Coexisting Type III Hyperlipoproteinemia and Familial Hypercholesterolemia: A Case Report

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A 39-year-old man presented with type III hyperlipoproteinemia in association with heterozygous familial hypercholesterolemia (FH). He had extensive tuberous xanthomas over the knees and elbows and xanthomas in the Achilles tendons. He also had palmar xanthomas. He exhibited severe hypercholesterolemia and hypertriglyceridemia. This patient was heterozygous for FH, as evidenced by low low-density lipoprotein (LDL) receptor function on lymphocytes, and had type III hyperlipoproteinemia, as determined by apolipoprotein (apo) E phenotype 2/2 in isoelectric focusing of the E isoproteins and the presence of a broad β band on electrophoresis. Because therapy consisting of diet restrictions and lipid-lowering agents such as clinofibrate and niceritrol did not decrease serum total cholesterol ([TC] 15.26 mmol/L) and triglyceride ([TG] 10.79 mmol/L) levels effectively, the patient underwent plasmapheresis once every 2 weeks using a dextran sulfate–cellulose column. Repeated plasmapheresis markedly reduced serum TC and TG and induced complete regression of the palmar xanthoma after 6 months. The severity of tuberous xanthomas on the knees and elbows was reduced after 2.5 years. After plasmapheresis, TC decreased to 1.94 mmol/L from 10.40 mmol/L and TG decreased to 0.33 mmol/L from 7.90 mmol/L. Plasmapheresis performed with a dextran sulfate–cellulose column was highly effective in removing the lipoprotein-remnant particles in this patient, leading to generalized improvement in the lipoprotein profile.

TYPE III HYPERLIPOPROTEINEMIA is a relatively rare disease characterized by hypercholesterolemia and hypertriglyceridemia, which are thought to accelerate the development of atherosclerosis in affected patients.[1] Its primary cause is believed to be an aberration in the genetic makeup of apolipoprotein (apo) E.[2]

We observed a patient with apo E phenotype 2/2 who had type III hyperlipoproteinemia in association with familial hypercholesterolemia (FH). After he failed to respond to dietary and drug therapy, plasmapheresis was instituted.

CASE REPORT

History

A 39-year-old man was referred to the internal medicine clinic of Nagoya City University Medical School Hospital in 1980 for treatment of hyperlipidemia. He was 180 cm tall and weighed 75 kg. His body mass index (weight in kilograms divided by height in meters squared) was 23.2.

Palmar xanthoma was observed, appearing as yellowish deposits of lipid in the palmar creases. Bilateral xanthomas on the elbows and knees (Fig 1) and bilateral thickening of the Achilles tendons were observed. Xerography showed that both Achilles tendons measured 13 mm, which is twice the normal thickness (Fig 2). Electrocardiograms obtained at rest and after exercise by the Master two-step test were normal. There was no clinically detectable atherosclerotic disease in the carotid artery by ultrasonographic examination. Also, there was no detectable atherosclerotic disease in the aortic root to the bifurcation of the abdominal aorta, common iliac artery, and femoral artery by computed tomography. Systolic blood pressure and diastolic blood pressure were normal. Other physical findings were normal, and no abnormal features were noted on medical history. He was a nonsmoker. He did not have a habit of drinking alcohol. Fasting plasma glucose was 100 mg/dL. Glucose intolerance was not detected. Blood urea nitrogen and serum creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, calcium, phosphorus, uric acid, and electrolyte concentrations were normal. Thyroid-function tests were normal. The thyroxine level was 7 μg/dL, triiodothyronine was 1.5 ng/mL, and thyrotropin was 1.05 μU/mL (normal ranges: thyroxine, 4.5 to 12 μg/dL; triiodothyronine, 0.9 to 2 ng/mL; thyrotropin, 0.34 to 3.97 μU/mL).

