Immune Complex Hyperlipidemia Induced by an Apolipoprotein-reactive Immunoglobulin A Paraprotein from a Patient with Multiple Myeloma

Characterization of This Immunoglobulin

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Abstract

An antibodylike paraprotein has been isolated from a patient with multiple myeloma and autoimmune hyperlipoproteinemia. The paraprotein bound to apolipoprotein B (apo B)-containing lipoproteins that formed macromolecular aggregates, and globules thought to be aggregated complexes of lipoproteins and reactive immunoglobulins were observed circulating within the retinal blood vessels of this patient. This binding specificity permitted purification of the paraprotein from both the agglutinated immune complexes and from the plasma.

The protein is an IgA, κ-immunoglobulin which exists primarily in a polymeric state. Capillary immunoprecipitation demonstrated reactivity with very low density lipoproteins (VLDL) and low density proteins (LDL), but not with high density lipoproteins (HDL). Delipidated apo B and apo E, but not apo A or apo C, formed precipitates with this immunoglobulin. In using a radioimmunoassay format, the affinity of the immunoglobulin was greatest for VLDL and decreases sequentially for intermediate density lipoproteins and LDL. No binding occurred with a dispersion of LDL lipids or with HDL. Deglycosylation did not change the binding to LDL. The apolipoproteins B and E bound with similar affinity, but no binding occurred with apo A-I or apo A-II. Weak binding appeared to occur with apo C. This paraprotein immunoprecipitated apo B-containing lipoproteins from all classes of vertebrates tested. Displacement of the lipids of LDL by Triton X-100 resulted in the formation of an apo B–Triton complex which, however, did not bind to the immunoglobulin; apparently the binding site on apo B was lost.

Upon enzymatic digestion with the IgA-specific protease from *Streptococcus sanguis* the immunoglobulin was cleaved into Fc and Fab fragments, and the binding of LDL occurred only with the latter, consistent with the behavior of an immunoglobulin. The immunoreactivity of this paraprotein with apo B and apo E raises the interesting possibility that it may be binding to a site on these apolipoproteins which is reactive with the apo B, E receptor of the plasma membrane, a site which is conserved throughout the vertebrate phylum.

Introduction

The occurrence of hyperlipidemia accompanied by circulating immunoglobulins reactive against plasma lipoproteins has been observed most frequently, but not exclusively, in multiple myeloma. The concept of an autoimmune hyperlipidemia was proposed by Beaumont and Beaumont to explain this phenomenon (1), and numerous examples have been reported (2–5), with either IgG- or IgA-immunoreactive paraproteins predominating. The mechanisms of these diseases are becoming better understood and appear to involve a balance involving variable inhibition of the normal lipoprotein catabolic pathways, removal of lipoprotein immune complexes by macrophage-mediated processes or via the bile, and compensatory acceleration of cellular lipoprotein synthesis and secretion (5–11). Hyperlipidemia may also occur in the presence of a circulating immunoglobulin which binds heparin, thus blocking activation of lipoprotein lipase (9).

This report concerns a subject with autoimmune hyperlipidemia, associated with an IgA myeloma, whose lipoprotein immune complexes formed aggregates that comprised ~20% of the blood volume and were thought to constitute the globules visible in the retinal circulation. We report the characterization of the circulating paraprotein and describe its immunoreactivity with specific apolipoproteins.

Clinical summary

A 74-yr-old man with a 10-yr history of elevated cholesterol was diagnosed 2 yr previously as having a plasma cell myeloma with extensive bone marrow infiltration. Serum protein electrophoresis revealed a monoclonal spike and serum IgA was elevated to 2,720 mg/dl; IgG and IgM were normal. The serum cholesterol was 1,370 mg/dl and triglycerides 3,620 mg/dl. He was not diabetic and had no symptoms of ischemic heart disease. The patient was treated with alkeran and prednisone with an apparent good response, his cholesterol and triglycerides falling to 150 and 457 mg/dl.

4 d before the patient was first seen at The Shand's Hospital of the University of Florida, Gainesville, FL, he had developed lower extremity weakness with bladder incontinence, and his mental status had begun to deteriorate. On admission the patient was lethargic but arousable. He had no xanthomas, arcus cornea, or evidence of retinal lipid deposits. A striking finding on ophthalmoscopic examination of the retinal vessels was the presence of many discrete, white globules flowing rapidly through the arteries and veins (Fig. 1). There were no hemorrhages or exudates. Cardiovascular exam was positive for bruits in the left carotid and bilateral femoral arteries but with good peripheral pulses. The liver and spleen were not enlarged. Neurologically he was oriented only to person and place, and there was diffuse weakness of the distal muscles in the legs with asymmetric tendon reflexes and negative Babinski reflexes. During the next several days he lapsed into unconsciousness.

The important laboratory findings were in the blood. As venous blood was drawn into a syringe it flowed in mottled patterns of white, pink, and red. Upon low-speed centrifugation blood separated into three layers: red cells, clear plasma, and

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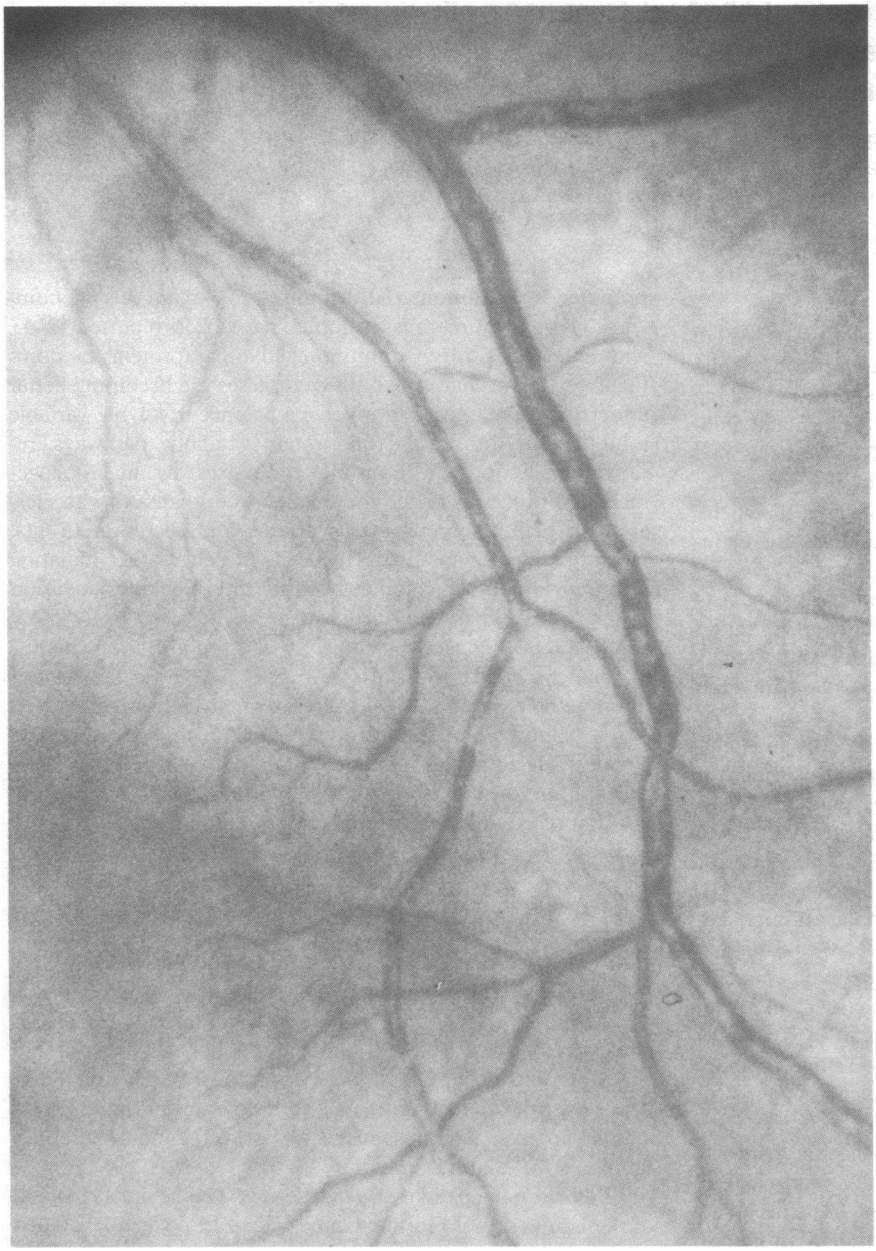

