MYELOMATOSIS WITH TYPE III HYPERLIPOPROTEINEMIA

Clinical and Metabolic Studies

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Abstract We investigated the metabolism of intermediate-density lipoproteins (IDL [1.006 to 1.019 g per milliliter]) and low-density lipoproteins (LDL [1.019 to 1.063 g per milliliter]) in two men with Type III hyperlipoproteinemia associated with myelomatosis. In vivo kinetic studies using radiolabeled autologous lipoproteins demonstrated a greatly reduced fractional catabolic rate of IDL, relative to control values (patients vs. normal, 0.006 and 0.025 per hour vs. 0.20±0.08 per hour [mean ±S.E.M.]) and a greatly prolonged IDL-to-LDL conversion time (45 and 17 hours vs. 5.4±1.6 hours). In studies in vitro, LDL from both patients failed to bind to the LDL receptor of normal blood lymphocytes, whereas LDL from subjects with familial Type III hyperlipoproteinemia bound normally to the receptor. In one patient immunoglobulin was shown to be associated with IDL and LDL. Thus, hyperlipoproteinemia reflected an impaired metabolism of IDL, probably secondary to the binding of immunoglobulin to the lipoproteins. A similar impairment of receptor-mediated LDL catabolism did not elevate the plasma LDL concentration because of the low IDL-to-LDL conversion rate. (N Engl J Med. 1982; 307:79-83.)

PRONOUNCED hyperlipidemia, often with cutaneous xanthomas, is an infrequent but well-documented finding in myelomatosis.[1-7] Hyperlipidemia is also an occasional finding in other disorders associated with hypergammaglobulinemia.[7-11] The lipid abnormalities may be an incidental finding, but they are clinically important in some patients with severe hypertriglyceridemia, in whom acute pancreatitis may result.[8] Associated atherosclerotic disease has been reported,[1,10] but it is not certain that atherosclerotic cardiovascular problems are overrepresented among patients with this condition.

The metabolic bases of immunoglobulin-induced hyperlipidemias are of considerable interest. Autoimmune mechanisms, including inhibition of the lipoprotein lipase system[6,8] and antibodies to lipoprotein,[3-5,9,10] have been considered. Since receptor-mediated catabolism of lipoproteins depends on the recognition of their apolipoproteins,[12-16] impairment of lipoprotein interconversion and degradation due to blocking of recognition sites by immunoglobulins could be responsible. We have had the opportunity to study lipoprotein metabolism in two patients with myelomatosis and Type III hyperlipoproteinemia, and we report below the findings supporting this hypothesis.

CASE REPORTS

Patient 1 was a 52-year-old man (weight, 60 kg; height, 160 cm) with a nine-year history of yellow streaks on the palms and yellow swellings over the elbows, knees, and knuckles. For four years he had had lumbar backache, and more recently intermittent claudication. The family history was not contributory, and no relative was available for study. He drank little alcohol. Before referral he had been treated with clofibrate, without regression of the xanthomas, but no details about his lipid levels were available. He was lean, with tuberous and eruptive xanthomas over extensor surfaces, linear xanthomas in the palmar and neck creases, large xanthelasmas, and tendon and subperiosteal xanthomas. The foot pulses were absent. There was no endocrine abnormality, and renal and hepatic functions were normal. Radiologic examination showed multiple lytic lesions in the skull and spinal demineralization. The IgA level was raised (31 g per liter) because of the presence of a monoclonal IgA kappa paraprotein, with suppression of IgG (2.4 g per liter) and IgM (0.14 g per liter). The bone marrow contained many atypical plasma cells.

Plasma lipoprotein-lipid concentrations, assayed by enzymatic procedures after preparative ultracentrifugation,[17] are given in Table 1. More detailed lipoprotein fractionation was performed by density-gradient ultracentrifugation[19] (Fig. 1). The patient had severe hyperlipidemia due to greatly elevated levels of cholesterol-enriched very-low-density lipoproteins (VLDL [<1.006 g per milliliter]) and intermediate-density lipoproteins (IDL [1.006 to 1.019 g per milliliter]), with a low concentration of low-density lipoproteins (LDL [1.019 to 1.063 g per milliliter]). The VLDL migrated as a broad beta band on agarose-gel electrophoresis.[20] This is the pattern of Type III hyperlipoproteinemia.[21]

In Patient 2, a 50-year-old man (weight, 68 kg; height, 172 cm), hyperlipidemia had been detected 10 years before admission. The patient had had a partial response to clofibrate (1 g per day) and a fat-modified diet before referral to St. Thomas' Hospital. His two siblings and three children had normal serum lipid concentrations. He was lean, with linear xanthomas in the palmar creases but with no other xanthomas or xanthelasma. Moderate hyperlipidemia of the Type III pattern was present (Table 1). Renal and hepatic functions and the serum thyroxine level were normal. Normochromic anemia with marked rouleaux formation (hemoglobin, 9.5 g per

Table 1. Fasting Plasma Lipid Concentrations in the Patients and Controls.

