

Immunochemical study of the plasma low and high density lipoproteins in Tangier disease

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Major disturbances of the lipoproteins in Tangier serum have been investigated using electrophoretic and immunochemical techniques. Previously described anomalies concerning the striking deficiency in HDL and the very low levels of apo A-I and apo A-II in Tangier patients are illustrated and explained. Anomalies concerning the fast LDL of Tangier serum are attributed to different forms of apo B not previously described. These data are strengthened by the features of a 2-dimensional electrophoresis method elaborated in the laboratory which allows apoproteins to separate in the second dimension. These apoproteins are obtained by the delipidation of the lipoproteins fractionated in a first polyacrylamide discontinuous gel. This method clearly shows the distribution of apoproteins in the first lipoprotein track and is in perfect accordance with the new concept of lipoprotein particles.

Apolipoprotein Tangier disease 2-Dimensional electrophoresis Immunochemistry Lipoprotein

1. INTRODUCTION

Analphalipoproteinemia is the characteristic biochemical trait in homozygous forms of Tangier disease. Present knowledge suggests that heavy disturbances of other plasmatic lipoproteins may be associated with this defect. Some of these have been underlined by Heinen et al. [1]. Thirty cases at the most have been described in the world [2] from 1961 [3] to our recent observation [4]. During this period, new immunochemical techniques have been used to explore Tangier lipoproteins, principally HDL and their major apoproteins A-I and A-II. Thus, Tangier HDL (HDL_T) have been found as trace components among LDL in electrophoretic patterns [5] or in unexpected ultracentrifugation density fractions [6]. Furthermore, abnormally dissociated lipoproteins marked by apoproteins A-I and A-II have been demonstrated.

First, we describe the immunochemical pattern of the whole serum lipoproteins and major

apoproteins (apo) in a new case of Tangier disease. Second, we compare these immunochemical features to the data obtained with a 2-dimensional (2D) electrophoretic technique developed in our laboratory for lipoprotein apoprotein analysis.

2. MATERIALS AND METHODS

2.1. Serum

Patient serum was obtained after 12 h fast. The diagnosis of Tangier disease (homozygous form) was established with the clinical and biochemical features reported in [4].

2.2. Reagents

The chemicals used were purchased from Sigma and were of analytical reagent grade.

2.3. One-dimensional electrophoresis

One-dimensional (1D) electrophoresis was run on a plastic sheet (8 × 10 cm) supporting a film of discontinuous polyacrylamide gel (1 mm thick): 2% cathodic, where chylomicrons were trapped,

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and 3% anodic into which VLDL could not enter, thus allowing LDL and HDL to be separated. This gel was purchased from Sebia (92130, Issy-les-Moulineaux, France). The gel was soaked for 2 h just before use in a 47 mmol sodium barbital, 80 mmol sodium acetate buffer, pH 8.6, containing bovine albumin (10 g/l). A small well (0.5 × 7 mm) was punched on the cathodic side of the gel for 5 μ l of normal serum, and a large well was made (1 × 17 mm) for 25 μ l of Tangier serum to be injected. Migration was performed with the same buffer (8 mA per film, for 80 min). A lipidogram was performed with succinylated Sudan black pre-stained serum in a single 1D assay. Crude serum was used for the other following experiments.

2.4. Immunofixation

Immunofixation was performed with serum antiapo A-I, A-II and B from Immuno AG (Wien). A strip of Whatman no.1 paper (1 × 7 cm for normal serum, 2 × 7 cm for Tangier serum) was carefully applied on the gel to cover the lipoprotein migrating track in the 1D assay. Antiserum was laid along each strip (30 μ l for narrow ones, 60 μ l for wide ones) and incubated for 18 h in a wet container at 20°C. The gels were thoroughly washed with 0.15 M NaCl (24 h), H₂O (12 h) and then stained with Coomassie blue R 250 (1 g/l). Gels were destained in 50 ml/l methanol and 75 ml/l acetic acid, until a clear background was obtained.

2.5. 2D electroimmunoassay

2D electroimmunoassay (EIA), first performed by Laurell [7], was used to detail the distribution of apoproteins within the complex mixture of lipoproteins in the serum. After 1D electrophoresis, the gel was covered with a sheet of Whatman no.1 paper (8 × 10 cm) impregnated with diluted antiserum (200 μ l anti A-I, A-II or B from Immuno AG in 200 μ l of 0.15 M NaCl or 100 μ l anti A-I and 100 μ l anti B in 200 μ l of 0.15 M NaCl, and then incubated for 24 h at 4°C in a wet container. This sheet was then removed and the second dimension was run on a cooling plate at 4°C at constant voltage (15 V/cm) for 3 h using the same buffer (pH 8.6) as above, but without albumin. After the experiment was stopped, the gel was immediately washed 3 times in distilled water for 5 h each time, and then Coomassie stained and destained as described above.

