A potential complication in the use of monoclonal antibodies: inhibition of apoB-mediated receptor binding by an anti-apoE antibody

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Abstract Monoclonal antibody (Mab) 1D7 is specific for human low density lipoprotein (LDL) receptor. We report here that Mab 1D7 can also block the binding of apoB-free LDL to the LDL receptor. The inhibition of LDL-receptor binding is not due to immunological cross-reactivity between the anti-apoE Mab and apoB, the ligand responsible for the interaction of LDL with the LDL receptor: 1) Mab 1D7 did not react with apoE-depleted LDL; 2) the LDL receptor binding inhibitory activity of 1D7 immunoglobulin G (IgG) preparations could be dissociated from the anti-apoE activity; 3) the inhibition was maintained when the fibroblasts were preincubated with 1D7 IgG, that mouse apoE-1D7 immune complexes contaminate 1D7 IgG preparations and that the contaminating mouse apoE can compete with 125I-labeled LDL for the LDL receptor. We have demonstrated mouse apoE in IgG preparations of 1D7 but not in those of other anti-apoE Mabs that do not influence LDL-receptor binding. Precipitation of 1D7 IgG with NH₄SO₄ eliminates both apoE and the capacity of 1D7 to block LDL receptor binding. Finally, mouse apoE can be isolated by immunoaffinity chromatography of mouse serum on immobilized 1D7 Mab. As this is probably not a unique case, the observation has important implications for the use of Mabs as structural probes. - Maurice, R., Y. L. Marcel, T. L. Innerarity, and R. W. Milne. A potential complication in the use of monoclonal antibodies: inhibition of apoB-mediated receptor binding by an anti-apoE antibody. J. Lipid Res. 1989. 30: 587-596.

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The low density lipoprotein (LDL) receptor, present on the surface of most mammalian cell types, plays a key role in the maintenance of cholesterol homeostasis. In their lipoprotein-bound forms, two apolipoproteins, apolipoprotein (apo) E and apoB can compete with each other for binding to the LDL receptor (1, 2). The receptor-binding domain of apoE has been localized to a region encompassing apoE residues 140 to 160, and an anti-apoE Mab, 1D7, whose epitope is situated between residues 139-169, blocks the binding of apoE-containing lipoproteins to the LDL receptor. While the receptor-binding domain of apoB has not been identified, Mabs that block LDL binding to the LDL receptor have epitopes that are clustered around a thrombin cleavage site at apoB residue 3249 (3-5). Analysis of the primary structure of apoE and apoB indicates little sequence homology between the two proteins. Nevertheless, common consensus sequences for the binding domains of apoB and apoE have been proposed (6, 7). These have been based on the identification of sequences that are similar in apoE and apoB with respect to the relative positions of basic amino acids. The basic amino acids in the consensus sequence could be approximately aligned with acidic residues in the putative ligand-binding domains of the LDL receptor.

In this study, we report that immunoglobulin G (IgG) preparations of the anti-apoE Mab 1D7 are capable of blocking the binding of apoE-free LDL to the LDL receptor. This observation has both practical and theoretical importance. In practical terms, the Mab 1D7 has been used to discriminate between apoE and apoB-mediated binding of lipoproteins to cell surface receptors (8-12) and as a probe for the identification of the receptor-binding (13) and heparin-binding (14) domains of apoE. It is therefore essential to determine the true specificity of this Mab. Furthermore, it would be of theoretical interest if it could

Abbreviations: Mab, monoclonal antibody; apo, apolipoprotein; LDL, low density lipoprotein; IgG, immunoglobulin G; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified Eagle's medium; BSA, bovine serum albumin; Mab-Sepharose, monoclonal antibody covalently coupled with Sepharose; LDL(apoE), LDL immunodepleted of endogenous apoE; LDL(apoE), LDL passed over a control immunoaffinity column; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NCP, nitrilotriacetic acid paper; apoVLDL, delipidated very low density lipoprotein; HDL, high density lipoprotein.