Serum total cholesterol (TC) was 12.08 mmol/L, triglyceride (TG) was 7.54 mmol/L, and high-density lipoprotein cholesterol was 1.22 mmol/L. The patient's family tree is illustrated in Fig 3. He was unmarried and had no siblings. His 68-year-old mother was healthy and normolipidemic, with a TC of 4.86 mmol/L and a TG of 1.55 mmol/L. His father had died at the age of 70 years from lung cancer; he had a TG of 1.33 mmol/L and a TC of 7.49 mmol/L before he became ill. A paternal uncle had died at age 76 years from myocardial infarction; he had a TC of 8.66 mmol/L and a TG of 1.37 mmol/L before he became ill.

Laboratory Investigation

Fasting (12 to 14 hours) blood samples were drawn from an antecubital vein. Very–low-density lipoprotein ([VLDL] d < 1.006 g/mL) and low-density lipoprotein ([LDL] d = 1.019 to 1.063 g/mL) were isolated by sequential ultracentrifugation in NaBr solutions.[3]

The cholesterol and TG content of serum and of lipoproteins in the serum were measured enzymatically. The ratio of VLDL cholesterol to total TG was greater than 0.9 (abnormal ratio, >0.7[1]). A significant reduction of LDL cholesterol was observed: the level was 1.22 mmol/L.

Agarose electrophoresis of serum lipoprotein showed a broad β-band pattern. Also, as determined by agarose electrophoresis, there is a prominent β band (β-VLDL) in the fraction d < 1.006 g/mL[4] (Fig 4-2, 4-3). The apo E phenotype 2/2 of this patient was identified by isoelectric focusing of E isoapoproteins obtained from VLDL separated on polyacrylamide gels[5] (Fig 5).

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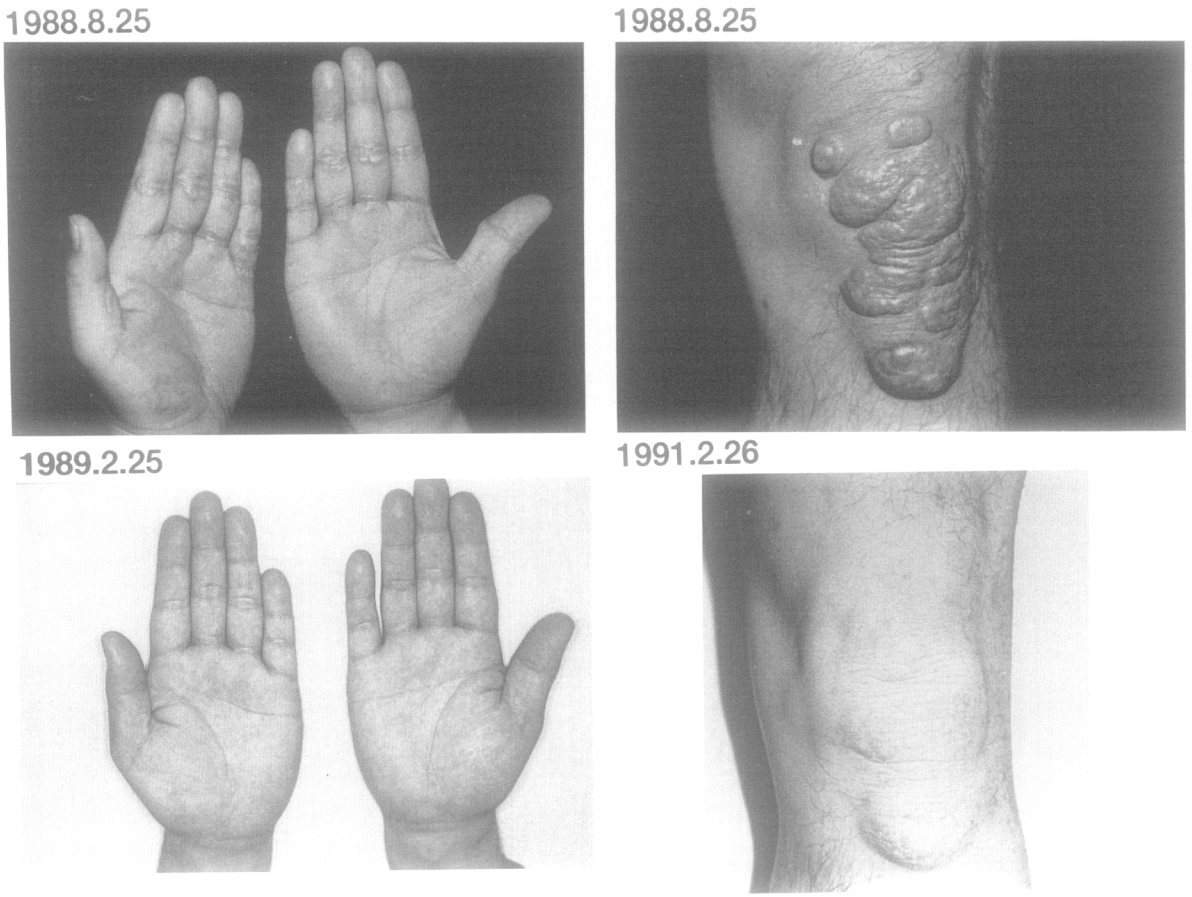