LIPOPROTEIN FRACTION	LIPID	PATIENT 1	PATIENT 2	CONTROLS †
			mmol/liter ‡	
Whole plasma	Cholesterol	34.1	10.9	5.3±0.10
	Triglyceride	26.3	5.6	1.4±0.10
VLDL (<1.006 g/ml)	Cholesterol	23.0	4.9	0.65±0.04
	Triglyceride	24.0	4.2	0.80±0.07
IDL + LDL (1.006–1.063 g/ml)	Cholesterol	9.9	4.3	3.6±0.11
HDL (>1.063 g/ml)	Cholesterol	1.2	1.7	1.1±0.04

*Each value is the mean of several determinations.

†Means ±S.E.M. in 140 men 20 to 69 years old. These values were derived from those of Lewis et al.[18] and have been corrected for the change in the cholesterol-assay procedure from Technicon N-24a to Boehringer-Mannheim 187313.

‡To convert to milligrams per deciliter, multiply cholesterol values by 0.026 and triglyceride values by 0.0113.

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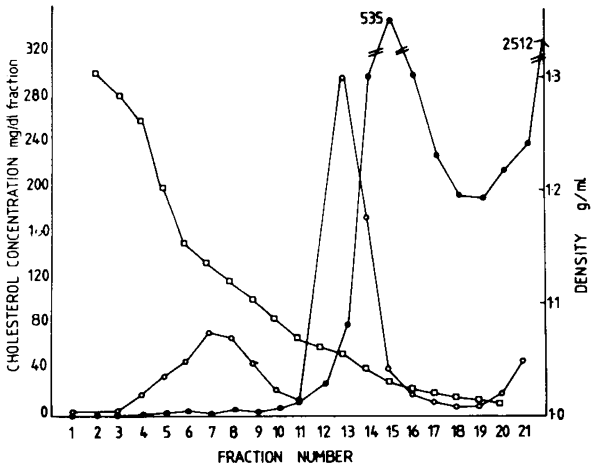

Figure 1. Density-Gradient Ultracentrifugation of Plasma Lipoproteins from Patient 1 (Solid Circles) and a Healthy Normolipidemic Subject (Open Circles).

The open squares represent background density. In the control patient the plasma cholesterol was 4.0 mmol per liter (156 mg per deciliter) the VLDL cholesterol 0.28 mmol per liter (11 mg per deciliter), the IDL-plus-LDL cholesterol 2.5 mmol per liter (95 mg per deciliter), and the HDL cholesterol 1.3 mmol per liter (50 mg per deciliter).

To convert cholesterol concentrations to millimoles per liter, multiply by 0.026.

deciliter) was present; the white-cell count was 3.7×10^8 per liter (34 per cent neutrophils). The serum IgG level was very high (54 g per liter) because of an IgG kappa paraprotein, with subnormal levels of IgA (0.75 g per liter) and IgM (0.12 g per liter). The bone marrow contained numerous immature and binucleate plasma cells. Radiologic examination showed marked bone demineralization and a compression fracture of a thoracic vertebra.

Results

Lipoprotein Kinetics in Vivo

The heritable form of Type III hyperlipoproteinemia represents the accumulation in plasma of partially catabolized VLDL.[21] Such VLDL remnants are normally extracted from plasma in the liver[22-24] after they have bound to hepatocyte cell-surface receptors that recognize the apoproteins B and E of the particles.[15,16,25] Most subjects with familial Type III hyperlipoproteinemia are homozygous for a mutant allele — E^d — that specifies an abnormal form of apoprotein E. The apoprotein E phenotype in such patients has been designated apoprotein E-D/D[26] or the β-IV subclass.[27] Recent studies on the in vitro binding of apoprotein E to cell-surface receptors,[28] on the uptake and degradation of human apoprotein E by estrogen-stimulated perfused rat liver,[29] and on the in vivo catabolism of apoprotein E in human beings[30] all indicate that the apoprotein in patients with familial Type III hyperlipoproteinemia represents a nonfunctional mutant, providing a molecular basis for the apparent impairment of remnant catabolism[31] in the disorder.