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be shown that the inhibition of LDL binding by 1D7 was due to an epitope that is common to apoB and apoE. Such an immunological cross-reactivity may reflect a structural relationship between apoE and apoB that, in turn, could explain the dual specificity of the LDL receptor. In view of the potential significance of the observation, we have determined the mechanism by which Mab 1D7 inhibits LDL-receptor binding. The results have important implications for the use of Mabs as structural probes of protein function.

MATERIALS AND METHODS

Lipoproteins and antibodies

Blood from fasting normolipemic subjects was collected in tubes containing EDTA, and red blood cells were removed by centrifugation. The plasma obtained was mixed with NaCl (0.02%) and phenylmethylsulfonyl fluoride (1 mM). Lipoprotein subfractions were prepared in a Beckman L8-M ultracentrifuge with a 50.2 Ti rotor (Beckman Instruments, Spinco Division, Palo Alto, CA). LDL was isolated at 10°C by successive preparative ultracentrifugations between densities of 1.020 and 1.050 g/ml (15), dialyzed against phosphate-buffered saline (PBS) containing 1 mM EDTA and 0.02% NaN3 (pH 7.4), sterilized by ultrafiltration, and stored at 4°C. Total mouse lipoproteins used as positive controls for Western blots were obtained in tubes containing EDTA, and red blood cells were removed by centrifugation. The plasma obtained was mixed with NaCl (0.02%) and phenylmethylsulfonyl fluoride (1 mM). Lipoprotein subfractions were prepared in a Beckman L8-M ultracentrifuge with a 50.2 Ti rotor (Beckman Instruments, Spinco Division, Palo Alto, CA). LDL was isolated at 10°C by successive preparative ultracentrifugations between densities of 1.020 and 1.050 g/ml (15), dialyzed against phosphate-buffered saline (PBS) containing 1 mM EDTA and 0.02% NaN3 (pH 7.4), sterilized by ultrafiltration, and stored at 4°C. Total mouse lipoproteins used as positive controls for Western blots were obtained at d < 1.210 g/ml.

Production and characterization of Mabs against human apoE and apoB have been described elsewhere (3, 16). The Mabs were isolated from a mixture of plasma and ascitic fluid from hybridoma-bearing mice by affinity chromatography on Protein A-Sepharose 4B (Pharmacia Inc., Uppsala, Sweden) using CNBr-activated Sepharose has been described elsewhere (20). To obtain apoE-containing lipoproteins, variable amounts of normal mouse serum or normal human serum were applied to 10-ml columns of either 3B7, 1D7, 6C5, or 7C9 IgG covalently coupled to Sepharose (Mab-Sepharose). The column was washed with PBS, and bound lipoproteins that were eluted with 0.05 M citric acid (pH 2.5) containing 1 M NaCl were dialyzed against PBS that contained 1 mM EDTA and 0.02% NaN3. ApoE-depleted LDL (LDL(apoE-)) or lZ5I-labeled LDL over a 3-ml column of 3B7-Sepharose. In the case of lZ5I-labeled LDL, the sample was diluted in PBS containing 1% BSA before application to the column to minimize nonspecific losses. lZ5I-Labeled LDL that contain endogenous apoE (125I-labeled LDL(apoE)) were prepared by passing lZ5I-labeled LDL over a 3-ml column of 3B7-Sepharose. In the case of 125I-labeled LDL, the sample was diluted in PBS containing 1% BSA before application to the column to minimize nonspecific losses. 125I-Labeled LDL that contain endogenous apoE (125I-labeled LDL(apoE)) were prepared by passing 125I-labeled LDL over Sepharose that was coupled to an irrelevant Mab (anti-rat natriuretic factor) (21).

