Immunogenicity of homologous low density lipoprotein after methylation, ethylation, acetylation, or carbamylation: generation of antibodies specific for derivatized lysine[1]

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Abstract We previously showed that immunization of guinea pigs with reductively glucosylated guinea pig low density lipoprotein (LDL) or albumin resulted in the formation of antibodies specific for the glucosylated protein. The present studies were done to determine if modifications of homologous LDL or albumin, other than addition of carbohydrate, would also render these proteins immunogenic. We found that derivatization of lysine residues of guinea pig LDL or albumin by carbamylation, acetylation, ethylation, or even methylation rendered them immunogenic in guinea pigs. In addition, the specificity of the antibodies was strikingly influenced by whether modified homologous LDL or modified homologous albumin was used as the immunogen. Antibodies generated against modified LDL were directed almost exclusively against the derivatized lysine residues (i.e., carbamyllysine, acetyllysine, or methyllysine) and hence reacted equivalently with other modified proteins that contained the same lysine derivative. However, antibodies generated against guinea pig albumin (or fibrinogen) modified in the same ways reacted primarily with the modified protein used as immunogen, and not with the free lysine derivative, or with other similarly modified proteins. Each of the modifications referred to above could potentially occur in vivo. Therefore, the findings presented may be relevant to autoantibody formation and immunopathogenetic mechanisms in certain diseases.—**Steinbrecher, U. P., M. Fisher, J. L. Witztum, and L. K. Curtiss.** Immunogenicity of homologous low density lipoprotein after methylation, ethylation, acetylation, or carbamylation: generation of antibodies specific for derivatized lysine. *J. Lipid Res.* 1984. **25:** 1109–1116.

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Autantibodies are believed to play a pathogenetic role in a considerable number of diseases, but the precise mechanism by which autoantibodies form is in most cases obscure (1). An immune response directed against self antigens could arise in immunoregulatory disorders (2), or as a consequence of cross-reactivity between a foreign antigen and host tissues, as has been implicated in the pathogenesis of rheumatic fever or autoimmune endocrine disease (3, 4). Alternatively, if certain host proteins were modified in some fashion as a consequence of disease or drug administration, an immune response directed against the modified protein might result. Our previous studies have established a precedent for this latter mechanism by demonstrating that nonenzymatic glucosylation of homologous low density lipoprotein (LDL) or albumin renders them immunogenic in guinea pigs (5). A limited degree of glucosylation of plasma and structural proteins occurs in normoglycemic individuals, but this may be greatly enhanced by hyperglycemia and hence potentially immunogenic modified proteins may be present in diabetic individuals. In fact, we have demonstrated the presence of antibodies to glucosylated LDL in the plasma of some diabetic individuals (6). It is noteworthy that the antibodies that developed in the immunized guinea pigs, as well as those present in the plasma of diabetics, failed to react with native proteins but were all directed specifically against glucitollysine, which is the glucose-lysine adduct formed by reductive glucosylation. Because of their specificity toward this remarkably small epitope, the antibodies were capable of reacting with many other glucosylated proteins. Similarly, Bassiouny, Rosenberg, and McDonald (7) recently showed that immunization of rats with reductively glucosylated rat collagen also results in the formation of antibodies specific for glucitollysine.

The studies described above indicated that derivatization of lysine residues of homologous proteins with

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Abbreviations: LDL, low density lipoprotein; PAGE, polyacrylamide gel electrophoresis.

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even a monosaccharide could lead to formation of specific antibodies. The present studies were undertaken to determine if modifications of homologous proteins other than addition of carbohydrate would also lead to specific antibody formation in guinea pigs. The results indicate that a modification as small as methylation of epsilon-amino groups of lysine residues of homologous LDL immunogen can lead to specific antibody formation. Ethylation, acetylation, or carbamylation of homologous LDL also resulted in antibody production. In every instance the antibodies were directed against the derivatized lysine moiety, and hence were capable of reacting with a structurally unrelated protein such as albumin that had been similarly modified and thus contained the same lysine derivative. In contrast to these findings with modified LDL, when similarly modified homologous albumin or fibrinogen was used as the immunogen, the antibodies that formed were directed primarily against the immunogen, and not against other similarly modified proteins or the free lysine derivative.