VLDL remnants taken up by the splanchnic bed are partly catabolized and partly converted to LDL,[24] and a reduced rate of remnant-to-LDL conversion probably accounts for the low LDL levels in patients with primary Type III disease.[21] The "terminal" VLDL remnants (i.e., the immediate precursor of LDL) are of the IDL class, and several aspects of IDL apoprotein B metabolism can be examined in vivo: the rate of synthesis, the fractional catabolic rate, the fractional conversion to LDL apoprotein B, the time required for conversion of IDL apoprotein B to LDL apoprotein B, and the absolute rate of transfer of apoprotein B mass from IDL to LDL. Since the apoprotein B of lipoproteins is conserved during their interconversions,[32] its kinetic behavior is an index of the metabolism of the particles. These indexes were measured in both patients by deconvolution analysis of the specific activity:time curves of IDL and LDL apoprotein B after simultaneous intravenous injection of autologous [^{131}I]IDL and [^{125}I]LDL according to laboratory procedures previously described.[33] In both patients clofibrate had been stopped at least eight weeks before admission to the ward. In Patient 2 discontinuation of the drug raised the plasma levels of cholesterol and triglyceride by approximately 10 per cent; no similar information was available about Patient 1, since therapy had been stopped before his arrival in England. The metabolic studies lasted 61 to 72 hours from the time of injection, during which time 10 to 14 blood samples were collected. Plasma IDL and LDL apoprotein B concentrations remained within 10 per cent of initial values; IDL apoprotein B was 42 mg per deciliter in Patient 1 and 9 mg per deciliter in Patient 2, and LDL apoprotein B was 26 mg per deciliter in Patient 1 and 11 mg per deciliter in Patient 2. Body weights changed by less than 2 kg. The diets provided 40, 45, and 15 per cent of derived calories from fat, carbohydrate, and protein, respectively.

The results are compared with values obtained in five control subjects of similar age (Table 2) (plasma cholesterol, 7.8±0.8 mmol per liter [301±31 mg per deciliter]; plasma triglyceride, 3.2±0.9 mmol per liter

Table 2. Metabolism of IDL Apoprotein B in the Patients and Controls.

Group	IDL Apoprotein B Metabolism				
	Rate of Synthesis	Fractional Catabolic Rate	Conversion to LDL Apoprotein B		
	mg/kg/day	*per hr*	*per cent converted*	*mean conversion time (hr)*	*absolute conversion rate (mg/kg/day)*
Patients					
1	2.7	0.006	15	45	0.41
2	2.4	0.025	44	17	1.06
Controls Means ±S.E.M.; n = 5	6.4±0.9	0.20±0.08	73±22	5.4±1.6	5.3±1.6
P value *	0.047	0.047	0.57	0.047	0.095

*Mann-Whitney U test (comparison of values for both patients with control values).

[283±80 mg per deciliter]; IDL apoprotein B, 4.5±1.2 mg per deciliter). The major defects in both patients were a low fractional catabolic rate of IDL apoprotein B and a greatly reduced rate of conversion of IDL to LDL. The rate of synthesis of IDL apoprotein B was also significantly lower in the patients than in the controls ($P = 0.047$).

LDL Metabolism in Vitro

The kinetic studies indicated that the catabolism of IDL and its conversion to LDL were greatly impaired in both patients. The possibility was considered that this impairment was due to the formation of immunoglobulin-IDL complexes, with masking of the apoprotein sequences in IDL that are normally recognized by hepatocyte receptors. It was not possible to test this hypothesis directly, since an in vitro assay for human hepatocyte-IDL interaction was not available in this laboratory. However, the possibility that the receptor-mediated degradation of LDL by peripheral cells might be similarly impaired was explored with the use of blood lymphocytes.