Fig 1. Extensive tuberous xanthomas over the knees and palms were observed in 1988. The palmar xanthoma was resolved within 6 months of initiation of plasmapheresis; 2.5 years after initiation of plasmapheresis, xanthomas on knees had regressed markedly.

Type III hyperlipoproteinemia was diagnosed. The patient's mother had the apo E phenotype 2/2. The apo E phenotypes of his father and uncle were unknown because they had passed away already. We assessed the function of LDL receptors on lymphocytes using a modification[6] of the method reported by Cuthbert et al.[7] This method of detecting LDL receptor abnormalities is based on the observation that lymphocytic proliferation induced in culture with mitogenic lectin phytohemagglutinin (PHA) is dependent on a source of cholesterol for synthesis of plasma membranes. When endogenous sterol is provided only as LDL, lymphocyte proliferation is dependent on functional LDL receptors. This ideal model system was used to assess LDL receptor function.

Mononuclear cells were isolated from 40 mL anticoagulated venous blood as previously described,[8] and were suspended at 2.5×10^5 cells/mL in RPMI 1640 containing 10% (vol/vol) lipoprotein-depleted fetal calf serum (d > 1.230 g/mL) prepared ultracentrifugally.[3] Cells were incubated with 0.025% PHA in triplicate sterile microtiter wells (Becton Dickinson, Oxnard, CA).

Human LDL (d = 1.020 to 1.050 g/mL) was isolated by ultracentrifugation from the serum of seven normal, fasting adults.[3] Cultures were untreated or treated with 0.3 mmol/L pravastatin at various concentrations of LDL cholesterol or 10 mmol/L mevalonate at 37°C. After 72 hours, [^{3}H]thymidine (370 kBq/mL) was added to the culture medium. After 18 hours, the cells were removed and then lymphocyte DNA synthesis was assessed by measuring [^{3}H]thymidine incorporation with a Beckman LS-5800 scintillation counter (Beckman Instruments, Fullerton, CA). DNA synthesis was expressed as the average counts per minute (cpm) of triplicate runs. The percent inhibition by pravastatin was calculated by the equation: % inhibition = [1 − (cpm experimental/cpm control)] × 100, where cpm experimental is the PHA-induced incorporation of [^{3}H]thymidine in the presence of pravastatin at various concentrations of LDL cholesterol or 10 mmol/L mevalonate. Cpm control is the PHA-induced incorporation of [^{3}H]thymidine in the absence of pravastatin but in the presence of an identical concentration of LDL cholesterol or mevalonate.

Data on the percent inhibition in lymphocytes from 10 normolipidemic subjects served as control data. When the concentration of LDL cholesterol in the culture was 26 μmol/L, the percent inhibition of proliferation of lymphocytes in normolipidemic subjects was 12.9 ± 8.9% (mean ± SD), as compared with 51% inhibition in the patient's lymphocytes (Fig 6), indicating that the uptake of LDL via LDL receptors was lower in the lymphocytes of the patient than in those of controls.[7] No LDL receptor function abnormality was observed in lymphocytes from the patient's mother; LDL receptor function in his father and paternal uncle was unknown, as well as their apo E phenotype, because plasma was not obtained before their deaths. However, because the father and uncle had hypercholesterolemia, we assumed that this patient had heterozygous FH related to an abnormal LDL receptor gene inherited from the father.

