Autoimmune Hyperlipidemia with Inhibitory Monoclonal Antibodies against Low Density Lipoprotein Binding to Fibroblasts in a Case with Multiple Myeloma

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The mechanism of severe hyperlipidemia in a 54-year-old woman with multiple myeloma and monoclonal immunoglobulin A (IgA) gammopathy was investigated. Her plasma total cholesterol and triglyceride concentrations were 29.7 mmol/l (1,150 mg/dl) and 11.9 mmol/l (1,060 mg/dl), respectively. Western blot analysis demonstrated that her low density lipoproteins (LDL) contained IgA. This IgA also was shown to bind to control LDL and inhibited 125I-LDL binding to fibroblasts, suggesting that by binding to the LDL, IgA interfered with LDL binding to LDL receptors. These findings indicate that an inhibitory monoclonal IgA against LDL binding may explain, at least in part, the severe hyperlipidemia observed in this case.

(Key words: xanthomatosis, immunoglobulin A (IgA) gammopathy, low density lipoproteins (LDL) receptor, mechanism, lipoprotein)

Introduction

Severe hyperlipoproteinemia with increased plasma concentrations of cholesterol and triglyceride is caused by various kinds of primary and secondary etiologies such as apo E2/E2 homozygotes (1, 2) or apoE deficiency (3, 4) as the primary cause or hypothyroidism or diabetic hyperlipidemia as the secondary cause. Among these, the presence of autoimmunity has been noted as a rare cause of severe hyperlipidemia. The concept of autoimmune hyperlipidemia was proposed originally by Beaumont et al (5). Since then, hyperlipidemic patients with autoantibodies to circulating lipoproteins as well as enzymes related to lipoprotein metabolism have been documented. However, the mechanism remains speculative except in a few cases. Corsini et al have reported one patient with autoantibodies to the low density lipoproteins (LDL) receptor (6) and, we have previously described a patient with autoimmune hyperchylomicronemia due to autoantibodies against lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) (7).

The current report presents a patient with multiple myeloma associated with severe hyperlipoproteinemia, xanthomatosis, and monoclonal immunoglobulin A (IgA) gammopathy, whose clinical findings have been previously described (8). Although the mechanism in which antibody inhibits the LDL binding to the LDL receptor in cells is suspected as a cause of hyperlipidemia, the presence of such antibodies has not been clarified in similar cases previously reported (9–11). Here, for the first time, we have identified an inhibitory antibody against LDL binding to fibroblasts in the present case.

Case Report

The proband was a 54-year-old Japanese woman, who was initially admitted to the National Fukuoka Central Hospital in 1987 at the age of 48 years with a pathologic fracture of the neck of the left femur due to an osseous xanthoma (8). Physical examination on the first admission revealed widespread xanthomatosis involving the elbows, knees, palms, interdigital regions, inguinal region, chest, and hips. In addition, the ocular fundi were filled with xanthomas. Examination of a bone marrow specimen demonstrated that foam cells accounted for 8% of the cell population and plasma cells for 6%. No hepatomegaly or splenomegaly was observed. Laboratory tests revealed a normocytic anemia (hemoglobin: 92 g/l) and severe hyperlipidemia (total cholesterol: 29.7 mmol/l (1,150 mg/dl), triglyceride 11.9 mmol/l (1,060 mg/dl), high-density lipoprotein (HDL) cholesterol: 1.24 mmol/l (48 mg/dl)). The electrophoretic separation of serum lipoproteins on agarose gel on
admission had revealed no definite broad beta band. Analysis of
the apo E phenotype showed E3/E2. LPL and HTGL activities
were both normal. There were no signs of atherosclerotic
cardiovascular diseases, diabetes mellitus, thyroid dysfunction,
or renal disease.