Each of the modifications of native proteins discussed in the present report might be expected to occur in vivo under appropriate conditions (8–15). Our results show that such modified homologous proteins can elicit an antibody response. Such antibodies directed against modified homologous proteins may have important pathophysiologic consequences.

MATERIALS AND METHODS

Materials

Male Hartley guinea pigs were supplied by Charles River Breeding Laboratories, Wilmington, MA. Guinea pig albumin, human albumin, bovine albumin (RIA grade), guinea pig fibrinogen, lysine, polylysine, methyllysine, acetyllysine, valine, citrulline, and trypsin immobilized on Sepharose 4B were all obtained from Sigma Chemical Corp., St. Louis, MO. Homocitrulline was purchased from United States Biochemical Corp., Cleveland, OH, and dimethyllysine from Vega Biochemicals, Tucson, AZ. Sodium cyanoborohydride ($NaCNBH_3$) was from Aldrich Chemical Co., Milwaukee, WI, acetic anhydride from J. T. Baker, Phillipsburg, NJ, and acetaldehyde and formaldehyde from Mallinckrodt, Paris, KY. Goat anti-guinea pig IgG was supplied by United States Biochemical Corp., and carrier-free $Na^{125}I$ by Amersham Corp., Arlington Heights, IL.

Lipoprotein preparation

Human LDL (d 1.019–1.063 g/ml) or guinea pig LDL (d 1.019–1.070 g/ml) was isolated from fasting plasma containing 1 mg/ml ethylenediamine tetraacetic acid (EDTA) by sequential ultracentrifugation as previously described (16). Purity of the LDL was verified by agarose gel electrophoresis, and by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS/PAGE). LDL protein was estimated using the method of Lowry et al. (17), with bovine albumin as the standard. Throughout this report, amounts of LDL will be expressed in terms of the mass of protein.

Modification of proteins

Methylation of proteins was carried out by a modification of the method described by Weisgraber, Innerarity, and Mahley (18). Typically, solid $NaCNBH_3$ (final concentration 50 mM) was added to 10 mg of native protein in phosphate-buffered saline with 0.1% EDTA, pH 7.4 (PBS) at 0°C. Then, a total of 30 μmoles of formaldehyde was added in six aliquots at 10-min intervals with gentle magnetic stirring; excess reagents were removed by dialysis against PBS. Ethylation of LDL, albumin, polylysine, and valine was accomplished using the same procedure except that acetaldehyde was used in place of formaldehyde. Ethylvaline was purified from reactants by chromatography on Dowex 50 (Bio-Rad, Richmond, CA) and quantified by ninhydrin assay (19). Acetylation was performed by sequential addition of acetic anhydride as previously described (20). Proteins were carbamylated by exposure to potassium cyanate (18). The extent of derivatization of LDL or albumin for each type of modification was greater than 60% of lysine residues, as estimated with the trinitrobenzenesulfonic acid assay (21). The modified proteins were used for immunization and for determination of antibody titer and specificity as described below.

To determine if homologous LDL subjected to limited proteolysis would be immunogenic in our model system, we exposed guinea pig LDL (7 mg) to Sepharose-immobilized trypsin for 10 min at 24°C and removed fragments by chromatography on Sephacryl S-300. The material which eluted in the void volume was used for immunization and for antibody titration studies. When this trypsin-treated LDL was analyzed by SDS/PAGE in a 3.5% polyacrylamide gel, 75% of the stainable protein comigrated with native apoB-100 and the remainder appeared in a single band of slightly lower molecular weight. The effect on LDL of digestion by intrinsic serum proteases was tested by mixing 7 mg of LDL with freshly clotted serum and incubating overnight at 37°C. This serum-incubated LDL was then reisolated by ultracentrifugation and used for immunization and antibody titration studies. No discrete low-molecular weight bands were detected by SDS/PAGE in the reisolated serum-incubated LDL.