These findings suggested that the patient had type III hyperlipoproteinemia combined with FH.

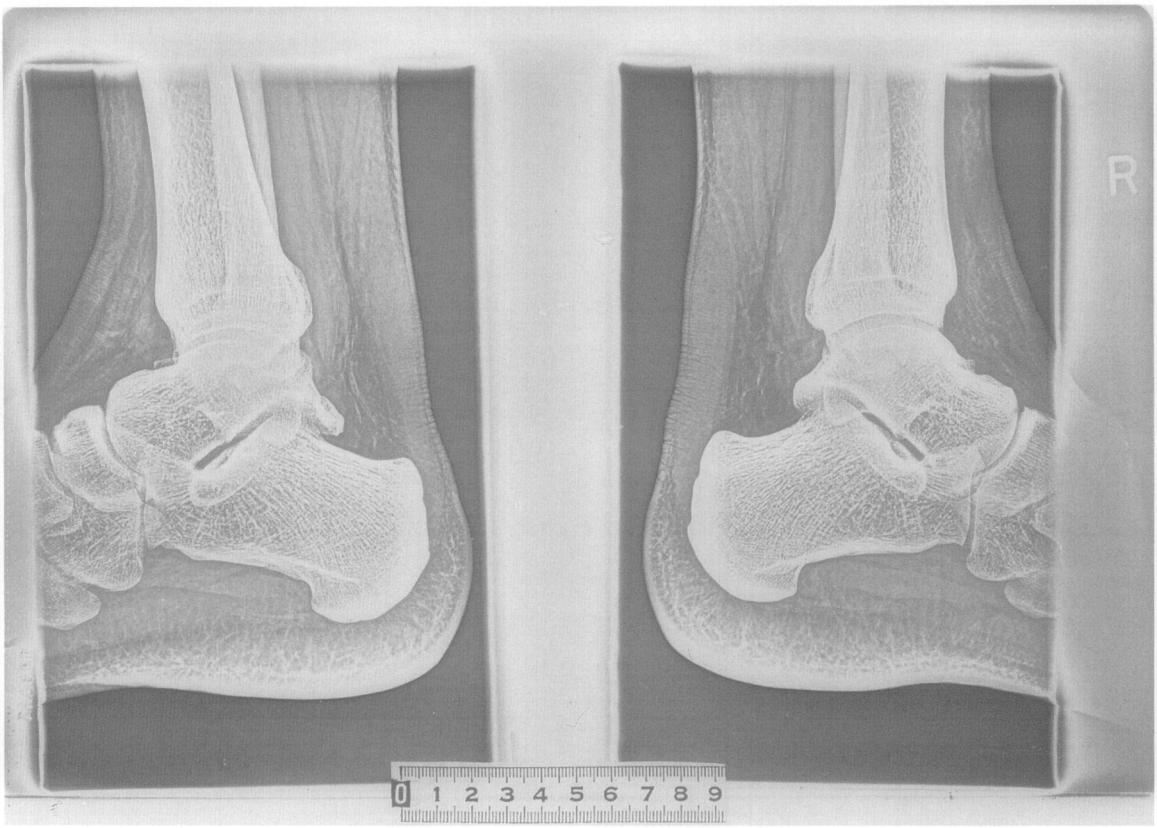

Fig 2. Xanthomatous thickening was observed in the Achilles tendons, which measured 13 mm on xerography.

TREATMENT

The patient was placed on a restricted diet with a total calorie content of approximately 2,400 kcal/d, with 25% of total calories as fat, 55% as carbohydrate, and 15% as