In the first experiment LDL from Patient 1 and a normal person was labeled with ^{125}I and incubated (for six hours at 37°C at a concentration of 10 μg of LDL protein per milliliter) with the patient's or normal blood lymphocytes (2×10^6 per milliliter) in a crossover design, after derepression of the cells by incubation in lipoprotein-free medium for 72 hours. The extent of [^{125}I]LDL degradation was measured from the accumulation of non-iodide trichloroacetic acid-soluble radioactivity in the medium.[17] Normal [^{125}I]LDL was degraded normally by the patient's lymphocytes (Table 3, Experiment I). In contrast, there was no detectable degradation of the patient's [^{125}I]LDL by his own or normal lymphocytes. Thus, an abnormality in the LDL prevented its catabolism by derepressed lymphocytes. That this finding reflected a failure of LDL to bind to the LDL receptor was supported by the results of competition assays, in which LDL from the patient and LDL from a normal subject were compared for their ability to compete with normal [^{125}I]LDL for degradation by normal lymphocytes. Although the addition of normal LDL to the medium substantially reduced the rate of degradation of the ^{125}I-labeled normal LDL, addition of the patient's LDL had little or no effect (Table 3, Experiment I). Similar but less detailed studies were performed with the LDL and lymphocytes from Patient 2, with concordant results (Table 3, Experiment II).

In another experiment it was found that LDL from four patients with familial Type III hyperlipoproteinemia (diagnosed by the presence of palmar xanthomas, high levels of cholesterol-rich VLDL, and the D/D phenotype of VLDL apoprotein E) was just as effective as LDL from a normal subject in competing with ^{125}I-labeled normal LDL for the receptor (Table 3, Experiment III).

Other Investigations

To detect the presence of IgA-lipoprotein complexes in Patient 1, an immunoelectrophoretic procedure was employed.[34] VLDL, IDL, LDL, and high-density lipoprotein (HDL [1.063 to 1.21 g per milliliter]) from the patient were introduced into the wells of a 1.5 per cent agarose plate (barbital sodium buffer, concentration of 50 mM, and pH of 8.6) containing rabbit anti-

Table 3. [^{125}I]LDL Degradation by Derepressed Blood Lymphocytes.

Experiment *	Incubation Conditions & Source of Material			Amount Degraded †
	Lymphocytes (2×10^6/ml)	[^{125}I]LDL (10 μg/ml)	Unlabeled LDL (μg/ml)	
I	Normal subject	Normal subject	None	3413
	Normal subject	Patient 1	None	0
	Patient 1	Normal subject	None	2915
	Patient 1	Patient 1	None	0
	Normal subject	Normal subject	Normal subject (10)	1950
	Normal subject	Normal subject	Normal subject (20)	992
	Normal subject	Normal subject	Patient 1 (10)	3191
	Normal subject	Normal subject	Patient 1 (20)	3276
II	Normal subject	Normal subject	None	1119
	Patient 2	Normal subject	None	1768
	Normal subject	Normal subject	Normal subject (100)	270
	Normal subject	Normal subject	Patient 2 (100)	1579
III	Normal subject	Normal subject	None	3199
	Normal subject	Normal subject	Normal subject (25)	937
	Normal subject	Normal subject	Hyperlipoproteinemic ‡ subject (25)	470
	Normal subject	Normal subject	Hyperlipoproteinemic ‡ subject (25)	598
	Normal subject	Normal subject	Hyperlipoproteinemic ‡ subject (25)	707
	Normal subject	Normal subject	Hyperlipoproteinemic ‡ subject (25)	1046

*See text for details.

†Nanograms of [^{125}I]LDL protein degraded per milligram of cell protein per six hours. (Means of two to four determinations).

‡Familial Type III hyperlipoproteinemia.

serum to human IgA (Dako, Copenhagen), and electrophoresis was performed at 8.6 V per centimeter for 16 hours. During electrophoresis "rockets" developed from the VLDL, IDL, and LDL samples but not from the HDL. Analogous studies with agarose plates containing anti-human IgG (Dako, Copenhagen) failed to demonstrate IgG-lipoprotein complexes in the serum of Patient 2.

The VLDL apoprotein E phenotypes in both patients were assessed with isoelectric focusing according to the method of Utermann et al.[26] Patient 1 had the heterozygous apoprotein E-N/D phenotype, and Patient 2 had the common apoprotein E-N/N phenotype.