Immunization protocol

Guinea pigs were immunized by intradermal and foot-pad injection of a total of 150 μg of antigen suspended in 0.8 ml of complete Freund's adjuvant. Two booster doses of 125 μg of antigen suspended in incomplete Freund's adjuvant were given subcutaneously and intramuscularly at 14-day intervals. Serum was obtained for antibody studies 10 days after the final injection. In some cases, animals were immunized with antigen suspended in PBS, without Freund's adjuvant. It should be emphasized that in all cases, only *homologous* proteins, i.e., guinea pig LDL or guinea pig albumin, were used for immunization.

Determination of antibody titer and specificity

Solid-phase radioimmunoassay (RIA) techniques similar to those previously described (4) were utilized for antibody studies. Briefly, 96-well polyvinylchloride microtitration plates (Dynatech, Alexandria, VA) were coated by incubation with 50 μl per well of antigen solution (10 μg/ml in PBS). After 2 hr, wells were aspirated and remaining binding sites on the plastic were blocked with PBS containing 3% goat serum and 3% bovine albumin. For determination of antibody titer, 50 μl of serially diluted serum was added to duplicate wells and incubated overnight. The wells were then aspirated, washed four times, and the amount of immunoglobulin bound was quantified using goat anti-guinea pig IgG that had been radioiodinated to a specific activity of approximately 10,000 cpm/ng with carrier-free $Na^{125}I$ using lactoperoxidase/glucose oxidase (Enzymobeads, Bio-Rad Laboratories, Richmond, CA). The antibody titer was defined as the reciprocal of the greatest dilution of plasma which gave binding three times greater than assay background (determined using nonimmune guinea pig serum at an equivalent dilution). Specificity of the antisera was determined by competition assays in which a fixed, limiting dilution of antiserum was incubated overnight with varying amounts of competitor in duplicate antigen-coated wells; subsequently the amount of antibody binding was determined as above. Results of competition assays are presented as the ratio B/B_0 where B is the radioactivity bound in the presence of the indicated amount of competitor, and B_0 the amount bound in the absence of competitor.

RESULTS

Each of the various chemically or enzymatically modified guinea pig LDL preparations, as well as native guinea pig LDL, were used to immunize three guinea pigs by the standard protocol described in Methods. To examine the importance of the carrier protein in influencing antibody specificity, we also immunized two animals each with carbamylated guinea pig albumin or methylated guinea pig albumin. Finally, to determine if modified LDL or albumin was immunogenic even in the absence of Freund's adjuvant, we immunized two animals each with carbamylated LDL or carbamylated albumin in PBS without adjuvant.

Fig. 1 shows antibody dilution curves with sera from three animals immunized with carbamylated guinea pig LDL emulsified in Freund's adjuvant, and from two animals immunized with carbamylated LDL without adjuvant. The titers (binding 3-fold greater than nonimmune serum) against carbamylated guinea pig LDL were greater than 500,000 in all three animals immunized with carbamylated LDL in Freund's adjuvant, and somewhat lower (100,000) in two animals immunized without adjuvant.

Similar high-titered antisera were also obtained in animals immunized with acetylated, methylated, or ethylated guinea pig LDL, in animals immunized with carbamylated or methylated guinea pig albumin, and in animals immunized with carbamylated guinea pig fibrinogen (**Table 1**). In contrast, no antibodies were detected