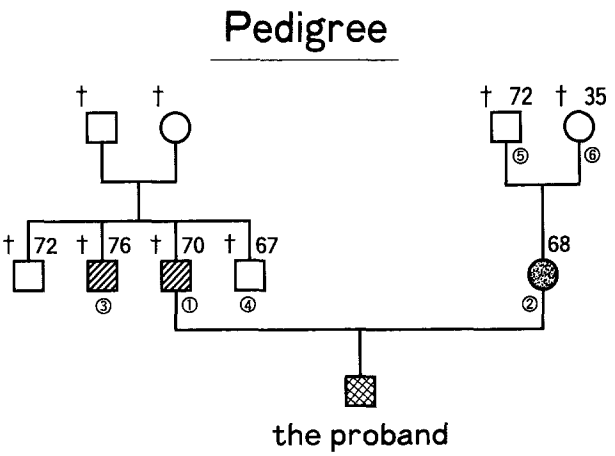

Fig 3. Patient's pedigree. †Deceased (age at death); (▨) hypercholesterolemia; (▩) hypercholesterolemia + hypertriglyceridemia; (♿) normolipidemia; (□) unknown lipid status. Complications in members of the patient's family: (1) father, hypercholesterolemia and lung cancer; (2) mother, apo E-2 homozygote (normolipidemia); (3) paternal uncle, hypercholesterolemia and myocardial infarction; (4) paternal uncle, myocardial infarction; (5) maternal grandfather, cerebral infarction; (6) maternal grandmother, complications of childbirth. (□) Male, (○) female.

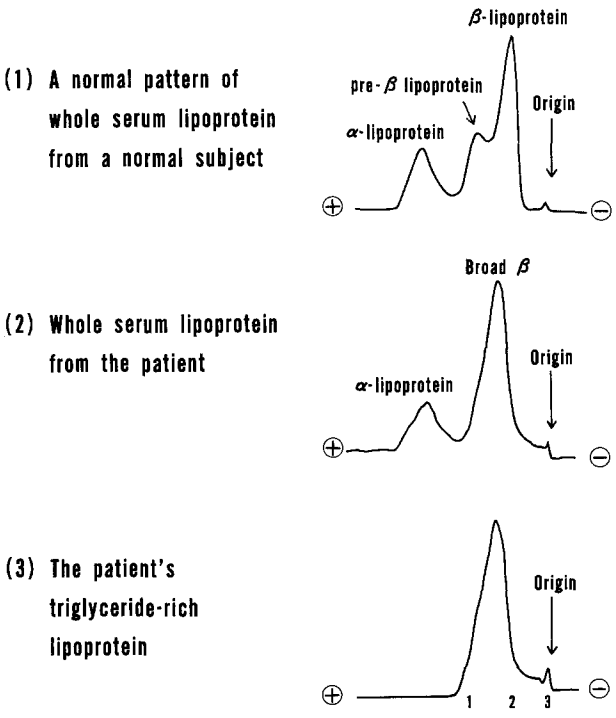

Fig 4. (1) Whole-serum lipoprotein electrophoresis of normal subject not related to the patient is shown for control. (2) A broad β band was demonstrated in whole-serum lipoprotein electrophoresis of patient. (3) There is a prominent β band (β-migrating lipoproteins) in the fraction d < 1.006 g/mL obtained ultracentrifugally.