Figure 1. Retinal photograph showing the particulate nature of the globules circulating in the arteries and veins in this subject.

at the top of the tube, a congealed mass of white lipid which would not disperse upon mixing (Fig. 2). This lipid coagulum comprised ~20% of the blood volume. Total plasma triglycerides measured ~3,500 and cholesterol 800 mg/dl. The coagulum-free plasma had triglyceride and cholesterol concentrations of 396 and 85 mg/dl, respectively, and immunoglobulin quantitation revealed IgA 1,118 mg/dl, IgG 270 mg/dl, and IgM 21 mg/dl. The plasma creatinine and liver function parameters were normal.

On the assumption that lipoprotein aggregates were producing a hyperviscous blood, plasmaphereses were performed over several days followed by reinstitution of alkeran and prednisone. The patient sustained a drop in the volume of his plasma lipid coagulum to <10% with a gradual return to a normal mental status; however, his lower extremity paralysis and neurogenic bladder persisted and was thought to be the result of a spinal cord infarction.

A description of the isolation and characterization of the immune complexes follows.

Methods

Large and small very low density, intermediate density, low density, and high density lipoproteins (VLDL, IDL, LDL, and HDL)[1] were isolated from human plasma by sequential differential-density ultracentrifugation as described (12). LDL was delipidated by ether/ethanol

1. *Abbreviations used in this paper:* apo, apolipoprotein; apo B, apolipoprotein B, the major protein component of low density lipoproteins; apo A, apo C, apo E, apo A-I, apo A-II, apo C-II, and apo C-III, apolipoproteins A, C, E, A-I, A-II, C-II, and C-III; HDL, HDL isolated at $1.06 < d < 1.21$ g/ml; IDL isolated at $1.006 < d < 1.02$ g/ml; LDL isolated at $1.02 < d < 1.06$ g/ml; Sie IgA-C, IgA paraprotein recovered from the coagulum; Sie IgA-P, IgA paraprotein recovered from the plasma.

extraction in the presence of 6 M guanidine as described (13), and apolipoprotein B (apo B) was made soluble in 10 mM Tris by the method of Cardin et al. (14). Homogeneity of the myeloma paraprotein and of apo B was assessed by SDS-polyacrylamide gel electrophoresis (15). Protein concentrations were determined by a modification of the method of Lowry described by Markwell et al. (16).

Isolation of IgA from myeloma plasma. After centrifugation of the patient's plasma at 4,000 rpm, the IgA/lipoprotein coagulum was recovered at the meniscus and washed repeatedly in 0.1 M sodium phosphate, 0.15 M NaCl, 0.02% NaN_3, pH 7.2 (PBS). The washed IgA/lipoprotein coagulum was dissociated in 0.1 M glycine, 0.1 M NaCl, 0.02% NaN_3, pH 10.8. The solution density was adjusted to 1.20 g/ml by addition of solid KBr before a 20-h centrifugation at 40,000 rpm. The supernatant lipoprotein fraction was titrated to neutrality, made 20% with sucrose, and frozen. The infranatant fraction, which is very nearly pure IgA recovered from the coagulum (hereafter Sie IgA-C), was dialyzed against PBS and frozen.

A second fraction of IgA was isolated from the coagulum-free plasma. The plasma was adjusted to a density of 1.08 g/ml with KBr, then diluted 1:1 with phosphate-buffered KBr, pH 7.2, of the same density, and filtered. 1 mg of exogenous LDL protein was added for each milliliter of original plasma. The mixture was made 5% with sucrose, then centrifuged for 20 h at 40,000 rpm. The supernatant fraction, which consisted of soluble IgA/LDL complexes, was dialyzed against glycine buffer, pH 10.8, and adjusted to density 1.20 g/ml with KBr prior to a second centrifugation at 40,000 rpm. The lipoprotein supernatant was discarded and the free IgA from plasma, now in the infranatant fraction (hereafter Sie IgA-P) was dialyzed into PBS. IgA was further purified for some experiments on LDL-Sepharose 4B immunoadsorption columns (17).

Preparation of apolipoproteins. The lipoprotein fraction isolated from the IgA/lipoprotein coagulum at a density of 1.20 g/ml was delipidated by either tetramethyl urea or ether/ethanol extraction, and the apolipoproteins that remained soluble were examined by 7.5% polyacrylamide gel electrophoresis in the presence of 10 M urea (18) or by isoelectric focusing in the presence of 6 M urea using pH 4–6 ampholytes (Bio-Rad Laboratories, Richmond, CA). The insoluble apoprotein fraction was resolubilized in 3% SDS and examined on 4% polyacrylamide/SDS gels (15).