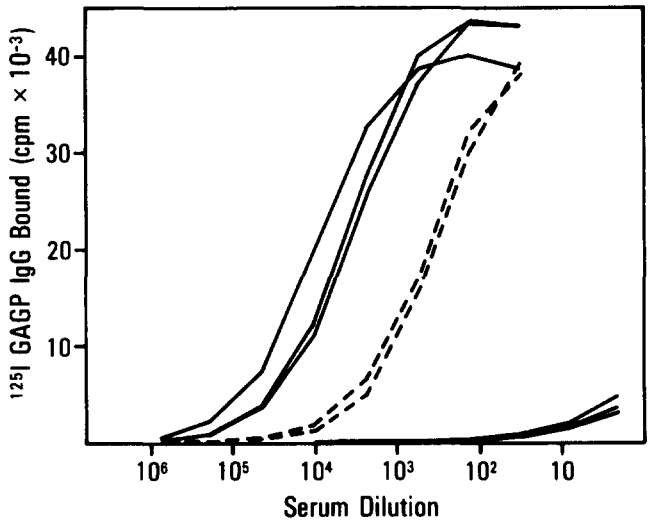


Fig. 1. Antibody dilution curves for antisera to carbamylated guinea pig LDL. Guinea pigs were immunized as described in the methods with carbamylated guinea pig LDL. Serum was obtained 10 days after the second booster immunization. Serial dilutions of serum were incubated in microtitration wells coated with carbamylated guinea pig LDL, and the amount of bound antibody was quantified as described using ^{125}I-labeled goat anti-guinea pig IgG (GAGP IgG). Data are shown for three animals immunized with carbamylated guinea pig LDL emulsified in Freund's adjuvant (solid lines, top tracings), for two animals immunized with carbamylated LDL without adjuvant (broken lines), and for preimmune serum of three animals (bottom tracings). Identical results were obtained using plates coated with carbamylated human LDL.

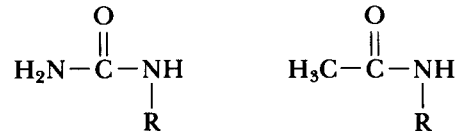
TABLE 1. Antibody titers in guinea pigs immunized with homologous modified protein

Immunizing Agent	Titer
Native LDL (3)	nil
Methyl LDL (3)	$>5 \times 10^5$
Ethyl LDL (3)	$>5 \times 10^5$
Acetyl LDL (3)	$>5 \times 10^5$
Carbamyl LDL (3)	$>5 \times 10^5$
Carbamyl LDL, no adjuvant (2)	1.3×10^5, 3.3×10^4
Trypsin-treated LDL (3)	nil
Serum-incubated LDL (3)	nil
Native albumin (2)	nil
Carbamyl albumin (2)	$>5 \times 10^5$
Methyl albumin (2)	$>5 \times 10^5$
Methyl albumin, no adjuvant (2)	8×10^3
Native fibrinogen (2)	nil
Carbamyl fibrinogen (2)	3×10^5, 8×10^4

Guinea pigs were immunized according to the described protocol with native guinea pig proteins (LDL, albumin, or fibrinogen) or with various modified homologous LDL, albumin, or fibrinogen preparations. Antibody dilution assays were performed with antiserum from each animal titrated against the corresponding immunogen, as illustrated in Fig. 1 for carbamylated LDL antisera. Antibody titer was determined as the reciprocal of the greatest dilution of antiserum which gave binding three times that of assay background (equivalent dilution of nonimmune serum). The number of animals that received a particular immunogen is indicated in parentheses. The titers shown apply to every animal in the group, except that individual values are shown for animals immunized with carbamylated LDL without adjuvant, and with carbamylated fibrinogen.

in animals after immunization with native guinea pig proteins (LDL, albumin, or fibrinogen), or with guinea pig LDL previously subjected to proteolysis with trypsin or serum proteases. These results indicate that simple chemical substituents on the free amino group of lysine residues of homologous proteins can lead to a brisk antibody response.

Competition assays were performed to determine the specificity of the antibodies in all 22 animals with positive titers. Because the antibodies in animals immunized with modified guinea pig LDL were found to react equally with similarly modified guinea pig or human LDL, in some cases modified human LDL was used for coating microtitration plates simply because of its greater availability. However, antisera from animals immunized with modified guinea pig albumin were always tested on plates coated with the same modified guinea pig albumin preparation. Results of typical competition experiments with antisera against chemically modified LDL are shown in **Fig. 2** and **Fig. 3.** As shown in the left panel of Fig. 2, carbamylated guinea pig LDL and carbamylated human LDL were equally effective competitors for antiserum to carbamylated guinea pig LDL whereas native human or guinea pig LDL did not compete. Carbamylated albumin and homocitrulline (which is epsilon-carbamyllysine) were also effective competitors. Citrulline, which differs from homocitrulline by a single CH_2 unit, was a very weak competitor, and acetylated LDL did not compete at all in spite of the structural similarities between acetylated and carbamylated proteins.



carbamylated protein acetylated protein

The specificity of the antisera of each of the other animals immunized with carbamylated LDL was similar. In addition, there was no difference in the specificity of