2.6. 2D electrophoresis of lipoprotein apoproteins

Lipoproteins of crude serum separated as above were delipidated and their apoprotein components analyzed electrophoretically in the second dimension. To delipidate the lipoproteins, a strip (1 × 7 cm for normal serum, 2 × 7 cm for Tangier serum) corresponding to the first dimension run was cut out and soaked in a mixture of 80 mmol SDS, 1 M glycerol, 0.6 M β -mercaptoethanol, 62.5 mmol Tris-HCl, pH 6.8, for 20 min at 20°C and then loaded on top of the discontinuous polyacrylamide gel. The gel was placed between two glass plates measuring 160 (height) × 180 mm (width) which were assembled with a 2 cm wide gasket of 1.5 mm thick soft poly(vinyl chloride). A stock solution of 29.2% (w/v) acrylamide and 0.8% (w/v) methylenebisacrylamide was used to prepare a lower gel (9 cm height) in 1.5 M Tris-HCl (pH 8.8) of a final concentration of acrylamide $T = 15\%$ (w/v) and $C = 2.6\%$ (w/v), and an upper gel (4 cm height) in 0.5 M Tris-HCl (pH 6.8) of a final concentration of acrylamide $T = 4.5\%$ (w/v) and $C = 2.6\%$ (w/v).

The upper part of the mould containing the strip of delipidated lipoproteins was sealed with a buffer of 125 mmol Tris, 192 mmol glycine, 3.5 mmol SDS containing 1.5% agarose. Two gels (one for normal serum and the other for Tangier serum) were clamped to an LKB 2001 vertical electrophoresis unit. The lower and upper reservoirs were filled with the same buffer as that for preparing agarose, and electrophoresis was started at 350 V (constant voltage) for 5 h. The gels were silver stained using the ultrasensitive technique of Oakley [8].

3. RESULTS

The 1D electrophoresis of lipoproteins in patient serum showed two anomalies: first, a near absence of the α -lipoprotein band (HDL material) which is the major biochemical trait of the diagnosis of Tangier disease, and second, the presence of a fast-moving β -lipoprotein band (LDL material) and a light band at the cathodic edge of LDL (fig.1).

The immunoprints corresponding to the 1D electrophoresis of lipoproteins revealed numerous differences between normal and Tangier serum in the distribution of major apoproteins (fig.1). Apo A-I appeared clearly in the HDL bands of normal