Apolipoproteins were isolated from normal human plasma. Apo B, in a soluble state was prepared from LDL by the method of Cardin et al. (14). Apolipoproteins apo A-I and apo A-II were prepared from HDL and apo C and apo E from VLDL by ethanol/diethylether delipidation in a 0.1 M Tris, pH 8 buffer containing guanidine-HCl, 3 or 6 M, respectively. The proteins were then isolated in the same Tris buffer but with 6 M urea using, respectively, either Sephacryl S-200 or Sepharose CL-6B columns, and the purity of the proteins was determined by polyacrylamide gel electrophoresis (18). Apolipoproteins from VLDL and HDL were also purified directly from polyacrylamide gels by electrophoretic elution as recently described (19).

Radioiodination. LDL, and Sie Fab and Sie Fc fragments were iodinated enzymatically according to directions from the manufacturer (Enzymobead Reagent, Bio-Rad Laboratories). Enzymatic iodination yielded an incorporation of ^{125}I into LDL with specific activities on the order of 110–200 cpm/ng of protein. The Fab and Fc fragments of Sie IgA iodinated much less efficiently; the specific activities of both were ~30 cpm/ng.

Production of antiserum. Antisera to IgA and LDL were raised in female New Zealand white rabbits. All antisera and normal human plasma used in the immunoassays were cleared of lipoproteins by ultracentrifugation. Rabbit polyvalent anti-human LDL-apo B antiserum has been described elsewhere (Kilgore, L. L., B. W. Patterson, and W. R. Fisher, submitted for publication.)

Capillary precipitation. Capillary precipitation of Sie IgA, 2–5 mg/ml, with apo B, apo E, and the various classes of lipoproteins, 1–3 mg/ml, was performed in microcapillary tubes.

Radioimmunoassay. The working buffer was 0.04 M Tris, 0.09 M NaCl, 0.001 M EDTA, 0.02% NaN_3, pH 8.1, and all IgA and lipoprotein samples were dialyzed against it. The final assay buffer contained 2% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO; fatty acid and globulin free).

Dilutions of Sie IgA-C and Sie IgA-P from 100 ng/ml to 100 μg/ml, were titrated against ^{125}I-LDL. Approximately 50 ng of radiolabeled LDL (~6,500 cpm) and 5 μl of lipoprotein-free human plasma in a 70-μl total volume were added to 200-μl aliquots of IgA at the appropriate concentrations. Samples and controls for nonspecific binding, made up to a final volume of 370 μl, were incubated 72 h at 4°C. The IgA–^{125}I-LDL complexes were precipitated by the addition of 50 μl of lipoprotein-free rabbit anti-human IgA, in a 1:10 dilution. After an additional 24-h incubation, the samples were centrifuged, and the supernates were discarded. The precipitates were washed with 0.01 M Tris, 0.15 M NaCl, pH 8.1, then counted in a gamma counter. All experiments were run in triplicate. The results were plotted as $(B - B_{ns})/(TC - B_{ns})$ vs. log IgA, where B = counts bound in the presence of IgA, B_{ns} = counts bound in the absence of IgA, which are <2% of total counts, and TC = total counts present.

The concentration of Sie IgA-C yielding a 60–65% binding of radiolabeled LDL was utilized for the displacement assay. In this assay, progressive amounts of unlabeled lipoprotein or apolipoprotein in 100-μl vol were added to 200-μl aliquots of IgA at the appropriate concentration, followed by a 70-μl vol containing 50 ng of ^{125}I-LDL and 5 μl of lipoprotein-free human plasma. Controls for the binding of ^{125}I-LDL in the absence of IgA were included. The samples, run in triplicate, were incubated and processed as outlined above for the titration assay. The results were plotted as $(B - B_{ns})/(B_0 - B_{ns})$ vs. log unlabeled apolipoprotein concentration, where B = counts bound in the presence of unlabeled lipoprotein, B_0 = counts bound in the absence of unlabeled lipoprotein, and B_{ns} = counts precipitated nonspecifically.

The influence of the nonionic detergent Triton X-100 (Sigma Chemical Co.) on the reactivity of Sie IgA with ^{125}I-LDL was assessed by measuring the formation of precipitable immune complex in the presence of varying concentrations of Triton. Specifically, a 200-μl aliquot containing ~6,000 ng of Sie IgA-C was added to 100 μl of diluted Triton X-100, and followed by 70 μl containing 50 ng of ^{125}I-LDL and 5 μl of lipoprotein-free human plasma. Final Triton X-100 dilutions ranged from 0.002% to 0.2%. The samples were incubated for 72 h, anti-human IgA was added, and they were processed as outlined above. The results were plotted as $(B - B_{ns})/(TC - B_{ns})$ vs. % Triton X-100.

To examine in greater detail how Triton X-100 alters the interaction of Sie IgA with ^{125}I-LDL, samples of each reactant were incubated with 0.2% Triton X-100 at 4°C overnight. Triton treated Sie IgA-C was then titrated against untreated ^{125}I-LDL in the 2% BSA/Tris buffer as outlined above, or untreated Sie IgA was titrated against Triton treated ^{125}I-LDL. In either case the final concentrations of Triton X-100 remained well below the critical micellar concentration for the surfactant, ~0.02% (20). A parallel titration, employing nonpretreated reactants, was performed in the presence of 0.2% Triton X-100 in the assay buffer and a fourth titration was performed in the total absence of the detergent.

Binding studies with Fab and Fc fragments. Fab and Fc fragments of the paraprotein were prepared by hydrolysis of the protein with *Streptococcus sanguis* IgA protease isolated as previously described by Plaut et al. (21, 22). 15 mg of Sie IgA myeloma protein in 3 ml of PBS, pH 7.0 was incubated for 48 h with 300 μl of the *S. sanguis* IgA protease preparation.

The IgA myeloma digest was dialyzed against Tris-phosphate, 0.015 M, pH 8.0, and chromatographed on a DEAE cellulose column (2 × 10 cm, Whatman Inc., Clifton, NJ), starting in the same buffer. Proteins were eluted with a continuous pH/salt gradient to Tris-phosphate, 0.3 M, pH 4.0. Two peaks were detected and pooled as separate fractions; these contained Fab and Fc fragments as identified by their reactions to anti-κ and anti-IgA antisera (Atlantic Antibodies, Scarborough, ME) in Ouchterlony analysis (22).

The abilities of the Fab and Fc fragments to bind LDL were

assessed. A 50-μl vol of 2% BSA/Tris buffer containing either 1 μg of ^{125}I-Fab or 1 μg ^{125}I-Fc was added to 100 μg of LDL protein in a 100-μl vol. The samples were incubated for 18 h at 4°C before the addition of 100 μl of lipoprotein-free rabbit anti-human LDL serum in a 1:4 dilution. After an additional 6-h incubation at 4°C, the samples were centrifuged, and the pellets were washed once and counted for 5 min. The results were expressed as percentages of total counts recovered in the precipitate.

