouse apoB-100 in apoB-100-only mice (*Apob*$^{100/100}$) and apoE-deficient apoB-100-only mice (*Apoe*$^{-/-}$*Apob*$^{100/100}$). Each plasma sample represents a pooled plasma sample prepared from 4–6 female mice of the same genotype. The competitive RIA was designed to test the ability of the apoB-100 in different mouse plasma samples to compete with immobilized mouse LDL for binding to ^{125}I-labeled LF3 (see Methods for a complete description of this RIA). The variability within this RIA was low; in a typical assay, the amount of ^{125}I binding to triplicate wells varied by only 5%. The results shown in panels A and B have been verified in several independent experiments with different groups of mice.

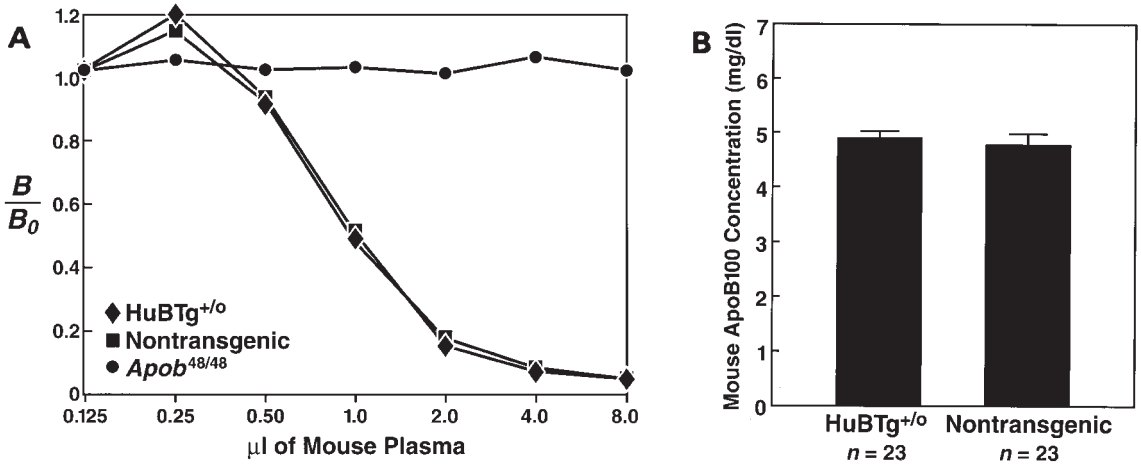

Fig. 7. (A) A competitive RIA demonstrating equivalent concentrations of mouse apoB-100 in chow-fed wild-type mice (*Apob*$^{+/+}$) and human apoB transgenic mice (HuBTg$^{+/o}$). This RIA was formatted as described in Methods. Plasma was pooled from groups of 23 chow-fed female human apoB transgenic mice (30) and wild-type littermate controls, and the two samples were then tested in the competitive RIA for their ability to compete with immobilized mouse LDL for binding to ^{125}I-labeled LF3. (B) Mean apoB-100 concentrations (±SEM) in the plasma of 23 female p158-human apoB transgenic mice (HuBTg$^{+/o}$) and 23 female wild-type littermate controls.

clonal antibodies against mouse tissue plasminogen activator (tPA) in tPA-knockout mice and documented that many of those antibodies bound to antigenic determinants that were highly conserved throughout mammalian evolution.

ApoB-deficient mice die early in embryonic development (19, 20), making it impossible to use those animals to develop monoclonal antibodies. However, our study demonstrates that a lethal embryonic phenotype in a gene knockout mouse does not pose an impassable roadblock for development of monoclonal antibodies against mouse proteins. To develop monoclonal antibodies against mouse apoB-100, we utilized gene-targeted mice (*Apob*$^{48/48}$ mice) that expressed a truncated apoB (apoB-48) as well as apoB knockout mice carrying a human apoB transgene (HuBTg$^{+/o}$*Apob*$^{-/-}$ mice). In our experiments, we identified 10 hybridomas producing mouse apoB-specific monoclonal antibodies from fusions with the splenocytes of *Apob*$^{48/48}$ mice, but only one in fusions with HuBTg$^{+/o}$ *Apob*$^{-/-}$ splenocytes. The fact that more monoclonal antibodies were identified in the *Apob*$^{48/48}$ mice was not surprising, inasmuch as the polyclonal antibody response was far more robust in those mice than in HuBTg$^{+/o}$*Apob*$^{-/-}$ mice. The reason for the weak immune response in the HuBTg$^{+/o}$*Apob*$^{-/-}$ mice is not entirely clear, but we suspect that it relates to the overall health and immune function in those animals. HuBTg$^{+/o}$*Apob*$^{-/-}$ mice lack the ability to synthesize chylomicrons in the intestine, and consequently have intestinal fat malabsorption, growth retardation, and vitamin E deficiency (25). A deficiency in vitamin E is known to adversely affect the function of the immune system (38). More recently, we have developed completely healthy HuBTg$^{+/o}$*Apob*$^{-/-}$ mice by using a different human apoB transgene that was expressed at high levels in both the intestine and liver (39). We suspect that those animals would develop a higher titer of antibodies against mouse apoB and therefore would be more suitable for use in developing monoclonal antibodies. Using HuBTg$^{+/o}$*Apob*$^{-/-}$ mice to develop monoclonal antibodies is appealing because those animals should generate antibodies against the entire length of the apoB molecule, including the amino-terminal region shared by apoB-48 and apoB-100. An alternate means of generating monoclonal antibodies that would bind to both mouse apoB-100 and apoB-48 would be to use homozygous apoB-39-only mice (E. Kim and S. G. Young, unpublished observations) that had been immunized with mouse apoB-48.

The antibodies and immunoassays described in this paper are entirely specific for apoB-100 and therefore cannot be used to create an immunoassay to measure the 'total apoB concentration' (i.e., the concentration of both apoB-48 and apoB-100). For most mouse studies, we suspect that it is actually an advantage to use immunoassays that detect exclusively apoB-100, as we are skeptical whether it would be possible to design a single immunoassay that would measure apoB-48 and apoB-100 with equal efficiency. Also, lipid and apolipoprotein levels are typically measured after a 4–16 h fast, and the plasma levels of apoB-48 after a fast are generally quite low (30, 40). The one situation in which it might be desirable to measure apoB-48 levels is apoE deficiency. ApoE-deficient mice have high levels of cholesterol-rich apoB-48-containing lipoproteins and develop spontaneous atherosclerotic lesions on a chow diet (41, 42). Consequently, those animals have been widely used to study atherogenesis. However, investigators who are interested in studying atherogenesis in chow-fed mice do not need to abandon the idea of rigorously quantifying apoB levels with the immunoassays described in this paper. ApoB-100-only mice, when bred onto the background of apoE-deficiency or LDL receptor deficiency, develop severe atherosclerotic lesions on a chow diet (21). Both of those animal models are available from The Jackson Laboratory.

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