Discussion

These patients had in common the presenting feature of xanthomatous hyperlipidemia; their myelomatosis was recognized in the course of the clinical investigation. The random coexistence of two independent disorders — myelomatosis and familial Type III hyperlipoproteinemia — is statistically improbable. Furthermore, several features of the hyperlipidemia were atypical of the primary Type III disorder: the patients had not responded well to clofibrate, probably the most effective treatment for the primary disorder,[21] and on isoelectric focusing neither patient had the apoprotein E isopeptide pattern that conforms with that usually found in patients with the heritable condition.[26,27]

The metabolic findings provided some clarification of the mechanisms by which monoclonal hypergammaglobulinemia may disturb lipoprotein metabolism. The kinetic studies established that impairment of catabolism, rather than overproduction, was the common feature explaining the elevated concentrations of IDL. With respect to LDL it was shown that ^{125}I-labeled LDL from Patient 1 was not degraded by derepressed normal human lymphocytes in vitro. Subsequent competition studies carried out with unlabeled LDL from both patients indicated that an abnormality of the lipoprotein prevented its binding to the LDL receptor. The possibility that LDL from subjects with familial Type III hyperlipoproteinemia might also have a diminished affinity for the receptor was excluded by an additional experiment, in which LDL from each of four patients with the primary disorder was found to compete effectively with normal [^{125}I]LDL for degradation by lymphocytes.

In Patient 1 it was shown by electroimmunoassay that the lipoprotein fractions VLDL, IDL, and LDL contained IgA. It is unlikely that the presence of the immunoglobulin reflected contamination of the fractions by free IgA, since none could be demonstrated in HDL. VLDL, IDL, and LDL have in common the presence of apoprotein B.[31] Although it was not possible to demonstrate the presence of IgG-lipoprotein complexes in Patient 2, this could have been due to masking of antigenic sites[11]; it seems likely that the defective catabolism of IDL in vivo and of LDL in vitro was due in both patients to the formation of immunoglobulin-lipoprotein complexes, resulting in the blocking of apoprotein sequences in IDL and LDL that are normally recognized by hepatic and peripheral cell-surface receptors. Evidence for the presence of immunoglobulin-lipoprotein complexes has been provided in previous studies of patients with myeloma.[3,5,9,10]

Although we obtained evidence for impairment of both IDL catabolism and LDL catabolism, only the former contributed to the hyperlipidemia, since the LDL concentration was normal or low. In human beings the catabolism of remnant lipoproteins occurs within the splanchnic bed,[24] and in animals it is clearly a function of the liver.[15,16,22,23] Presumably, the reduced rate of IDL-to-LDL conversion in the patients described here prevented the excessive intravascular accumulation of LDL, which would have otherwise resulted from the impairment of the receptor-mediated degradation of LDL.

The recognition of remnant lipoproteins by hepatocyte receptors has been attributed to the presence of apoprotein E.[15,16] In most patients with primary Type III hyperlipoproteinemia, impaired recognition of remnants appears to result from an inherited defect in the composition of apoprotein E, the so-called apoprotein E-D/D phenotype.[26,30] The present cases, in which the presence of apoprotein E-D/D was excluded by isoelectric focusing, illustrate an acquired mechanism for reduced remnant recognition.

It has been shown that recognition of LDL by the receptors of fibroblasts can be blocked by chemical modification of the arginyl residues of its apoprotein B.[14] Our results provide evidence of a naturally acquired modification of LDL that similarly inhibits receptor-mediated binding of the lipoprotein. Although Baudet et al. have described two patients with myeloma in whom IgA antagonized the suppression of cholesterol synthesis normally produced in fibroblasts by LDL, in neither patient was the antagonism due to an effect on the surface binding of the lipoprotein.[5] A similar reduction of the receptor-mediated uptake and degradation of LDL may have been responsible for the massive increase in whole-body cholesterol synthesis found in one patient with autoimmune hyperlipidemia.[35]

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MEDICAL PROGRESS

THE NORMAL MICROBIAL FLORA

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CONTROVERSY has surrounded the role of the normal microbial flora in health since the early days of microbiology. Pasteur, for one, hypothesized that the normal flora was essential to life.[1] Some years later, his hypothesis was tested and convincingly refuted by Reyniers and others at the Lobund Laboratory, University of Notre Dame, who demonstrated that the germ-free state could be maintained for successive generations in various laboratory animals.[2] Metchnikoff, in one of many differences with Pasteur, argued that indigenous microorganisms were antagonists that competed with the host for factors essential to life.[3]

Metchnikoff's proposal has not been so easily rejected. Clearly, there are animals within nature that profit from the activities of their indigenous microbial burden. Cattle and other ruminants are able to use cellulose as a food source because of the fermentation activity of symbiotic organisms colonizing their gastrointestinal tracts[4]; ambrosia beetles cultivate fungi originating in their normal flora as a chief source of food[5]; bioluminescent fish harbor photobacteria in various light-generating organs that are used to attract prey, assist in escaping predators, or function as a means of communication.[6] These examples would seem to vitiate Metchnikoff's contention that the normal flora is detrimental to higher animals, except that

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