Results

Physical studies. The physical properties of the coagulum recovered from the patient's plasma (Fig. 2) made it possible to recover his coagulated immune complexes in an almost pure state upon repeated washings with PBS and low-speed centrifugation. The coagulum was readily dissociable at pH 10.8, and further purification could be achieved through a series of cycles in which the coagulum was dispersed at pH 10.8 and permitted to reaggregate at pH 6.5. The density of the solution of dispersed coagulum at pH 10.8 was increased to 1.20 g/ml by addition of KBr and the lipoprotein, and immunoglobulin components were readily separated by ultracentrifugation.

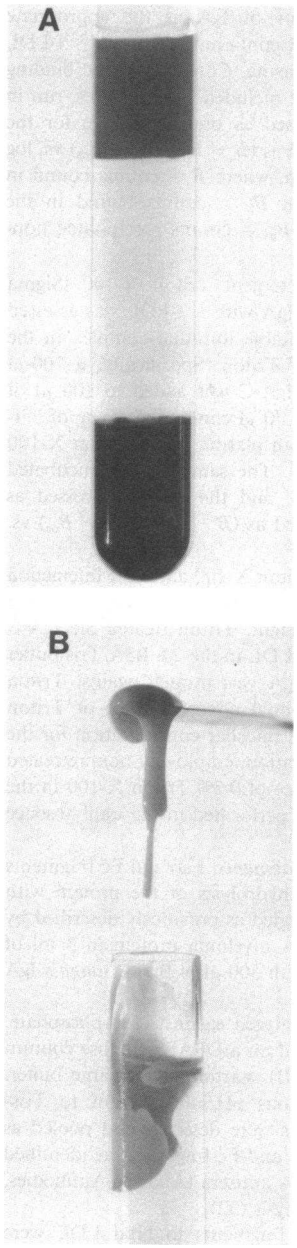

Figure 2. (*A*) Low-speed centrifugation of the subject's heparinized plasma showing the accumulated lipoprotein coagulum at the top of the tube. (*B*) Demonstration of the gellike properties of the coagulum.

The patient's plasma was also processed. After adjusting it to pH 10.8 and adding KBr to *d* 1.20 g/ml, the plasma lipoproteins were floated ultracentrifugally and recovered. Reactive immunoglobulins from the plasma were also recovered by ultracentrifugational flotation subsequent to addition of exogenous LDL. The largely soluble IgA–LDL complexes recovered were dispersed at pH 10.8 and separated as described above.

Each of these fractions was characterized. The lipoproteins isolated from the coagulum and from the plasma were studied by analytical ultracentrifugation. The total lipoprotein fraction from plasma was centrifuged in a solvent of *d* 1.20 g/ml and showed a floating LDL peak with an $s_{1.20}$ value of −39 S, typical of normal LDL (Fig. 3 *A*) (23). This peak was preceded by a small quantity of VLDL which is heterogeneous in size. Of interest is the absence of any HDL in this or any other fraction.

The lipoproteins isolated from the coagulum are shown in Fig. 3 *B* during flotation in a 1.06-g/ml solvent. The peak fraction seen in the Schlieren pattern floats with an s_f of 10 S, and this LDL is preceded by a large zone of VLDL, and perhaps chylomicrons, much of which accumulated at the meniscus. In the coagulum, VLDL (and chylomicrons) comprise the major recovered lipoproteins.

Electrophoresis on 4% polyacrylamide gels run in SDS demonstrated the presence of apo B-100 in the lipoproteins recovered from the coagulum. Small amounts of a protein having the mobility of intestinally derived apo B-48 were also present.

When the coagulum-derived lipoproteins were extracted with ethanol/diethylether and electrophoresed in 9.5% acrylamide gels in a urea buffer, the C apoproteins were identified

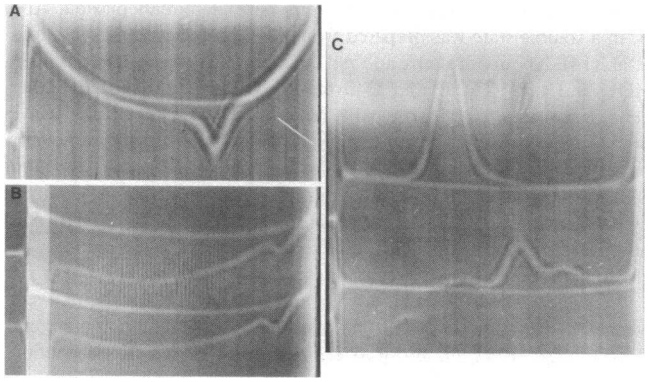

Figure 3. Analytical ultracentrifugation of lipoproteins and immunoglobulins. (*A*) Lipoproteins recovered from the coagulum-free plasma by ultracentrifugation as described. The flotation run was made in a KBr buffer, pH 6.5, of *d* 1.20 g/ml. Speed was 42,040 rpm at 25°C. The photograph was taken at 12 min. (*B*) Lipoproteins recovered from the coagulum after its dissociation at pH 10.8, as described. This analytical run was made with the solution at a density of 1.06 g/ml. The upper sample was run in a solution at pH 10.8, the lower at pH 6.5. The photograph was taken at 24 min. (*C*) Sedimentation velocity run on Sie IgA recovered from the coagulum as described. The solvent was 0.2 M sodium phosphate, pH 7.6, and protein concentration was 10 mg/ml. The upper sample was in 0.02 M dithiothreitol. Speed 42,040 rpm at 25°C. The photograph was taken at 98 min.

(18). Similarly extracted proteins were also isoelectrically focused on single-dimensional 7% gels with the demonstration of apo E isoforms as well as apo A-I.[2]

The immunoglobulins of the coagulum were examined by immunoelectrophoresis which demonstrated reactivity only with anti-IgA and anti-κ chain serum (performed at The Shand's Hospital). This paraprotein was designated Sie IgA, taking the first three letters from the name of the subject. Upon electrophoresis in 5% polyacrylamide gels in SDS (Fig. 4 *A*), Sie IgA-C and Sie IgA-P each showed multiple bands with the former appearing enriched in larger molecular weight components. Upon reduction both samples resolved into two bands which migrated as expected for heavy and light chains.

Fig. 3 *C* demonstrates the Schlieren pattern obtained during sedimentation velocity studies of Sie IgA-C demonstrating its polymeric state, the peaks sedimenting as 7, 10, and 13 S. Upon reduction a single peak is seen. By contrast, in IgA from plasma, the 7-S component was the most prominent, preceded by a large 11-S peak but no resolved 13-S material.

Sie IgA was further purified on a Sepharose 4B column to which LDL had been coupled (17). After being adsorbed to the column, washed with saline, and eluted with pH 10.8 glycine buffer, Sie IgA-C was again shown to be polymeric with predominant 10- and 13-S species upon analytical ultracentrifugation.