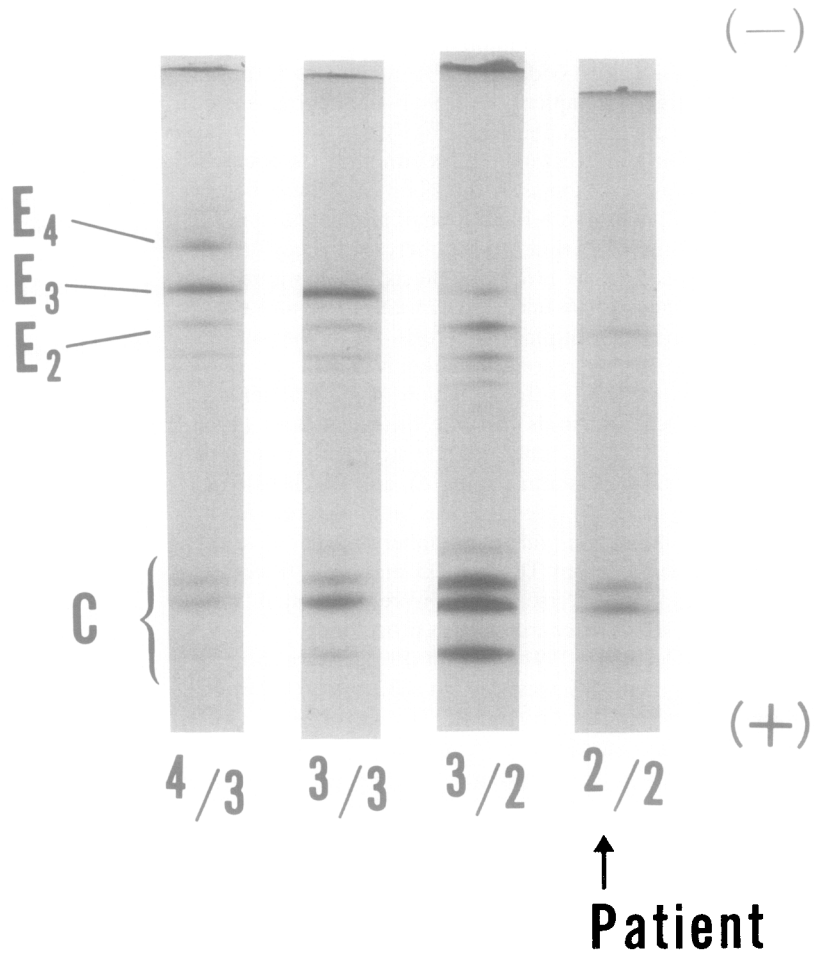

Fig 5. Isoelectric focusing patterns of VLDL apo E. The patient's apo E phenotype was 2/2 (arrow). Disk gels shown for comparison of apo E phenotypes 4/3, 3/3, and 3/2 were from subjects not related to the patient.

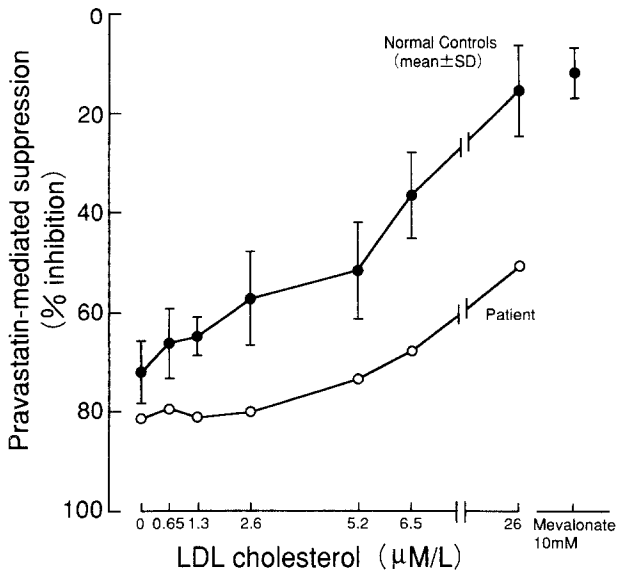

Fig 6. Assessment of functional LDL receptors on lymphocytes. The function of LDL receptors on lymphocytes from the proband decreased as compared with that of subjects with normolipidemia. Lines represent mean values and bars indicate standard deviation in the normal controls.

protein. Cholesterol intake was less than 300 mg/d. The polyunsaturated fatty acid to saturated fatty acid ratio was 1.0. Clinofibrate and niceritrol (lipid-lowering drugs) were administered. The patient's serum TC decreased from 10.97 to 8.07 mmol/L and TG from 4.76 to 2.54 mmol/L. Even though the effects of the restricted diet and drugs were not good enough to decrease serum lipids, xanthomas of the palms disappeared; however, xanthomas of the elbows and knees did not disappear. Between August 1986 and February 1987, the patient did not return for follow-up appointments. During this term, his hyperlipidemia worsened and xanthomas reappeared on his palms. Laboratory test results in September 1987 were as follows: serum TC, 15.26 mmol/L; TG, 10.79 mmol/L; high-density lipoprotein cholesterol, 0.78 mmol/L; apo B, 144 mg/dL; and apo E, 39.9 mg/dL. In the summer of 1988, we initiated plasmapheresis to adsorb apo B–containing lipoprotein without concomitant drug therapy. Plasmapheresis was conducted once every 2 weeks using a dextran sulfate–cellulose column (Liposorber, Kanegafuchi Chemical Industry, Osaka, Japan) in combination with polysulfone hollow fiber (Sulflux, Kanegafuchi) as a plasma separator in the LDL apheresis unit (model KEM-21, Nikkiso, Tokyo, Japan). On each occasion, 3.6 to 4.9 L plasma was treated by plasmapheresis. The hyperlipidemia began to improve. Via plasmapheresis,