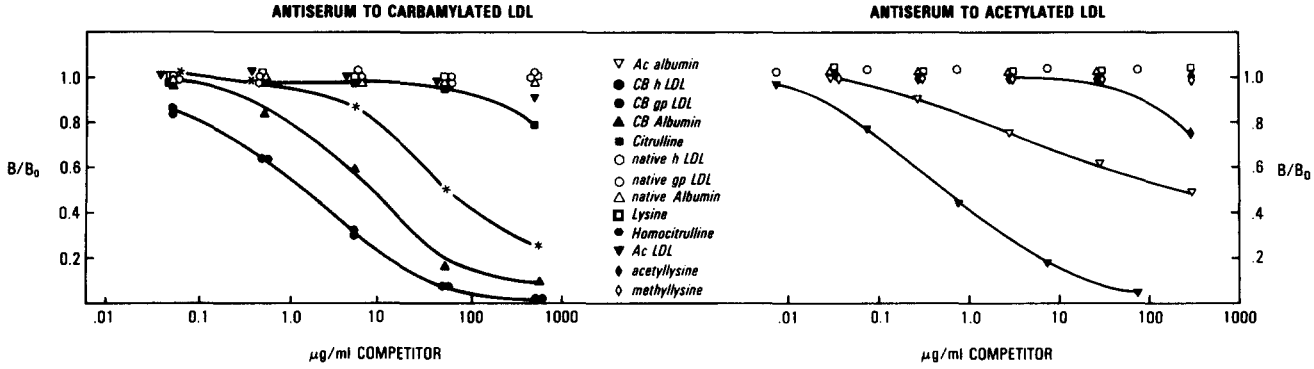


Fig. 2. Specificity of antisera to carbamylated or acetylated guinea pig LDL. Guinea pigs were immunized with carbamylated or acetylated homologous LDL in Freund's adjuvant as described. Antiserum obtained 10 days after the second booster was added to microtitration wells coated with carbamylated human LDL (left panel, antiserum dilution 1:1000) or acetylated guinea pig LDL (right panel, antiserum dilution 1:6000), in the presence of the indicated concentrations of various competitors. After overnight incubation, wells were aspirated, washed four times, and bound immunoglobulin was quantified as described. Results are presented as the ratio B/B_0 where B is the amount of antibody bound in the presence of a given concentration of competitor and B_0 is the amount bound in the absence of competitor. Actual values of B_0 were 30,300 cpm for carbamylated LDL antiserum and 14,200 cpm for acetylated LDL antiserum. Each panel shows results with antiserum from a single animal; very similar patterns of specificity were observed with two antisera from the other animals in each group.