Analytical procedures

Electrophoresis of proteins and lipoproteins in the presence of sodium dodecyl sulfate on polyacrylamide gels (SDS-PAGE) was performed according to the method of Laemmli (22). Electrophoretic transfer of the migrated proteins to nitrocellulose paper (NCP) and immunodetection of the transferred proteins on the NCP replicas has been described previously (23).
To test for reactivity of anti-apoE Mabs with apoB, aliquots of dilutions of LDL or LDL(apoE-) were adsorbed to polystyrene wells (Dynatech Corp., Alexandria, VA) (16). After the wells were washed and then saturated with 1% BSA, they were exposed to the appropriately diluted Mab overnight and washed. The bound Mab was revealed by the addition of 125I-labeled anti-mouse IgG. ApoE was measured as previously described (16). To measure the anti-apoE titre of IgG preparations, delipidated very low density lipoproteins (apoVLDL) (24) at a concentration of 5 µg/ml was adsorbed to polystyrene wells. Dilutions of the IgG to be tested were incubated with the immobilized apoVLDL and washed; the bound IgG was detected with 125I-labeled anti-mouse IgG as described previously (3). At low dilutions of Mab, the bound radioactivity reached a plateau. The concentration of IgG that resulted in 50% of the plateau value of bound radioactivity was defined as the titre of the IgG preparation.

Protein was measured according to Lowry et al. (25) using BSA as standard.

Iodination of proteins and lipoproteins

Antibodies were labeled by a modification of the chloramine T method (26), while LDL was iodinated as described by Bilheimer, Eisenberg, and Levy (27). Specific activities obtained for the IgG were 5–10 µCi/µg of protein and 400–500 cpm/µg of protein for the LDL.

Results

Inhibition of binding of LDL to the LDL receptor by anti-apoE Mabs

The ability of anti-apoE Mabs 1D7, and 6C5 and anti-apoB 4G3 to inhibit the binding of 125I-labeled LDL to human fibroblasts is shown in Fig. 1. As expected, Mab 4G3 was able to inhibit 90% of the binding of LDL at a concentration of 10 µg/ml, whereas 6C5 displayed only a slight inhibition at 250 µg/ml. However, Mab 1D7 showed a concentration-dependent inhibition of LDL binding, which, at 300 µg/ml, approached that of 4G3. As apoB is thought to be the ligand responsible for binding of LDL to the LDL receptor (1), this was a surprising and unexpected finding. Inhibition of binding by Mab 1D7 was at least as great with LDL that had been immunodepleted of apoE-containing particles (125I-labeled LDL(apoE-)) as with 125I-labeled LDL(apoE*). Thus, the ability of 1D7 to inhibit the binding of LDL is independent of the presence of apoE in the LDL. Contamination of the 1D7-IgG preparation with anti-apoB Mabs was excluded by antibody competition studies (data not shown).

Cross-reactivity of anti-apoE Mab with apoB

The inhibition of LDL binding shown in Fig. 1 could result from a low-affinity cross-reactivity of Mab 1D7 with the apoB of LDL. In our initial characterization of Mab...
1D7, we reported that it did not cross-react with apoB (16). Because our conclusion was based on experiments in which LDL(apoE−) failed to compete with 125I-labeled apoE in radioimmunoassay, we may not have detected a low-affinity cross-reactivity of Mab 1D7 with apoB. To test this possibility, we adsorbed dilutions of LDL or LDL(apoE−) to polystyrene and then tested the Mabs for their ability to directly bind to the immobilized lipoproteins. As can be seen in Fig. 2, binding to LDL of the anti-apoE Mabs, including 1D7, was due to the endogenous apoE in the LDL preparation.