Since her first admission, the patient stopped drinking alco-
hol and she has been treated with a low calorie, low cholesterol
diet (1,600 kcal/day, fat 20 g/day, cholesterol 250 mg/day) and
lipid-lowering agents including probucol, cholestyramine, clofi-
brate, niceritrol and pravastatin. These treatments reduced the
patient’s serum total cholesterol concentrations to 6.5–7.7
mmol/l and her triglyceride concentrations to 1.5–3.4 mmol/l.
The size and number of xanthomas also decreased. In 1989, a
monoclonal IgA gammopathy was diagnosed. The levels of
IgA, immunoglobulin G (IgG), and immunoglobulin M (IgM)
were 6.23, 8.40, and 0.88 g/l, respectively. Bone marrow
examination revealed that the percentage of plasma cells was
23% and the subject was diagnosed with multiple myeloma.
In accordance with the increase in monoclonal IgA, plasma lipopro-
tein concentrations gradually increased. In 1993, her concentra-
tions of IgA (kappa type), serum cholesterol and serum triglyceride were 26.1 g/l, 19.8 mmol/l and 3.7 mmol/l, respec-
tively despite the continuation of hypolipidemic therapy. Then,
MP treatment (melphalan 6 mg, prednisolone 30 mg for 5
consecutive days) was started principally once in one month in
1993. Subsequently, serum IgA levels decreased and total
cholesterol and triglyceride also decreased in spite of stopping
the hyperlipidemic treatment. The serum levels of IgA, total
cholesterol, triglyceride were followed in the period from
1983–1997 (Fig. 1). As shown in Fig. 1, the levels of serum
cholesterol and triglyceride were parallel with the levels of IgA.

Methods

Plasma lipoprotein characterization
The patient’s blood was collected in tubes containing
Na₂EDTA after 12 h of fasting. The plasma was recovered after
centrifugation at 3,000 rpm for 15 minutes at 4°C. Plasma
cholesterol and triglyceride values were determined by
enzymatic methods (12). HDL-cholesterol was quantitated by
the heparin-manganese precipitation method (12). Apolipoprotein concentrations were measured using the single
radial immunodiffusion method (13). Plasma lipoproteins were
separated by ultracentrifugation at densities of 1.006, 1.063,
1.125 and 1.21 for the very low density lipoprotein (VLDL),
LDL, HDL₂, and HDL₃ fractions, respectively.

Western blot analysis
Five mg of LDL (d: 1.019–1.063) from a control and the
patient were subjected to a non-denaturing polyacrylamide
gradient gel (PAGG) electrophoresis with a linear gradient of 4
to 30% (PAA4/30, Pharmacia-LKB, Uppsala, Sweden). The
gels were stained with 50% methanol/9% acetic acid/0.1%
Coomassie blue R250 (wt/vol). The separated proteins were
also transferred to a nitrocellulose membrane (Bio-Rad, Rich-
mond, CA) according to a method reported previously (14). The
blots were incubated with peroxidase-conjugated goat anti-
human IgA antibody (Zymed, South San Francisco, CA) for 1
hour. After extensive washing with 0.05% polysorbate 20
(Tween 20) in phosphate-buffered saline (PBS), the blots were

Figure 1. Serum levels of IgA, total cholesterol, and triglyceride in the period from 1983–1997. The levels of
serum cholesterol and triglyceride were parallel with the levels of IgA. The dosage per day of medications is
indicated in each box. MP Tx: melphalan 6 mg and prednisolone 30 mg per day for 5 consecutive days, T-CHOL:
colorized using 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Nacalai Tesque, Kyoto).

To study the binding of IgA to LDL, lipoprotein-deficient serum (LPDS) from the patient or a control subject was incubated with 5 μg of control LDL for 2 hours at room temperature, then subjected to non-denaturing polyacrylamide gradient gel electrophoresis (PAAAg/30). Next, the gels were transferred to a nitrocellulose membrane and incubated with anti-human IgA as described above.

**IgA purification**

IgA was partially purified from serum using Jacalin (15). In brief, serum was applied to Agarose-Jacalin (Vector, Burlingame, CA, USA), and then IgA fraction was eluted with 0.1 mol/l melibiose. Eluted IgA fractions were dialyzed in PBS for 48 hours. Control human IgA was obtained from Sigma Chemical Co. (St. Louis, MO). The purity of the patient’s IgA was ascertained by SDS-PAG electrophoresis and was almost the same as that of the control IgA. The concentrations of IgA were measured by nephelometric analysis.

**LDL receptor binding assay**

LDL receptor binding assay was performed as previously reported (16, 17). Briefly, the cultured normal skin fibroblasts were washed twice with MEM and preincubated for 48 hours with MEM containing 10% (v/v) LPDS. On the day of the experiment, the binding of 125I-LDL was measured by incubating cells at 4°C for 1 hour. Cell association and degradation of 125I-LDL in fibroblasts were determined after 4 hours of incubation at 37°C with 125I-LDL. After washing the cells with washing buffer (0.15 mol/l NaCl, 50 mmol/l Tris-HCl, pH 7.4) three times, the cells were dissolved in 1.0 ml of 0.1 N NaOH and the radioactivity was measured as cell association or binding of lipoproteins. The degradation of 125I-LDL was quantitated by the measurement of trichloroacetic acid-soluble noniodide radioactivity in the media after incubation. Specific values were determined by subtracting the values in the presence of 40-fold excess amounts of unlabeled LDL.