Immunologic studies with Sie IgA. As an initial assessment of the immunoreactivity of Sie IgA, capillary precipitation was utilized with VLDL, LDL, and HDL from normal donors and paraprotein recovered from the coagulum (Fig. 4 *B*). As seen, precipitation occurred with VLDL and LDL only. By contrast, IgA isolated from the patient's plasma did not form visible aggregates with any class of lipoproteins, perhaps reflecting its enrichment with monomeric IgA.

Capillary precipitation was used to screen the apolipoprotein reactivity of Sie IgA. Fig. 5 shows immune precipitates which formed with apo B and two different preparations of apo E isoproteins: E, 2:2 and E, 3:3 kindly provided by Dr. Robert Mahley, Gladstone Foundation, San Francisco, CA. No immune precipitates formed with the A or C apolipoproteins.

The lipoprotein-binding characteristics of Sie IgA were investigated by using an adaptation of the classical double-antibody radioimmunoassay in which ^{125}I-LDL served as the tracer, Sie IgA was the primary antibody and rabbit anti-human IgA was the second or precipitating antibody. The results of representative titrations of Sie IgA isolated from the coagulum and from the plasma are shown in Fig. 6 *A*. The titrations are very similar with 50% binding of the tracer achieved with ~630 ng of IgA.

Fig. 6 *B* illustrates the relative efficiency of each of the major classes of human serum lipoproteins in the displacement of ^{125}I-LDL tracer from Sie IgA-C. Large VLDL, at a protein concentration of 1×10^{-4} g/liter, displaced 50% of ^{125}I-LDL tracer at 1.25×10^{-4} g/liter, whereas $\sim 2.5 \times 10^{-4}$ g/liter of small VLDL and over 5×10^{-4} g/liter of LDL protein were required to achieve the same end. HDL did not displace in the range assayed, nor did a sonicated suspension of solvent extracted LDL lipids.

2. The subject's plasma contained essentially no appreciable HDL, even though the apo A-I concentration was >50% of normal and apo A-II was normal as measured by radioimmunoassay (courtesy of Dr. Angelo Scanu, University of Chicago, IL). An explanation for the lack of normal HDL is unclear.

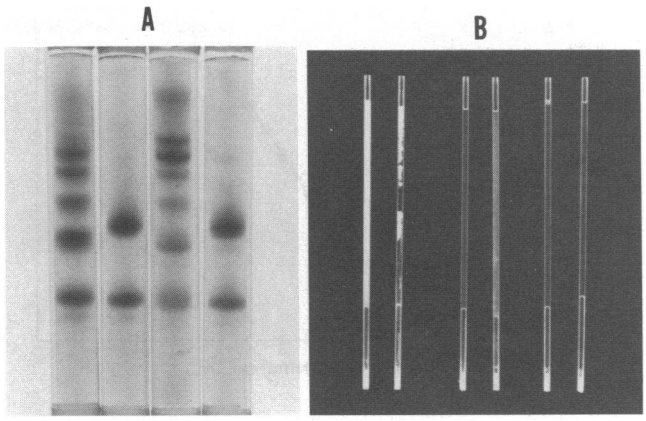

Figure 4. (*A*) Sie IgA electrophoresed in 5% acrylamide gels with SDS. From the left, the second and fourth gels were treated with 2-mercaptoethanol. Gels 1 and 2 were IgA from the plasma and gels 3 and 4 from the coagulum. The gels were stained with Coomassie Blue. (*B*) Capillary precipitation of normal plasma lipoproteins in 0.1 M $NaHCO_3$, 0.5 M NaCl upon the addition of Sie IgA-C to tubes 2, 4, and 6. Tubes 1 and 2 contain VLDL, tubes 3 and 4 contain LDL, and tubes 5 and 6 contain HDL.

Fig. 7 shows the displacement of ^{125}I-LDL from Sie IgA-C using purified apolipoproteins, the concentrations of the displacing apoproteins being expressed on a molar basis. The similar displacement with apo B and apo E was a consistent finding with many preparations of these proteins; however, because of uncertainty in the molecular weight of apo B and its aggregation in solution, the apparent identity of the displacement curves may be fortuitous. No displacement was ever observed with apo A-I or apo A-II. The reactivity of apo C with Sie IgA was examined in 11 experiments utilizing either column purified bulk apo C or apo C-II and apo C-III purified by electroelution from acrylamide gels. In each case weak displacement occurred with displacement curves shifted about two orders of magnitude to the right of those for apo B and apo E. In only one case did displacement approach completion (shown in Fig. 7).

If one calculates the difference in quantity of apo B needed to give 50% displacement using native LDL (Fig. 6) and after delipidation in guanidine-HCl and resuspension in an aqueous

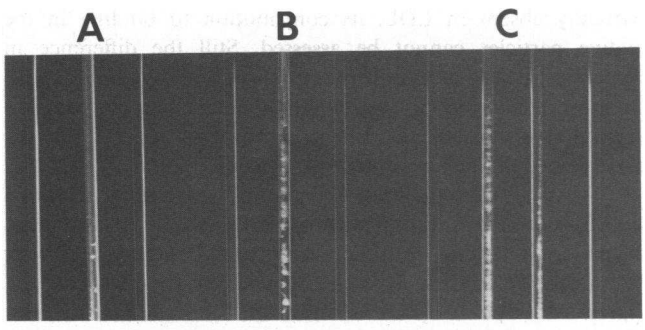

Figure 5. Capillary precipitation of (*A*) deglycosylated LDL plus Sie IgA-C, flanked on the left by deglycosylated LDL in buffer and on the right by Sie IgA-C in buffer; (*B*) delipidated apo B in 10 mM Tris buffer plus Sie IgA, flanked on the left by apo B in buffer and on the right side Sie IgA in buffer; (*C*) The four capillaries from the left are apo E, 2:2 in buffer; apo E, 2:2 plus Sie IgA; apo E, 3:3 plus Sie IgA, and Sie IgA in buffer.