Effect of time on the inhibition capacity of Mab 1D7

We have noted considerable variability amongst different preparations of 1D7 IgG with respect to their abilities to inhibit binding of LDL to the LDL receptor. When the results were compiled and analyzed, it became apparent that the loss of inhibitory activity appeared to be a function of the time elapsed between purification of the IgG and assessment of the ability of Mab 1D7 to block LDL binding (Fig. 3). In contrast to the loss in ability to block the interaction between LDL and its receptor, there was no consistent change with time in the anti-apoE titre of different preparations of 1D7 IgG (Fig. 3); as a consequence, it was possible to dissociate the anti-apoE activity in the 1D7 IgG preparation from the ability to block LDL-receptor binding. It was subsequently shown that the ability to block LDL-receptor binding could be maintained when the IgG preparations were either kept at 4°C in the presence of 1 mM EDTA or refrozen (data not shown).

Direct interaction of the 1D7 IgG preparation with the LDL receptor

As Mab 1D7 did not react with the ligand apoB, we next tested the possibility that the inhibition of LDL-receptor binding by Mab 1D7 results from a direct interaction of the IgG preparation with the LDL receptor. 1D7 IgG was added to cells for 2 hr, and the cells were then washed and exposed to 125I-labeled LDL for an additional 2 hr. Maximum binding was determined by replacing the IgG preparation by 0.5% BSA. As shown in Fig. 4, it was possible, with this approach, to obtain the same inhibition of LDL binding to the fibroblasts as was seen when Mab 1D7 was preincubated with 125I-labeled LDL prior to addition to the cells. In contrast, the anti-apoB Mab 4G3, which blocks LDL binding when added to cells in the presence of 125I-labeled LDL, had no effect on binding if incubated with the cells prior to 125I-labeled LDL. Thus, the inhibitory activity of 4G3 is due to an interaction of the Mab with the ligand apoB while the inhibition by 1D7 results from an interaction of the IgG preparation with the receptor itself. In subsequent experiments we were, however, unable to demonstrate anti-receptor antibody activity in the 1D7 IgG preparations (results not shown).

Contamination of anti-apoE Mab by apoE-containing lipoproteins

If 1D7 IgG preparations were contaminated with apoE- or apoB-containing lipoproteins of mouse origin, these murine lipoproteins could potentially compete with human LDL for the LDL receptor. IgG prepared by affinity chro-

Fig. 2. Reactivity of anti-apoE Mabs 1D7 (O), 3B7 (X), and anti-apoB Mab 4G3 (●) with apoB. Total LDL (——) or LDL(apoE−) (—) were diluted and adsorbed to polystyrene, and Mabs were added to immobilized LDL, as described in Materials and Methods. Bound antibodies were revealed by adding labeled anti-mouse IgG to the well. Results are expressed as bound radioactivity, in counts per min (cpm), per well. Each value represents the average of duplicate determinations.
Fig. 3. Anti-apoE reactivity (solid bars) of individual 1D7 IgG preparations and their respective abilities to inhibit specific binding of $^{125}$I-labeled LDL to cultured human fibroblasts (open bars). Different IgG preparations were purified from the same pool of ascites by affinity chromatography on Protein-A Sepharose and are referred to as 1D7 A, 1D7 C, 1D7 D, 1D7 F, 1D7 G, and 1D7 J. The preparations differed with respect to the time, in days, between the purification of the IgG and the evaluation of the IgG for its ability to influence LDL-receptor binding (see text). Purified IgG were kept at 4°C without EDTA until they were assessed in the binding study (see technical details in Fig. 1). Specific binding of $^{125}$I-labeled LDL to the LDL receptor was determined, and the results are shown in open bars. The 100% control value for the binding of $^{125}$I-labeled LDL was 100 ng of lipoprotein protein/mg cellular protein. Each value represents the mean of triplicate determinations. The immunoreactivity of the Mabs was determined by direct fixation of diluted Mabs to fixed amounts of immobilized apoVLDL, and results are expressed as the reciprocal of the anti-apoE titer (closed bars). Bound IgG was revealed with labeled anti-mouse IgG. Each value represents the average of duplicate determinations.