**Results**

**Lipoprotein profiles**

The administration of serum lipid-lowering agents could not be discontinued in this patient because of the severe hyperlipoproteinemia and xanthomatosis. Accordingly, the lipoprotein profile of this patient was obtained during the administration of a combination of multiple hypolipidemic agents (clofibrate, 600 mg/day; cholestyramine, 12 g/day; niacin, 1 g/day; probucol, 750 mg/day; and pravastatin, 10 mg/day). Serum cholesterol and triglyceride concentrations were 9.0 mmol/l (348 mg/dl) and 1.6 mmol/l (141 mg/dl), respectively. VLDL, LDL, HDL2, and HDL3 cholesterol levels were 0.23, 6.9, 0.74, and 1.11 mmol/l, respectively. Apolipoprotein A-I, A-II, B, C-II, C-III, and E levels were 0.88, 0.41, 1.53, 0.061, 0.108, and 0.105 g/l, respectively. The serum concentrations of LDL cholesterol, apo B, and apo E were resistant to such intensive treatment and remained elevated, while the level of apo A-I was below the level observed in 14 normal controls (mean ± SD control ranges for apo A-I, A-II, B, C-II, C-III, and E were 1.49 ± 0.22, 0.38 ± 0.06, 0.91 ± 0.25, 0.037 ± 0.012, 0.083 ± 0.027 and 0.041 ± 0.011 g/l, respectively).

**Detection of IgA in the LDL fraction of the patient**

The LDL fraction of the patient was shown by western blot analysis to contain IgA, while the LDL fraction from control subjects contained little IgA (Fig. 2). The VLDL fraction from the patient also contained IgA. However, the HDL fraction from both the patient and control did not contain IgA (data not shown).

**IgA binding to LDL**

LPDS from the patient or a control subject was incubated with control LDL for 2 hours at room temperature. The mixture was applied to a polyacrylamide gradient gel and transferred to a nitrocellulose membrane as described in the Methods. The membrane was analyzed using anti-human IgA, revealing that patient IgA bound to LDL after preincubation with patient LPDS and control LPDS, whereas LDL preincubated with control LPDS was not associated with IgA. These data suggest that the patient’s IgA bound to LDL (Fig. 3).

**Competition study on LDL binding to LDL receptors of fibroblasts by LPDS or purified IgA**

The biological function of the IgA was then analyzed. LPDS...
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Figure 3. Immunoblotting detection of the patient’s IgA bound to the LDL fraction. Five microgram of LDL was preincubated with LPDS from the patient or the control (IgA concentrations were adjusted to 4 \( \mu \)g) for 2 hours, then applied to PAGEG electrophoresis and transferred to a nitrocellulose membrane for 48 hours at 100 mA. The nitrocellulose membrane was incubated with peroxidase-conjugated anti-human IgA and colorized by DAB. The results show that IgA of the patient was associated with LDL after preincubation with the patient’s LPDS, whereas LDL preincubated with control LPDS was not associated with IgA. A: control LPDS, B: patient LPDS.

Figure 4. Effects of control and patient IgA on binding and degradation of \( ^{125} \)I-LDL in fibroblasts. Fibroblasts were preincubated with normal human LPDS for 48 hours. On the day of the experiment, the medium was changed. Next, 5 \( \mu \)g/mL of \( ^{125} \)I-LDL was added to the fibroblasts with or without the amounts of control or patient IgA as indicated in the figure and incubated for 1 hour for binding and 4 hours for degradation. Binding and degradation were determined as described in the Methods.

The current study has clarified the mechanism of severe hyperlipidemia in a patient who presented with monoclonal IgA gammopathy. Analysis of the patient’s IgA revealed that it from the patient was added to media containing 5 \( \mu \)g of \( ^{125} \)I-LDL. Both the binding and degradation of \( ^{125} \)I-LDL in fibroblasts were markedly reduced compared with those obtained by the addition of control LPDS. Partially purified IgA from the patient also inhibited the binding and degradation of \( ^{125} \)I-LDL in fibroblasts (Fig. 4).

