Abnormal lipoproteins in multiple myelomatosis

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SUMMARY In a study of the lipoprotein pattern in multiple myelomatosis electrophoresis on agarose gel showed abnormal lipoproteins, named paralipoproteins (p-Lp), in 24 out of 30 normo-lipidaemic patients. These paralipoproteins were grouped according to their mobility into one or another of the following types: (1) p-Lp₁ with a mobility identical with that of γ -globulin, (2) p-Lp₂ with a mobility between that of β - and γ -globulin, (3) p-Lp₃ with a mobility identical with that of β -globulin. On ultracentrifugation the abnormal lipoproteins were found to have a density above 1.063 g/ml.

Isolated cases of multiple myelomatosis with the appearance of abnormal lipoprotein fractions have been reported (Hollan, 1958; Lennard-Jones, 1960; Neufeld *et al.*, 1964; Koga *et al.*, 1974). In this paper we report the lipoprotein pattern in 30 patients with multiple myelomatosis.

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Table Plasma lipids in 30 patients with multiple myelomatosis

Case No.	Age (years) and sex		Class of M-Component	Electrophoresis		Cholesterol (mg/130 ml)*	Triglycerides (mg/100 ml)*
				Abnormal globulin	Abnormal lipoprotein		
1	58	F	IgG	γ	p-Lp ₁	143	130
2	58	M	IgG	γ	p-Lp ₁	186	160
3	53	F	IgG	γ	p-Lp ₁	180	100
4	51	F	IgG	γ	p-Lp ₁	140	110
5	63	M	IgG	γ	p-Lp ₁	176	124
6	67	M	IgG	γ	p-Lp ₁	206	165
7	65	F	IgG	γ	p-Lp ₁	210	70
8	62	F	IgG	γ	—	200	180
9	60	F	k-chain	γ	—	169	170
10	63	M	k-chain	γ	—	150	100
11	65	M	k-chain	β - γ	p-Lp ₂	165	150
12	56	M	IgG	β - γ	p-Lp ₂	168	120
13	58	M	IgG	β - γ	p-Lp ₂	215	82
14	64	F	IgG	β - γ	p-Lp ₂	225	78
15	55	M	IgG	β - γ	p-Lp ₂	195	70
16	70	M	IgG	β - γ	p-Lp ₂	100	110
17	72	M	IgG	β - γ	p-Lp ₂	140	93
18	77	F	IgG	β - γ	p-Lp ₂	194	102
19	71	M	IgA	β - γ	p-Lp ₂	135	43
20	76	M	IgA	β - γ	—	235	125
21	59	F	IgA	β	p-Lp ₃	180	105
22	48	F	IgG	β	—	158	95
23	88	F	IgG	β	p-Lp ₃	140	53
24	63	M	IgG	β	—	180	162
25	52	M	IgA	β	p-Lp ₂	120	187
26	59	M	IgA	β	p-Lp ₃	225	95
27	70	M	IgG	β	p-Lp ₃	165	74
28	65	F	IgG	β	p-Lp ₃	120	100
29	64	M	IgG	β	p-Lp ₃	145	60
30	64	M	IgG	β	p-Lp ₃	154	71

*Normal range according to data from our laboratory (Nikiforakis, 1976) for people aged over 40: plasma cholesterol 100-270 mg/100 ml. plasma triglycerides 41-200 mg/100 ml.
Conversion: traditional to SI units—Cholesterol: 1 mg/100 ml $\approx$ 0.025 mmol/l. Triglyceride: 1 mg/100 ml $\approx$ 0.012 mmol/l.

Patients and methods

Thirty patients (17 men and 13 women) aged 48 to 88 years suffering from multiple myelomatosis (MM) were studied (Table). All had the following clinical and laboratory findings: osteolytic changes characteristic of MM, anaemia, a sharp peak or narrow band of protein on electrophoresis, myeloma plasma cells in the bone marrow aspirate, and hyperimmunoglobulinaemia (detected by immunoelectrophoresis). Bence Jones proteinuria was present in only three patients (cases 9, 10, 11).

Thirty healthy individuals with normal lipoproteins and of about the same age and sex distribution as the patients served as controls.

Plasma lipids were measured in venous blood collected in tubes containing EDTA (1 mg/ml) after overnight fasting of 12-14 hours.

Triglycerides were measured by the method of Jover (1963) and cholesterol by the method of Abell *et al.* (1952).

Plasma lipoprotein and serum protein electrophoresis was carried out on fresh samples on agarose gel (Johanson, 1972).

Plasma ultracentrifugation was performed on three selected patients (see below). Lipoproteins were separated by the method of Carlson (1973) into VLDL at a density of 1.006 g/ml and into LDL at a density of 1.063 g/ml. The HDL was obtained from the bottom fraction by the tube-slicing technique after the second centrifugation. After ultracentrifugation the isolated fractions were submitted to electrophoresis on agarose gel.

In three selected patients the abnormal lipid-staining bands isolated by ultracentrifugation (see below) were subjected to electrophoresis on agarose gel. The area corresponded to that stained by Sudan Black. Abnormal material was extracted according to Folch *et al.* (1957) before staining. The lipid nature of the abnormal bands was confirmed by thin layer chromatography (TLC) on glass plates covered by silica gel (Pyrovolakis *et al.*, 1974). The lipids were developed in a mixture of petroleum ether, diethylether, and acetic acid (82:18:1). Three healthy individuals with normal lipoprotein pattern served as controls.

Results

QUANTITATIVE LIPID ASSAY

Plasma triglyceride and cholesterol levels were normal in all patients (Table).