Fig. 4. Ability of anti-apoB Mabs 1D7, 3B7 and anti-apoB Mab 4G3 to inhibit the specific binding of $^{125}$I-labeled LDL to the LDL receptor by an interaction of the IgG preparation with $^{125}$I-labeled LDL (open bars) or with cultured human fibroblasts (solid bars). For experiments in which the cells were pre-exposed to Mab before addition of $^{125}$I-labeled LDL, 200 μg of purified IgG/ml in 0.75 ml of DMEM-BSA was added to cultured human fibroblasts for 2 hr at 4°C. The cells were then washed (3) and incubated with 3 μg of $^{125}$I-labeled LDL in DMEM-BSA/ml for another 2 hr (solid bars). Maximum binding was determined by omitting the Mab during the first incubation. The inhibition of binding of $^{125}$I-labeled LDL to the LDL receptor following preincubation of Mabs with $^{125}$I-labeled LDL (open bars) was done as in Fig. 1 except for a 2-hr incubation of the reaction mixture with the cells. Maximum binding values were 75 ng and 130 ng of lipoprotein protein/mg of cellular protein for the experiments represented by solid and open bars, respectively. Each value represents the average of triplicate determinations.
matography on Protein A-Sepharose from ascites containing the anti-apoE Mabs 1D7, 3B7, 6C5, 7C9, and 3H1 were separated by SDS-PAGE, and the migrated proteins were electrophoretically transferred to NCP. The NCP replicas were exposed first to a rabbit anti-rat apoE antiserum, which shows a strong cross-reactivity with mouse apoE (R. LeBoeuf, personal communication) and then to 125I-labeled anti-rabbit IgG. The immunoblots revealed that mouse apoE was present in the preparation of Mab 1D7 but was absent from the IgG preparations of the other anti-apoE Mabs, 6C5, 7C9, 3H1, and 3B7 (Fig. 5). These results strongly suggest that apoE-containing lipoproteins from mouse origin copurify with the Mab 1D7 during its isolation from mouse ascites on a Protein A-Sepharose column. In addition to the 35-kDa band of apoE, the heavy and light chains of Mabs (50 kDa and 25 kDa, respectively) were revealed because of a cross-reactivity of the 125I-labeled anti-rabbit IgG with mouse IgG.

We have similarly tested 1D7 IgG preparations for the presence of other mouse apolipoproteins using anti-sera to rat apoA-I, apoA-IV, and apoB. No mouse apolipoproteins, with the exception of apoE, were detected (data not shown).

**Purification of apoE-containing lipoproteins from mouse serum by immunoaffinity chromatography on insolubilized anti-human apoE Mab 1D7**

The fact that mouse apoE was present in the 1D7 preparation and not in other anti-apoE Mab IgG preparations could indicate a cross-reactivity of 1D7 with mouse apoE. To confirm this, we coupled Mabs 1D7, 6C5, 7C9, and 3B7 to Sepharose and tested the insolubilized Mabs for their ability to purify apoE-containing lipoproteins from mouse serum. Each immunoadsorbent was first tested for its ability to bind apoE-containing lipoproteins from human serum. Measurement of apoE by radioimmunoassay in the retained and nonretained fractions showed that the respective capacities of the four immunoadsorbents were similar for binding of human apoE (data not shown). Each immunoadsorbent was then tested for its ability to bind mouse serum apoE. Immunoblots of the retained fractions obtained from 3B7- and 1D7-Sepharose are shown in Fig. 6. A 35-kDa band is present in the 1D7-Sepharose retained fraction. This band was absent from the 3B7-Sepharose retained fraction. This band was also absent from the 6C5- and 7C9-Sepharose retained fractions (data not shown).

We have tested the retained fractions of human or mouse serum that had been passed over 1D7- or 3B7-Sepharose columns in competition with human 125I-labeled LDL for binding to the LDL receptor of cultured human fibroblasts. The retained human serum fractions from both immunoadsorbents competed with 125I-labeled LDL for binding to the LDL receptor, whereas only the retained mouse serum fraction from the 1D7-Sepharose competed effectively (Fig. 7).