TC decreased to 3.47 mmol/L (mean value just after plasmapheresis) from 8.30 mmol/L (mean value just before plasmapheresis) and TG decreased to 1.76 mmol/L (mean value just after plasmapheresis) from 5.46 mmol/L (mean value just before plasmapheresis) (Fig 7).

After 6 months of treatment, the xanthomas disappeared from his palms. Xanthomas on the elbows and knees began to diminish 12 months after initiation of plasmapheresis.

Over a 12-month period, plasmapheresis was performed 26 times. Based on its favorable effects, we continued to perform plasmapheresis once every 2 weeks. Two and a half years after initiation of plasmapheresis, xanthomas on the elbows and knees had regressed markedly (Fig 1).

During the 3.5 years after initiation of plasmapheresis, the patient received trials of three drug regimens: (1) a combination of both clinofibrate (600 mg/d) and niceritrol (750 mg/d), (2) pravastatin (10 mg/d), and (3) cholestyramine (27 g/d). Each regimen was administered for 6 months in conjunction with plasmapheresis. These drugs did not decrease serum TC and TG and were therefore discontinued. No adverse effects were associated with plasmapheresis, so we continued this procedure.

Between December 1991 and June 1992, TC was 5.12 to 8.28 mmol/L and TG was 1.81 to 4.52 mmol/L before plasmapheresis. After treatment, TC was 1.94 to 3.93 mmol/L and TG was 0.57 to 1.76 mmol/L.

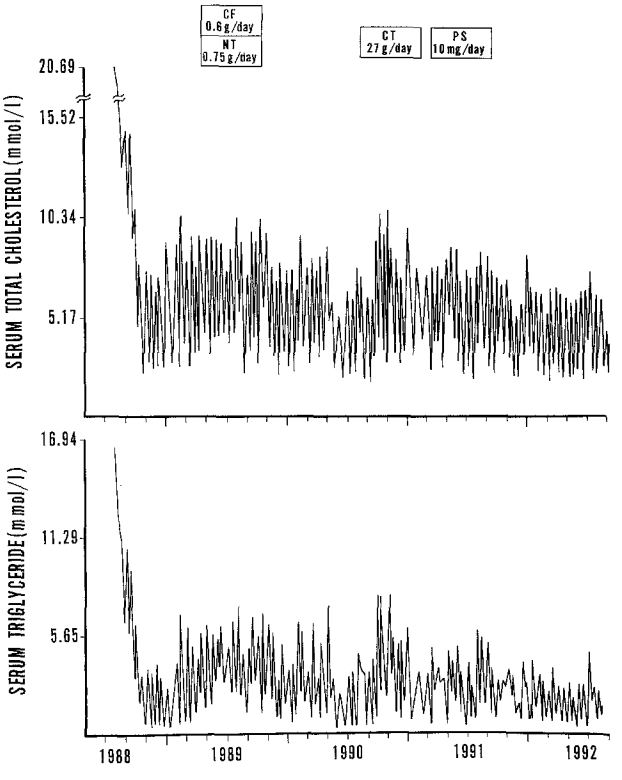

Fig 7. Lipid profiles by treatment of type III hyperlipoproteinemic subject. Serum lipid levels during the period of plasmapheresis are values obtained just before biweekly plasmapheresis and just after. No significant benefit was shown by any of these drug treatments, and all were discontinued after 6-month trials. Belts indicate the oral drug regimen and duration. CF, clinofibrate; NT, niceritrol; CT, cholestyramine; PS, pravastatin.