LIPOPROTEIN ELECTROPHORETIC STUDIES

Lipoprotein electrophoresis revealed the presence of abnormal lipid staining bands in 24 out of the 30

patients. These abnormal bands, which we have called paralipoproteins (p-Lp), were grouped according to their mobility into one or another of the following types.

(1) In seven patients the paralipoprotein band had a mobility identical with that of γ -globulin (p-Lp₁; Fig. 1a).

(2) In eight patients the paralipoprotein band had a mobility identical with that of β -globulin (p-Lp₂; Fig. 1c).

(3) In nine patients the paralipoprotein band had a mobility between β - and γ -globulin (p-Lp₃; Fig. 1b).

The mobility of these abnormal lipoprotein bands corresponded fully to that of the paraprotein in each case (Fig. 2).

Fig. 1 Electrophoresis on agarose gel of lipoproteins isolated from the plasma of myeloma patients. Note three abnormal lipoprotein fractions, p-Lp₁(a), p-Lp₂(b), p-Lp₃(c), marked by arrows.

Fig. 2 Electrophoresis of whole plasma (from a myeloma patient) as well as of isolated lipoprotein fractions after ultracentrifugation. (a) Lipoprotein electrophoresis of whole plasma. Note presence of abnormal p-Lp₁ fraction (arrow). (b) Lipoprotein electrophoresis of top fraction separated by ultracentrifugation at density of 1.006 g/ml. (c) Lipoprotein electrophoresis of bottom fraction at density of 1.006 g/ml. Note abnormal p-Lp₁ fraction (arrow). (d) Lipoprotein electrophoresis of top fraction separated by ultracentrifugation at density of 1.063 g/ml. (e) Lipoprotein electrophoresis of bottom fraction at density of 1.063 g/ml. As illustrated, the p-Lp₁ was isolated in the bottom fraction with HDL.

ULTRACENTRIFUGATION STUDIES

Further information on the abnormal lipoprotein bands of each type was obtained by ultracentrifugation. The paraproteins p-Lp₁, p-Lp₂, and p-Lp₃ were isolated in the bottom fraction with the HDL

after the second ultracentrifugation at a density of 1.063 g/ml (Fig. 2e).

QUALITATIVE LIPID ASSAY

Cholesterol, triglycerides, phospholipids, and free fatty acids were identified by TLC in the three p-Lp bands (p-Lp₁, p-Lp₂, p-Lp₃). No lipids were identified by TLC in the normal lipoprotein pattern in the area corresponding to the p-Lp bands.

LIPOPROTEIN PATTERN AFTER TREATMENT

In two patients with p-Lp₁, two with p-Lp₂, and three with p-Lp₃ the lipoprotein pattern was also studied electrophoretically after two to five courses of treatment by the regimen of Alexanian *et al.* (1958). The paraprotein disappeared completely

Fig. 3 Lipoprotein electrophoresis before and after treatment of myeloma. Note disappearance of abnormal lipoprotein and paraprotein bands after treatment (c, d).

only in one, a patient of the p-Lp₁ type. In this case the paraprotein also disappeared completely after treatment (Fig. 3).

Discussion

Sachs *et al.* (1954), using paper electrophoresis, noted an abnormal lipid staining band which migrated with the abnormal gamma-globulin in five out of 11 patients with multiple myelomatosis. In one with beta-myeloma and another with minor protein abnormalities a lipid band was found between beta- and gamma-globulin. As Sachs doubted that these abnormal lipid staining bands contained true fat he described them as 'lipid-like' and investigated no further. Later Pagé *et al.* (1974) found a lipid staining post-beta band in patients with multiple myelomatosis while performing a routine lipoprotein electrophoresis on cellulose. He also found this band in 18 out of 20 selected patients with multiple myelomatosis. The nature of these lipid stained bands was not investigated further. Other authors (Hollan, 1958; Lennard-Jones, 1960; Neufeld *et al.*, 1964; Lewis and Page, 1965; Beaumont *et al.*, 1967, 1970; Koga *et al.*, 1974) presented evidence in isolated cases of multiple myelomatosis that this material contained lipids. In all except one of these cases the patients were also hyperlipidaemic (Koga *et al.*, 1974).

Out of our 30 patients with multiple myelomatosis, all normolipidaemic, 24 had abnormal lipid stained bands, which were of three different types of mobility. We confirmed the lipid nature of the sudan-stained material by thin layer chromatography. We found that it was in fact a lipoprotein which was isolated by ultracentrifugation in the bottom fraction with the HDL. In each case the paraprotein had a mobility identical with that of the lipoprotein. These findings as well as the observation that in one case the disappearance of the abnormal lipoprotein coincided with the disappearance of the paraprotein after treatment favour the view that the lipid carrier was the abnormal protein. This hypothesis, however, fails to explain the absence of p-Lp band in six cases of multiple myelomatosis. We made no attempt to elucidate the nature of the abnormal lipoprotein bands.

Various authors (Beaumont *et al.*, 1967, 1970; Lewis and Page, 1965) presented evidence of a firm complex between the paraprotein and the normal lipoprotein fractions. Nevertheless, in our cases the different migration on the agarose gel of the p-Lp bands in correlation with the normal lipoprotein as well as their high density (> 1.063 g/ml) seem not to support this hypothesis. On the other hand, our

findings are compatible with Koga's view that the lipid-containing paraprotein is probably not the result of paraprotein/lipoprotein interaction.

We are continuing our research into the nature of the p-Lp fractions.

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