**Elimination of apoE-containing lipoproteins from 1D7 IgG preparations**

Mab 1D7 has been used to differentiate between the apoE and apoB components of lipoprotein binding to cell surface receptors. While the concentrations of Mab 1D7 required to totally eliminate apoE-mediated binding are normally sufficiently low so that effect of mouse apoE in 1D7 IgG preparation would be minimal, 1D7 IgG free of contaminating mouse apoE would certainly be preferable for use in receptor binding studies. It is well known that IgG precipitates in 33% saturated ammonium sulfate solutions (28). 1D7 ascites was therefore subjected to precipitation by 33% saturated ammonium sulfate and the pellet was redissolved and dialyzed. The IgG was then purified by affinity chromatography on Protein-A Sepharose and tested for the presence of mouse apoE (Fig. 8) and for its capacity to inhibit binding of 125I-labeled LDL to the LDL receptor (Fig. 9). Unlike the IgG prepared from 1D7 ascites not precipitated with ammonium sulfate that contains a 35-kDa protein which reacts with anti-rat apoE (lane 2, Fig. 8), the IgG fraction of ammonium sulfate-precipitated ascites contains no immunoreactive apoE (lane 4, Fig. 8). Likewise, the IgG from the non-precipitated ascites inhibits LDL binding to the LDL receptor, whereas there is no inhibition with 1D7 IgG prepared from precipitated ascites (Fig. 9). The precipitation does not change the anti-apoE titer of the purified IgG and the precipitated 1D7 continues to inhibit apoE-mediated lipoprotein binding to the LDL receptor (data not shown). These results, on the one hand, indicate again that it is the mouse apoE that is responsible for the inhibition of LDL-receptor binding and, on the other hand, provide a method to eliminate contaminating apoE from 1D7 IgG prepara-
Precipitation of the purified 1D7 IgG preparation is equally effective (data not shown).

DISCUSSION

When we first observed that the anti-apoE Mab 1D7 was able to inhibit the binding of 125I-labeled LDL to the LDL receptor, our initial interpretation was that the Mab was cross-reacting with apoB, the major protein of LDL and known to be the ligand responsible for LDL-receptor binding. This was an attractive hypothesis in that it would provide experimental evidence for a common structure on apoE and apoB which could account for the dual specificity of the LDL receptor. Moreover, if Mab 1D7 did recognize the binding domains of both apoE and apoB, identification of the corresponding epitope in apoB would also be an identification of the apoB receptor-binding domain. We have subsequently shown, however, that the ability of the 1D7 IgG preparation to block LDL-receptor binding is, in fact, due to a cross-reactivity with autologous mouse apoE rather than with human apoB, as we originally thought. Mab 1D7 binds mouse apoE and the resulting immune complexes of apoE-1D7 are co-purified with 1D7 IgG by Protein A-Sepharose immunoaffinity chromatography. The apoE present in the 1D7 IgG preparations can then compete with 125I-labeled LDL for the LDL receptor. We have shown that a 35-kDa protein that is immunoreactive...
Fig. 8. Elimination of apoE from 1D7 IgG preparations by precipitation with ammonium sulfate. Mouse IgG preparations, IgG that had been ammonium sulfate-precipitated, and mouse lipoproteins were immunoblotted as described in Fig. 5. From left to right, anti-apoE Mab 6C5; anti-apoE Mab 1D7; d < 1.210 g/ml mouse lipoproteins; and anti-apoE Mab 1D7 prepared from ascites which had been precipitated at 33% ammonium sulfate (1D7 P). Molecular weight markers are indicated in kDa.

with anti-rat apoE is present in the IgG fractions of 1D7 but not in the IgG preparations of other anti-apoE Mabs which do not influence LDL-receptor binding. According to all evidence, this protein is mouse apoE. Precipitation of 1D7 ascites at 33% saturated ammonium sulfate eliminates both the mouse apoE and the capacity of the IgG to inhibit LDL-receptor binding. Finally, when mouse serum is passed over 1D7-Sepharose, the retained fraction contains mouse apoE and is capable of blocking binding of LDL to the LDL receptor.