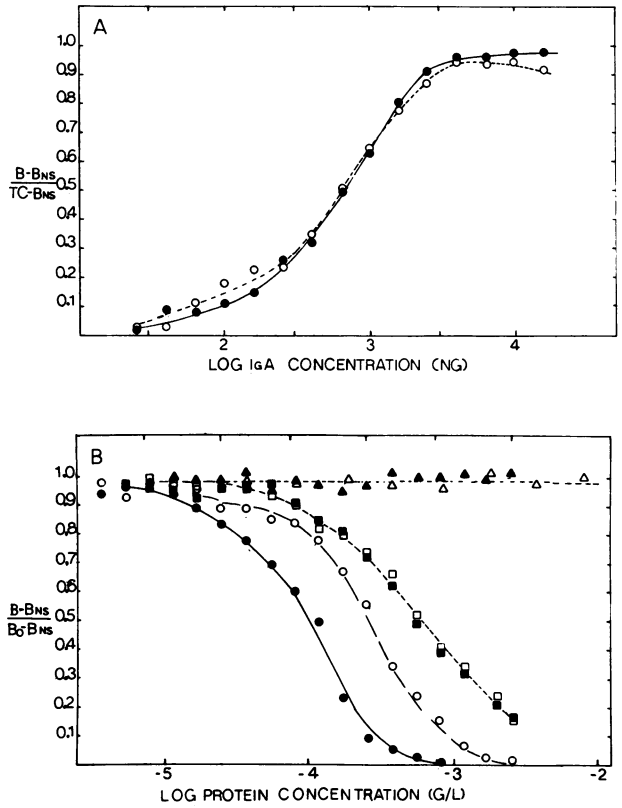

Figure 6. Examination of Sie IgA binding characteristics by using a radioimmunoassay protocol. (*A*) Titration of Sie IgA-C (●) and Sie IgA-P (○) against 50 ng of ^{125}I-LDL protein as described in the text. (*B*) Displacement of ^{125}I-LDL from Sie IgA-C by large VLDL (●), small VLDL (○), IDL (■), LDL (□), HDL (▲), and a sonicated suspension of ether/ethanol extracted LDL lipids (△).

buffer (Fig. 7) there is a two-order of magnitude increased efficiency of displacement by apo B in native LDL as compared with the delipidated and denatured protein. Uncertainties confound interpretation of this difference. It is unknown how many Sie IgA binding sites are present on LDL, and the apo B in the denatured, delipidated preparation is highly aggregated by viscometric and sedimentation equilibrium criteria (Fisher, W. R., unpublished observations). Because, by comparison to apo B, apo E is a minor component in chylomicron and virtually absent in LDL, its contribution to binding in the native particles cannot be assessed. Still the difference in apparent binding affinity between native and denatured apo B is most likely due to a change in antigenic conformation arising as a consequence of the removal of apo B from the lipid milieu of the lipoprotein particle.

To address this question, an attempt was made to examine apo B after reconstitution within a hydrophobic environment in which it retains immunoreactivity as demonstrated with polyvalent antibodies. This can be achieved by displacing the lipids from apo B by the addition of Triton X-100 to LDL (24, 25). In the presence of 0.2% Triton X-100 Apo B is delipidated, and its physical state is altered; however, it is virtually identical to native LDL from an immunologic standpoint (manuscript submitted for publication).

The effect of Triton X-100 on the activity of Sie IgA-C is summarized in Fig. 8 *A*. Triton X-100 at concentrations above the critical micellar concentration of the detergent completely

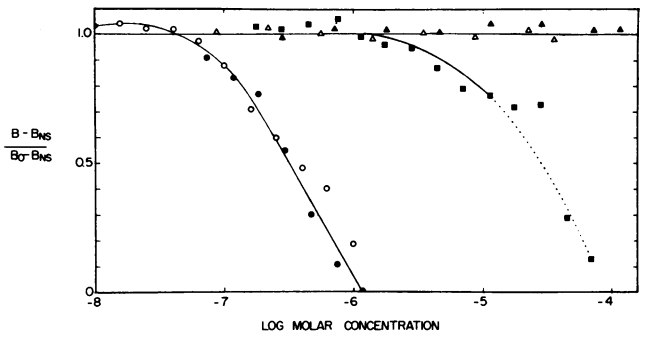

Figure 7. Displacement of ^{125}I-LDL from Sie IgA-C by delipidated apolipoproteins. (○) apo B,[3] (●) apo E, (■) apo C isolated in bulk from a Sephacryl S-200 column, (▲) apo A-I, and (△) apo A-II. Purified apo C-II and apo C-III mimic bulk apo C in this assay.

abrogated binding to an ^{125}I-LDL tracer. Pretreatment of Sie IgA-C with 0.2% Triton X-100 did not impair its recognition of the tracer as long as the final assay concentration of the detergent remained well below its CMC (Fig. 8 *B*). By contrast, similar pretreatment of the ^{125}I-LDL tracer with the detergent sharply reduced recognition by the paraprotein. Thus, the reactive conformation of the binding site on Sie IgA appears unperturbed after exposure to supramicellar concentration of Triton X-100. By contrast, these same conditions that remove >95% of the lipid on LDL, with profound conformational changes of the protein (25), give rise to a Triton–Apo B complex which is no longer a reactive ligand for the immunoprotein.

Thus, by contrast to the persisting reactivity with polyvalent antisera, the antigenic site of Sie IgA is lost after delipidation with Triton X-100, suggesting that this site is either readily disordered or buried by the surfactant.

To examine the nature of the Sie IgA binding site on apo B, LDL was incubated with a mixture of glycosidases from *Streptococcus pneumoniae* to remove essentially all of its carbohydrate, preparation II (26). This deglycosylated LDL was first tested with the Sie IgA in a capillary precipitation assay with the formation of a copious precipitate (Fig. 5 *A*). This modified LDL was also compared to native LDL as a displacing protein in a radioimmunoassay-type assay, and both apoproteins displaced the LDL tracer similarly. Apparently the carbohydrate is not an important participant in the binding of Sie IgA.

Sie IgA is a myeloma paraprotein with demonstrated binding specificity. To establish its similarity to a true antibody, it was necessary to determine whether its ligand binding site is located on the Fab fragment. Accordingly, Sie IgA was cleaved utilizing the *S. sanguis* IgA protease with recovery of Fab and Fc fragments. These fragments were radioiodinated, and 1 μg of radioiodinated fragment was incubated with 100 μg of LDL before the precipitation of the LDL with antiserum. Under these conditions ~47% of the radioactivity associated with ^{125}I-Sie Fab co-precipitated whereas only 3% of ^{125}I-Sie Fc associated counts were similarly recovered. Increasing the amount of LDL used in the assay did not increase the level of binding of ^{125}I-Fab; thus it appears that ~50% of the label is present on nonreactive components, presumably denatured Fab fragments.