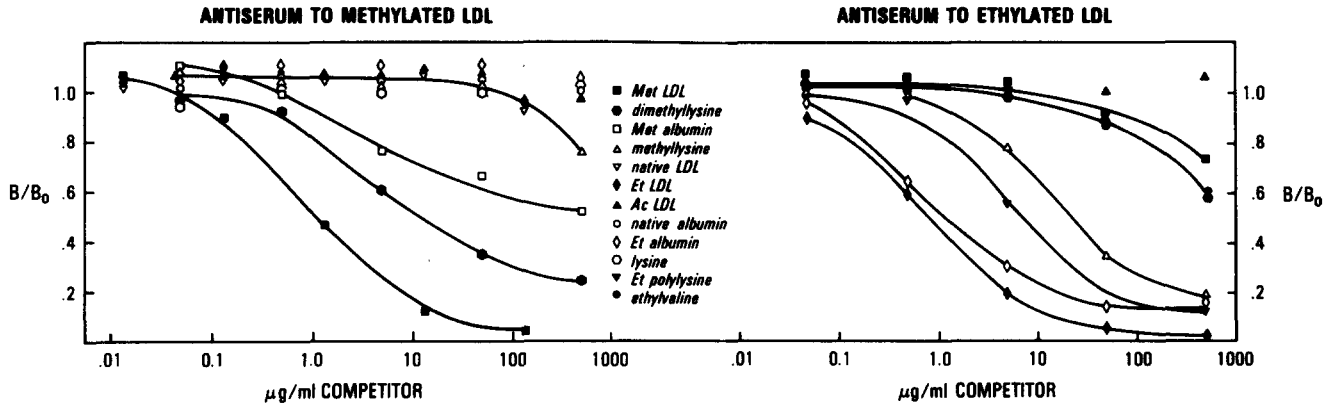


Fig. 3. Specificity of antisera to methylated or ethylated guinea pig LDL. Guinea pigs were immunized with methylated or ethylated homologous LDL in Freund's adjuvant. Antiserum obtained 10 days after the second booster was added at a final dilution of 1:6000 to microtitration wells coated with methylated guinea pig LDL (left panel) or ethylated guinea pig LDL (right panel), in the presence of the indicated concentrations of various competitors. Incubations were carried out as outlined in the methods and legend to Fig. 2. Actual values of B_0 were 8100 cpm for methylated LDL antiserum and 11,000 cpm for ethylated LDL antiserum. Each panel shows the specificity of antiserum from a single animal; very similar results were obtained using antiserum from the two other animals in each group. When antiserum to ethylated LDL was further tested on wells coated with ethylated polylysine rather than ethylated LDL, results were almost identical to those shown in the right panel above.

antisera obtained from animals immunized against carbamylated LDL with or without Freund's adjuvant.

Analogous results were obtained with antisera to acetylated LDL (right panel of Fig. 2), methylated LDL, and ethylated LDL (Fig. 3). In every case, antibodies to modified guinea pig LDL reacted with the free lysine derivative in question, and were capable of recognizing proteins other than guinea pig LDL carrying the same modification. With antisera to carbamylated, methylated, or ethylated LDL, appropriately derivatized lysine competed efficiently for antibody binding. However, with acetylated LDL antiserum, acetyllysine was a comparatively weak competitor. This is probably due in part to a low affinity of the antibodies for the isolated hapten. An additional explanation for this may be that acetylation with acetic anhydride is not absolutely specific for free amino groups, but may result in derivatization of tyrosine residues as well (22). Consequently, antisera to acetylated LDL may contain antibodies specific for acetyltyrosine as well as for acetyllysine.

It is also noteworthy that dimethyllysine was a better competitor than methyllysine for antibodies generated against methylated LDL (Fig. 3, left panel). This may reflect the fact that reductive methylation of LDL yields mainly dimethyllysine (18). Conversely, antibodies to ethylated LDL bound dimethyllysine less efficiently than methyllysine (Fig. 3, right panel). While the proportions of mono- to diethyllysine present on ethylated proteins have not been measured, from this finding we would predict that monoethyllysine predominates. The ability of the antisera to methylated or ethylated LDL to discriminate between methylated and ethylated competitors (Fig. 3) may reflect both this difference in number of substituents per lysine amino group, and the difference in the actual adduct (methyl versus ethyl groups).

In marked contrast to results obtained using modified LDL as the immunogen, when animals were immunized with methylated or carbamylated homologous guinea pig albumin, the resulting antibodies reacted only with the modified guinea pig albumin used for immunization, and did not react with other similarly modified proteins (**Fig. 4**). Antibodies to methylated guinea pig albumin did react slightly with ethylated guinea pig albumin, but not with methylated human albumin. Thus, these antibodies were directed not just to the derivatized lysine residue, but to a larger domain of the modified guinea pig albumin molecule.

To demonstrate that the restricted pattern of antibody specificity found with antisera to derivatized albumin was representative of most types of modified proteins, we examined the specificity of antisera generated against carbamylated guinea pig fibrinogen. The findings closely paralleled those described above for derivatized albumin, in that high-titered antisera were obtained that showed specificity primarily directed against the immunogen, carbamylated guinea pig fibrinogen. Homocitrulline was ineffective in competing for binding to these antisera, while other carbamylated proteins showed only partial competition even when added at very high concentrations (data not shown). This supports the conclusion that the generation of high affinity antibodies specific for derivatized lysine may be a characteristic of modified homologous LDL that is not shared by other modified homologous proteins.