It is known that lipid-free apoE is not recognized by the LDL receptor (29). The fact that the mouse apoE in the 1D7 IgG preparation can compete with human LDL for the LDL receptor led us to assume that it is an apoE-containing lipoprotein that is isolated with 1D7 IgG. We did not, however, detect mouse apoA-I, apoA-IV, or apoB in 1D7 IgG that contained both immunoreactive mouse apoE and activity that inhibited LDL-receptor binding. HDL₁, having apoE as the only detectable apolipoprotein, has been shown to represent a major lipoprotein subclass in mouse plasma (T. Innerarity, unpublished results). It may be such a lipoprotein that is the major contaminant of the 1D7 IgG preparations.

The cross-reactivity of Mab 1D7 with mouse apoE is probably of low affinity. Mab 1D7 apparently does not block binding of mouse apoE-containing lipoproteins to the human LDL receptor, presumably because the affinity of Mab 1D7 for mouse apoE is less than the affinity of mouse apoE-containing lipoproteins for the LDL receptor. The low affinity of Mab 1D7 for autologous mouse apoE could explain why we were unable to demonstrate 1D7 IgG in the serum lipoprotein fraction (d < 1.210 g/ml) of hybridoma-bearing mice or to show binding of Mab 1D7 to mouse apoE on Western blots (unpublished results). Nevertheless, the affinity is sufficient to allow the co-purification of mouse apoE with 1D7 IgG during affinity chromatography on Protein A-Sepharose.

The lack of species specificity in the interaction of lipoproteins with the LDL receptor would indicate that both
the ligand-binding domain of the LDL receptor and the receptor-binding domains of apoE and apoB have been maintained during mammalian evolution (30, 31). Mabs against human apoB that block the LDL binding to the LDL receptor have been shown to cross-react with a large panel of heterologous LDL, whereas cross-reactivity was much more limited with Mabs that failed to block LDL receptor binding (32, 33). It is, therefore, probably not by chance that Mab ID7, which reacts with the apoE receptor-binding domain, cross-reacts with mouse apoE and that Mabs specific for other regions of the protein do not show such cross-reactivity.

A question that naturally arises from these observations concerns the generality of the phenomenon and the conditions under which one might anticipate its occurrence. As the Mab forms an immune complex with the self antigen, the problem would presumably be restricted to Mabs specific for antigens that would be present in the ascitic fluid, e.g., plasma proteins or proteins produced locally in the peritoneal cavity. Secondly, antibodies directed against conserved regions of the antigen (e.g., functional domains) would be those most likely to detect a cross-reactivity with the equivalent mouse antigen, as was the case for apoE. While antibodies tend to be produced against epitopes that are not shared between the immunogen and the host, the production of antibodies with reactivity against self-components following immunization with self-antigens in adjuvant or with cross-reacting antigens does occur. Furthermore, it should be emphasized that the reactivity of ID7 with murine apoE was apparently of very low affinity; nevertheless, it resulted in contamination of the IgG preparations with mouse apoE.

We have demonstrated here that a Mab that detects an immunological cross-reactivity between the immunogen and the equivalent mouse protein can lead to the copurification of the mouse protein with the Mab. Furthermore, we have shown that, when one does not have the means to distinguish between the effects attributable to the Mab and those to the contaminating mouse antigen, this can complicate the interpretation of experiments in which the Mab is used as a probe of protein function. At present, there is little reason to believe that the phenomenon described here represents a unique case. However, until IgG preparations of Mabs are systematically tested for the presence of mouse antigen, it is difficult to predict how prevalent it may be.

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