Sie IgA is thus an immunoglobulin that binds apo B, and

3. A molecular weight of 250,000 was assumed for apo B (29).

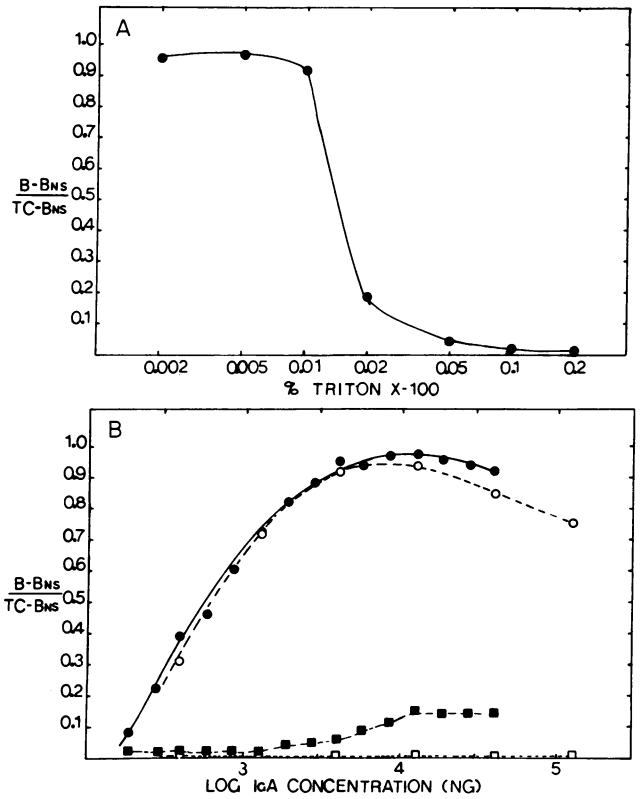

Figure 8. The effect of Triton X-100 on the binding of Sie IgA-C to ^{125}I-LDL using a radioimmunoassay protocol. (*A*) 6 μg of Sie IgA-C was incubated with 50 ng of ^{125}I-LDL in the presence of increasing concentrations of Triton X-100 as described in the text. (*B*) Titration of Sie IgA-C against ^{125}I-LDL in the presence of 2% BSA (●), after pretreatment of Sie IgA-C with 0.2% Triton X-100 (○), after similar pretreatment of ^{125}I-LDL (■), and in the presence of 0.2% Triton X-100 (□).

presumably apo E, through a site on the Fab fragment. This immunoglobulin is derived from a myeloma. Though disorders of lipoprotein metabolism are not a general feature of multiple myeloma, it seemed useful to document that myeloma derived immunoglobulins do not generally demonstrate the reactivity observed with Sie IgA. Accordingly, five additional IgA multiple myeloma sera were examined by immune titration in the same manner as with Sie IgA, carrying the titration over an IgA concentration range of 0.25–160 μg. None showed any reactivity with ^{125}I-LDL (not shown).

In that Sie IgA is an immunoreactive paraprotein though not a classic antibody, it was of interest to define the phylogenic span of this reactivity. Accordingly, blood was obtained from the following animals: mammals (human, horse, cow, goat, rabbit, rat, mouse), chicken, alligator, frog, shark, and fish (flounder). Total lipoproteins were recovered by ultracentrifugation after adjusting the density to 1.20 g/ml, and the lipoproteins were immunoprecipitated with Sie IgA-C. The immunoprecipitates were solubilized in SDS and electrophoresed on polyacrylamide gels in SDS. In each sample these gels demonstrated the presence of a major large molecular weight protein in the lipoprotein immune precipitate that comigrated with human apo B, in addition to several unidentified smaller proteins. This is strong presumptive evidence that Sie IgA recognizes a common structural determinant in an apo B-like protein of all animal species tested.

Discussion

The occurrence of aggregated complexes of lipoproteins and reactive immunoglobulins of large enough size so that they can be seen circulating through blood vessels is an uncommon finding. The presence of these circulating complexes was associated with deterioration in mental function, fortunately with a return to normal mentation as the concentration of circulating complexes decreased.

The composition of the immune complex has been determined to consist of IgA reactive with an apolipoprotein B and E containing lipoproteins; thus this patient is one of a growing number of individuals with an autoimmune hyperlipidemia. The strong propensity of this subject's IgA to form stable complexes in a specific manner with VLDL, IDL, and LDL was evidenced by the huge quantity of such complexes in the patient's circulation. The polymeric state of Sie IgA-C is consistent with that found in many other IgA myelomas (27).

The specificity of the binding of Sie IgA with apo B is independent of its carbohydrate moieties; however, lipid may play a role in fostering a favored antigen conformation for the apolipoproteins.

By contrast to its effective binding with apo B and E, there appears to be a very weak association between Sie IgA and the apo C. The nature of this affinity is unclear. Bulk apo C, apo C-II, and apo C-III, exhaustively purified by chromatography and electrophoresis, cause weak displacement of ^{125}I-LDL from Sie IgA (Fig. 7). In a further attempt to remove trace high-affinity immunoreactive fragments of apo B or apo E, apo C was passed over a Sie IgA-Sepharose 4B immunoadsorbent column that had sufficient binding capacity to remove small quantities of high-affinity contaminants in the amounts to be anticipated as judged by the total immune displacement of the apo C preparation. Nonetheless, the unbound apo C fraction retained immune displacement ability identical to Fig. 7. Despite these precautions, it is possible that a trace contaminant may be responsible for the weak displacement activity. Alternatively, Sie IgA may recognize apo C, weakly, perhaps through a conformational determinant, or the apo C may interact with the ^{125}I-LDL tracer to cause a loss of affinity for Sie IgA. These possibilities can not be distinguished at present.

Sie IgA has antibodylike properties which were exploited in the purification protocol for the paraprotein. The purified Sie IgA-C readily bound normal large and small VLDL, IDL, and LDL and failed to bind HDL. The observation that binding of Sie IgA to LDL occurs through the Fab portion provides added justification for considering it to be an antibodylike protein. The finding that triglyceride-rich lipoproteins, both large and small, interact more strongly than LDL suggests that the paraprotein recognizes differences in apolipoprotein structure among these various lipoproteins. Whether the enhanced binding seen with VLDL solely reflects conformational differences in apo B or some synergistic enhancement of binding brought about by the presence of apo E or even the apo C is unknown. Possible reactivity with the smaller form of apo B, apo B-48, which is present in chylomicrons, has not been tested (28).

One of the major differences distinguishing Sie IgA from a traditional antibody is its reactivity with an apo B-like protein found to exist in a sampling of animals throughout the vertebrate kingdom. Thus Sie IgA recognizes a common site expressed in all of these lipoproteins and conserved throughout much of vertebrate evolution. Apo E, the other ligand for Sie

IgA, apparently is a protein whose presence has only been reported in mammals. Its evolutionary relationship to apo B is conjectural, but the reactive domain seen by Sie IgA in both proteins appears in apo B at a much earlier evolutionary stage.

In concluding, it should be noted that though a number of immunoreactive paraproteins giving rise to autoimmune hyperlipidemias have been reported, in those instances where the lipoprotein ligand has been identified it has generally been a lipid, and to our knowledge this is only the second case where apo B has been specifically implicated as the reactive ligand (4). This is the only instance where a myeloma paraprotein is reactive with a domain that is common to apo B and apo E and appears to be conserved throughout the vertebrate animal kingdom. One wonders if Sie IgA may not be recognizing that site on these apoproteins which is reactive with the apo B, E receptor of the plasma membrane, and thus is conserved during evolution.