In order to study whether or not the patient’s IgA bound to LDL receptors on fibroblasts, LPDS from a control and the patient was preincubated with fibroblasts for 1 hour at 37°C; next, the medium was removed. \( ^{125} \)I-LDL was then added and fibroblasts were incubated at 37°C for 4 hours. There was no significant difference in the cell association of \( ^{125} \)I-LDL in the fibroblasts preincubated with LPDS from a control subject versus that from the patient (1,100 ± 85 vs 1,065 ± 76 ng LDL/mg of cell proteins, respectively). These findings suggest IgA does not act as an autoantibody to the LDL receptor as reported by Corsini et al (6).

Next, the binding site of IgA to LDL was investigated. Control VLDL was delipidated with ethanol/ether (v/v:3/1) and subjected to SDS-PAGE electrophoresis. The gel was then transferred to a nitrocellulose membrane. The membrane was incubated with 10% LPDS from the subject and a control for 24 hours. Proteins were subsequently detected with peroxidase-conjugated anti-human IgA. No bands were detected in membranes incubated with LPDS from the patient and a control, suggesting that the IgA did not bind apoVLDL (data not shown).

**Discussion**

The current study has clarified the mechanism of severe hyperlipidemia in a patient who presented with monoclonal IgA gammopathy. Analysis of the patient’s IgA revealed that it
inhibited LDL binding to the LDL receptor.

Before the administration of lipid-lowering agents, this patient exhibited markedly elevated serum total cholesterol and triglyceride concentrations with systemic xanthomatosis. Even when we analyzed the lipoprotein levels during the administration of hypolipidemic medications, the plasma LDL cholesterol concentrations were still high.

Furthermore, we investigated the role of monoclonal IgA in the hyperlipidemia of this patient. The presence of immunoglobulin and lipoprotein (Ig-Lp) complex in multiple myeloma or paraglobulinemia has been reported in several studies (1, 9–11, 18, 19). Autoantibodies to LP-receptors, their ligands, or lipolytic enzymes in plasma have been suggested as basic components of the mechanism of autoimmune hyperlipidemia. However, in most of these reports, it still is unclear why Ig-Lp complexes cause hyperlipidemia, and there have been very few reports addressing the role of the Ig-Lp complex in the pathophysiology of hyperlipidemia (2, 3). In some cases the Ig-Lp complex does not interfere with the metabolism of LDL (20). Baudet et al reported that IgG from a patient with multiple myeloma bound to LDL and inhibited LDL degradation in fibroblasts (21). However, they also reported that the LDL binding to fibroblasts was not inhibited by patient’s IgG. These data suggest that the Ig-Lp complex can bind to the LDL receptor because the patient’s IgG did not bind to the receptor binding site of the lipoproteins. Cortese et al have reported (22) two patients with type III hyperlipoproteinemia associated with myelomatisis and investigated the metabolism of LDL in a kinetic study. Although they reported that LDL separated from the subjects was not degraded in blood lymphocytes and did not interfere with the degradation of normal LDL, it was not clear whether or not the IgA from the subjects itself inhibited the LDL binding. Our case will be the first documentation of a monoclonal IgA that inhibits the binding of LDL to LDL receptors. VLDL from our patient also contained IgA, thus, interference of the VLDL metabolism by IgA was the cause of severe hypercholesterolemia and hypertriglyceridemia at the beginning of her disease.

Because the patient’s IgA significantly inhibited the binding of LDL to fibroblasts, the binding site of the IgA to LDL was estimated to be in apoB. However, the IgA in this case did not react to apoVLDL which includes apo B, apoE and apo C; therefore, IgA may react to the lipid component near the LDL receptor binding site.

Moriyama et al (23) recently reported the apoE gene abnormality of the apoE phenotype. Although they found that apo E is a novel variant (apoE2 Fukuoka) in this case, recombinant apoE2 Fukuoka produced in COS-1 cells showed almost the same binding activity to the LDL receptor on human skin fibroblasts as compared with recombinant apoE3; accordingly they concluded that apoE2 Fukuoka was not the primary cause of the hyperlipoproteinemia observed in this case.

Treatment of the multiple myeloma progressively reduced the concentrations of monoclonal IgA, total cholesterol and triglycerides in this patient without any medications for hyperlipidemia and the levels of serum cholesterol and triglyceride were parallel with the levels of IgA. This finding also suggests that the pathophysiology of hyperlipidemia in this patient involved the monoclonal IgA.

In summary, we reported a patient with autoimmune hyperlipidemia, whose immunoglobulin bound to the binding site for LDL on the LDL receptor. This mechanism may explain the undetermined cause of hyperlipidemia in other cases with autoimmune hyperlipidemia.

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