DISCUSSION

Patients with type III hyperlipoproteinemia generally have the apo E phenotype 2/2. The capacity of this apo E-2 to bind to LDL receptors (B/E receptors) of fibroblasts in vitro is estimated to be less than 2% when the capacity of apo E-3, the wild apo E phenotype, is accepted as 100%.[9] Common apo E-2 is the result of a substitution in apo E-3 of cysteine for arginine as the amino acid at position 158 (a 158 Arg to Cys substitution). Therefore, particles containing apo E-2 are difficult for receptors to process. As a result, chylomicron remnants or remnants of VLDL accumulate in the blood, producing hyperlipidemia.[10]

Not all individuals with the apo E phenotype 2/2 develop type III hyperlipoproteinemia.[2] Utermann et al,[11] hypothesized that type III hyperlipoproteinemia develops in individuals with apo E-2 who also have a mutant gene that causes familial combined hyperlipidemia or FH, or in those who have concomitant endocrine disease.

Type III hyperlipoproteinemia usually responds favorably to dietary control and drug therapy.[12] However, this patient did not respond to dietary control and drug therapy effectively. In Japan, pravastatin is restricted to a maximum dose of 20 mg/d and the other hepatic hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase inhibitor, simvastatin, is restricted to a maximum dose of 10 mg/d. Even though data for treatment with a maximum dose of HMG CoA reductase inhibitors were not shown in Fig 7, after June 1992, pravastatin 20 mg/d and simvastatin 10 mg/d were administered for 6 months, respectively. However, the patient did not respond to the higher-dose HMG CoA reductase inhibitor. His Achilles tendons were twice as thick as normal, suggesting the presence of FH. A diagnosis of FH was subsequently confirmed by an LDL receptor function assay.

In addition, his family history, specifically the finding that his father and paternal uncle both had hypercholesterolemia, suggested that he was heterozygous for FH related to an abnormal LDL receptor gene inherited from his father.

We chose to use the lymphocyte-proliferation test to help diagnose this patient's disease because it is much easier and faster than the traditional fibroblast testing.[13,14] Although the skin fibroblast assay gives much more complete information on binding, internalization, and degradation of radiolabeled LDL, information obtained from the simpler lymphocyte test is sufficient.[6,7]

The LDL receptor function of the patient in this report showed an abnormality, but his LDL cholesterol concentration was low. Low levels of LDL in this patient may be due to impaired conversion of VLDL to LDL. Ehnholm et al[15] reported that when hepatic β-VLDLs were hydrolyzed by lipoprotein lipase in the presence of d > 1.21 g/mL lipoprotein-deficient plasma, the addition of normal apo E (apo E-3) but not apo E-2 resulted in a shift of hepatic β-VLDL to the LDL range (≈1.05 g/mL). They suggested that apo E-3 plays a role in the normal conversion of VLDL to LDL, but the mutant form of apo E (apo E-2) found in β-VLDL from type III hyperlipoproteinemic subjects impedes this conversion. The inability of apo E-2–containing β-VLDL to be converted to LDL may be consistent with the

in vivo observation that the type III hyperlipoproteinemic patient in this report has low levels of LDL.

When his hyperlipidemia worsened and xanthomas reappeared on his palms, we initiated plasmapheresis using a dextran sulfate–cellulose column, with good results. This method is used primarily to adsorb LDL and to decrease elevated serum LDL levels in FH.[16-19]

This report is the first of a patient with combined type III hyperlipoproteinemia and FH treated with plasmapheresis. The results obtained with this patient indicate that plasmapheresis using the dextran sulfate–cellulose column is effective adsorption therapy for patients with type III hyperlipoproteinemia, which is characterized primarily by an increase in β-migrating VLDL (β-VLDL).

The prevalence of the apo E phenotype 2/2 is approximately 1%, and the prevalence of FH heterozygotes is approximately 0.2%.[20] Thus, heterozygous FH and the apo E phenotype 2/2 would be expected to coexist in two of 100,000 individuals. However, this report appears to be the first such case described in Japan. There are only three reports of similar patients outside Japan. One of the three was an FH heterozygote with type III hyperlipoproteinemia,[21] and the other two were FH homozygotes with type III hyperlipoproteinemia who had apo E phenotypes 2/2 and 2/3, respectively.[22] As techniques for diagnosing FH improve, reports of similar patients are likely to increase.

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