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References

1. Beaumont, J. L., and V. Beaumont. 1977. Autoimmune hyperlipidemia. *Atherosclerosis.* 26:405–418.

2. Riesen, W., and G. Noseda. 1975. Antibodies against lipoproteins in man, occurrence and biological significance. *Klin. Wochenschr.* 53: 353–361.

3. Taylor, J. S., L. A. Lewis, J. D. Battle, Jr., A. Butkus, A. L. Robertson, S. Deodhar, and H. H. Roenigk, Jr. 1978. Plane xanthoma and multiple myeloma with lipoprotein–paraprotein complexing. *Arch. Dermatol.* 114:425–431.

4. Riesen, W., G. Noseda, and R. Butler. 1971. Human myeloma proteins which bind B-lipoproteins. 19th Annual Colloquium Protides of the Biological Fluids. Brugge, Belgium, 273–278.

5. Groszek, E., J. J. Abrams, and S. M. Grundy. 1981. Normolipidemic planar xanthomatosis associated with benign monoclonal gammopathy. *Metab. Clin. Exp.* 30:927–935.

6. Baudet, M. F., C. Dachet, and J. L. Beaumont. 1980. Interaction between fibroblasts, lipoproteins and three antilipoproteins IgA kappa. *Clin. Exp. Immunol.* 39:455–460.

7. Dachet, C., M. F. Baudet, and J. L. Beaumont. 1979. Cholesterol synthesis by human fibroblasts in the presence of LDL and anti-LDL IgA. *Biomedicine (Paris).* 31:80–82.

8. Ho, K. J., V. G. deWolfe, W. Siler, and L. A. Lewis. 1976. Cholesterol dynamics in autoimmune hyperlipidemia. *J. Lab. Clin. Med.* 88:769–779.

9. Glueck, H. J., M. R. MacKenzie, and C. J. Glueck. 1972. Crystalline IgG protein in multiple myeloma: identification effects on coagulation and on lipoprotein metabolism. *J. Lab. Clin. Med.* 79: 731–744.

10. Cortese, C., B. L. Lewis, N. E. Miller, M. A. Peyman, S. N. Rao, B. Slavin, U. Sule, P. R. Turner, G. Utermann, A. J. Wing, M. Weight, and R. Wootton. 1982. Myelomatosis with type III hyperlipoproteinemia. Clinical and metabolic studies. *N. Engl. J. Med.* 307: 79–83.

11. Socken, D. J., E. S. Simms, B. R. Nagy, M. M. Fisher, and B. J. Underdown. 1981. Secretory component-dependent hepatic transport of IgA antibody–antigen complexes. *J. Immunol.* 127:316–319.

12. deLalla, O. F., and J. W. Gofman. 1954. Ultracentrifugal analysis of serum lipoproteins. *Methods Biochem. Anal.* 1:459–478.

13. Shireman, R., L. L. Kilgore, and W. R. Fisher. 1977. Solubilization of apolipoprotein B and its specific binding to the cellular receptor for low density lipoprotein. *Proc. Natl. Acad. Sci. USA.* 74: 5150–5154.

14. Cardin, A. D., K. R. Witt, C. L. Barnhart, and R. L. Jackson. 1982. Sulfhydryl chemistry and solubility properties of human plasma apolipoprotein B. *Biochemistry.* 21:4503–4511.

15. Fehrnstrom, H., and U. Moberg. 1977. SDS and conventional polyacrylamide gel electrophoresis with KBR 2117 multiphor. LKB Application Note 306:2–11. LKB-Produkter AB, Bromma, Sweden.

16. Markwell, M. A. K., S. M. Haas, L. L. Bieber, and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* 87:206–210.

17. Cuatrecasas, P., M. Wilchek, and C. B. Anfinsen. 1968. Selective enzyme purification by affinity chromatography. *Biochemistry.* 61:636–643.

18. Herbert, P. N., R. S. Shulman, R. I. Levy, and D. S. Fredrickson. 1973. Fractionation of the C-apoproteins from human plasma very low density lipoproteins: artifactual polymorphism from carbamylation in urea-containing solutions. *J. Biol. Chem.* 248:4941–4946.

19. Kilgore, L. L., J. L. Rogers, B. W. Patterson, N. H. Miller, and W. R. Fisher. 1985. The determination of specific radioactivity of proteins eluted from polyacrylamide gels, utilizing a fluorescamine assay. *Anal. Biochem.* 145:113–119.

20. Kushner, L. M., and W. D. Hubbard. 1954. Viscometric and turbidimetric measurements on dilute aqueous solutions of a nonionic detergent. *J. Phys. Chem.* 58:1163–1167.

21. Plaut, A. G., R. J. Genco, and T. B. Tomasi. 1974. Isolation of an enzyme from *Streptococcus sanguis* which specifically cleaves IgA. *J. Immunol.* 113:289–291.

22. Plaut, A. G., J. V. Gilbert, and I. Heller. 1978. Assay and properties of IgA protease of *Streptococcus sanguis. In* Secretory Immunity and Infection. J. McGee, editor. Plenum Press, New York. 489–495.

23. Fisher, W. R., M. E. Granade, and J. L. Mauldin. 1971. Hydrodynamic studies of human low density lipoproteins. Evaluation of the diffusion coefficient and the preferential hydration. *Biochemistry.* 10:1622–1629.

24. Ikai, A., and M. Hasegawa. 1978. Dimeric nature of apo-low density lipoprotein extracted with Triton X-100. *J. Biochem.* 83:755–759.

25. Patterson, B. W., L. L. Kilgore, P. W. Chun, and W. R. Fisher. 1984. Structural studies on apolipoprotein B: controllable heterogeneity of the complex formed with the surfactant, Triton X-100. *J. Lipid Res.* 25:763–769.

26. Shireman, R. B., and W. R. Fisher. 1979. The absence of a role for the carbohydrate moiety in the binding of apolipoprotein B to the Low density lipoprotein receptor. *Biochim. Biophys. Acta.* 572: 537–540.

27. Fine, J. M., P. Lambin, and D. Frommel. 1973. Size heterogeneity of human IgA myeloma proteins: relationships between polymers and "J" chains. *Biomedicine (Paris).* 18:145–151.

28. Krishnaiah, K. V., L. F. Walker, J. Borensztajn, G. Schonfeld, and G. S. Getz. 1980. ApoB variant derived from rat intestine. *Proc. Natl. Acad. Sci. USA.* 77:3806–3810.

29. Smith, R., J. R. Dawson, and C. Tanford. 1972. The size and number of polypeptide chains in human serum low density lipoproteins. *J. Biol. Chem.* 247:3376–3381.