Competition results are shown above with antiserum from only a single animal in each group, but very similar

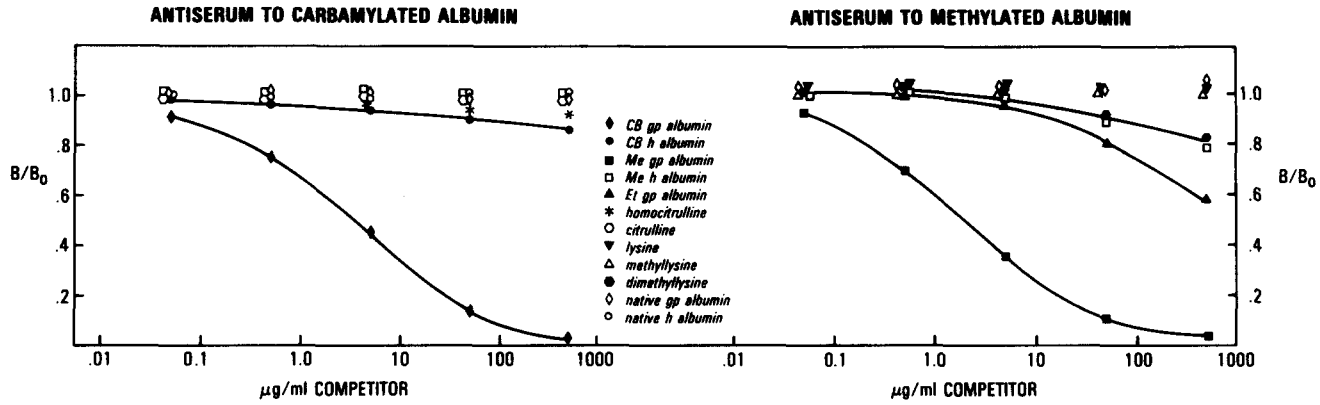


Fig. 4. Specificity of antisera to carbamylated or methylated guinea pig albumin. Guinea pigs were immunized with carbamylated or methylated guinea pig albumin in Freund's adjuvant. Antiserum obtained 10 days after the second booster was added to microtitration wells coated with carbamylated guinea pig albumin (left panel, antiserum dilution 1:1000) or methylated guinea pig albumin (right panel, antiserum dilution 1:4000) in the presence of the indicated concentrations of various competitors. Incubations were carried out as described in legend of Fig. 2. Values of B_0 were 31,100 cpm for carbamylated albumin antiserum and 18,200 cpm for methylated albumin antiserum. Similar competition results were obtained with antiserum from the second animal in each group.

results were obtained with the other animals in the same group indicating that both the immunogenicity of the modifications, and the specificity of the antisera were very reproducible.

DISCUSSION

The striking finding of the present studies was that addition of small chemical substituents to lysine residues of homologous proteins rendered the proteins immunogenic. Modifications as simple as carbamylation, acetylation, ethylation, or even methylation of lysine groups of homologous proteins resulted in the production of specific antibodies against the modified protein. In each case, the antibodies were directed against the modified protein, and not to epitopes of the native protein. We recently encountered a report of remarkably similar findings from as long ago as 1906 by Obermayer and Pick (23), who showed in rabbits that homologous albumin became immunogenic after derivatization of tyrosine residues by nitrosylation.

A second major finding was that the pattern of specificity of the antibodies depended on the modified homologous protein used as immunogen. When homologous LDL was modified and used for immunization, the resulting antibodies showed specificity restricted to the lysine derivative in question, and hence reacted very well with other similarly modified proteins that carried the same lysine derivative. On the other hand, antibodies against modified homologous albumin or modified homologous fibrinogen appeared to bind to a larger domain of the modified protein molecule, in that they recognized primarily the modified guinea pig protein used for immunization. They did not bind effectively to the free lysine derivative, or to other similarly modified proteins.

These results indicate that for a given lysine substituent, the specificity of antisera obtained is influenced by the type of homologous protein used for modification. However, the specificity of antisera to derivatized homologous proteins may also depend on the nature of the substituent. For example, Goodfriend, Levine, and Fasman (24) have shown that antisera from rabbits immunized with angiotensin or vasopressin coupled to homologous albumin were capable of reacting with the free peptide hormone, albeit less efficiently than with the hormone-albumin conjugate.

Several lines of reasoning suggest that these observations on the immunogenicity of modified homologous proteins may be relevant to the pathogenesis of certain disease states. Firstly, each of the modifications described could potentially occur in vivo; for instance, carbamylation of a variety of proteins occurs in renal failure through the action of cyanate derived from urea (8, 9), and ethylation could result from the reaction of acetaldehyde (formed during ethanol oxidation) with proteins (14). Enzyme systems have been described which are capable of transferring methyl or acetyl groups to a large variety of acceptors, including epsilon amino groups of lysine residues (12, 13, 25). Acetylation could also occur by nonenzymatic mechanisms, as a consequence of aspirin administration (10, 11). As yet no direct evidence for these particular modifications of LDL has been obtained in vivo, but we have previously shown that glucosylated LDL, which can be found in diabetics, is immunogenic (4), and furthermore, that glucitollysine-specific antibodies occur in the plasma of some diabetic subjects (5). By analogy, if the modifications of proteins discussed in the present report were to occur in vivo, formation of similar autoantibodies against the modified proteins might also result.

The physiologic consequences of such antibodies might include formation of circulating immune complexes with modified plasma proteins, resulting in vascular injury. In addition, the catabolic fate of proteins might be altered if the immune complexes were rapidly cleared by macrophages (26). In the case of LDL, such a process might lead to foam cell formation. It is also possible that an antibody-mediated attack on modified structural proteins such as basement membrane would ensue. This might occur if, for example, carbamylation of LDL (or other proteins) in uremia led to the formation of antibodies specific for homocitrulline (carbamyllysine). These antibodies could then attack any carbamylated structural or plasma protein, even though the modified "target" proteins were not identical to the protein that initiated antibody formation. Alternatively, antibodies specific to a given modified protein (analogous to the antibodies against modified homologous albumin described earlier) might account for selective tissue injury. For example, after ethanol ingestion, the formation of potentially immunogenic adducts would be favored in liver cells, as these cells would be exposed to the highest concentrations of acetaldehyde (27). If protein-specific antibodies against the adducts resulted, these antibodies could mediate an immune attack directed specifically against modified hepatocyte proteins. Some types of allergic reactions to drugs might also develop as a result of derivatization of lysine residues of proteins by the drug or its metabolites. Additional studies are required to directly address some of these possibilities.

The findings presented in this report are also of importance as examples of the remarkable specificity of the antibodies that are obtained when modified homologous LDL is used as an immunogen. In every animal immunized with chemically modified homologous LDL, a substantial population of antibodies in the resulting antisera reacted specifically with derivatized lysine itself (as the free amino acid). In many cases the antibodies bound equally well to modified albumin and to the modified LDL immunogen, indicating that neighboring sequences and local configurations did not greatly influence antibody binding to modified lysine residues. This technique may enable the facile production of high-titered antisera specific for relatively small molecules, for example, drugs or hormones, simply by conjugating them to *homologous* LDL. The complete absence of reactivity toward native proteins would be advantageous in immunoassay applications. Furthermore, this technique should also be very useful for monoclonal antibody production in mice, in that the use of the homologous LDL carrier would cause a high percentage of splenic B-cells from the immunized mice to produce antibodies specific only to the desired molecule, and not to other regions of the carrier protein. Hence, after fusion to form hybridomas, a large number of the resulting clones would be specific to the desired modification. Indeed, we have shown this to be the case with monoclonal antibodies to glucosylated LDL obtained from mice immunized with glucosylated murine LDL (28).

Our previous reports in guinea pigs (4), mice (28), rabbits (26), and man (5), together with the present studies have shown that immunization with lysine-derivatized homologous LDL leads to the formation of antibodies with specificity toward the lysine derivative and not toward a larger domain of the molecule, as was the case when derivatized homologous albumin or fibrinogen was the immunogen. Whether this effect of homologous LDL carrier is due to the lipid components of LDL (29), to the size of the immunogen, to the presence of apolipoprotein B, or to other factors, remains to be determined. Whatever the precise mechanism, the ability of LDL to effectively stimulate an immune response after subtle modifications of its structure might account for the numerous reports of autoantibodies directed against LDL in patients with myeloma, systemic lupus erythematosus, or diabetes, or in elderly subjects (30–33).

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