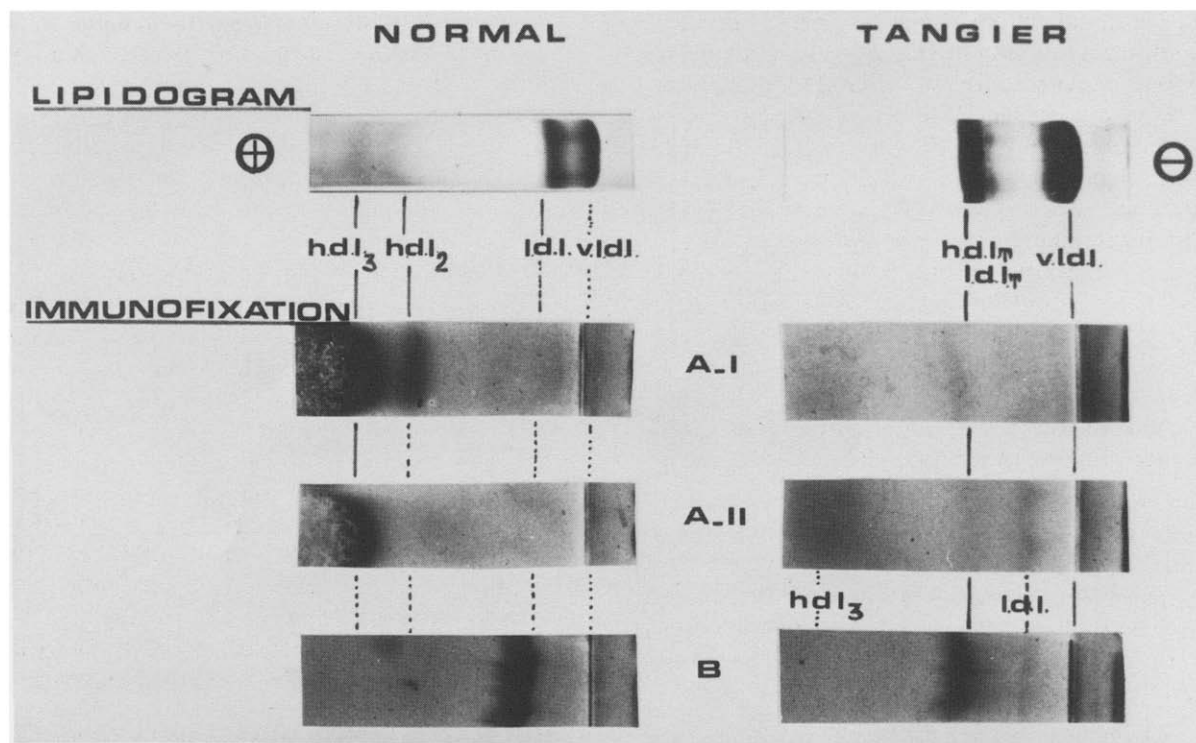


Fig.1. Lipidogram and immunofixation (see sections 2.3 and 2.4, respectively). Immunofixation with anti A-I (upper), anti A-II (middle), and anti-B (lower). The anode is on the left.

serum (abundant in HDL₃ with high mobility, less in the HDL₂ with low mobility), but in Tangier serum, it appeared as a very light band in the pre-LDL region, and was absent in the normal HDL region. Apo A-II corresponded only to HDL₃ of normal serum. On the other hand, it appeared in three different regions of Tangier serum: in the normal HDL₃ region, in the same region as apo A-I, and in the cathodic edge region of LDL Tangier. Apo B appeared as a single band in the LDL region of normal serum, but was distributed as A-I and A-II in the pre-LDL, LDL Tangier and cathodic post-LDL Tangier region in patient serum.

The 2D electroimmunoassays gave more information and revealed the heterogeneity of some bands (fig.2). In normal serum apo A-I appeared as a single peak which was 3-times higher in HDL₃ than in HDL₂. In Tangier serum, it appeared as a double peak of low mobility corresponding to HDL_T. In normal serum, apo A-II showed a similar distribution between normal HDL₃ and HDL₂, but appeared as a double peak in the HDL₃

fraction. In Tangier serum, it showed a small double peak in the HDL₃ region, a single one in the HDL_T region and another single one of slow mobility in the LDL region. Apo B showed high homogeneity in normal serum and a single peak in the normal LDL region. However, it exhibited great heterogeneity in Tangier serum. A single apo B component appeared in the normal LDL region, but 3 peaks were observed in the fast-moving LDL region (LDL_T) and another small peak in the forefront of the HDL_T region.

2D analysis of delipidated lipoproteins (fig.3) showed the distribution of the major apolipoproteins in the lipoproteic fractions of the first gel. The main observations are summarized in table 1.

4. DISCUSSION

The immunochemical analysis of major apoproteins of electrophoretically separated lipoproteins, and the electrophoretic analysis in a second dimension of apoproteins obtained after delipidation of

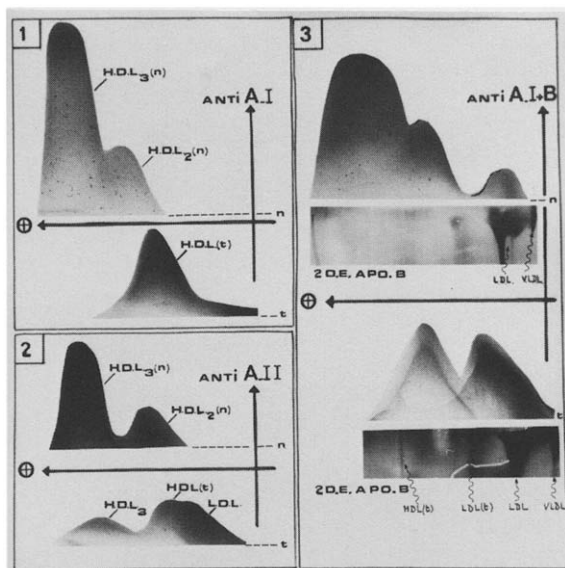


Fig.2. 2D EIA (see section 2.5). The anode is on the left (1st dimension) and on the top (2nd dimension). n, normal serum; t, Tangier serum. Serum anti A-I (1), anti A-II (2), anti A-I + B (3). 2D electrophoresis of apo B (see section 2.6): the gel of the 2nd dimension showing the distribution of apo B is placed under the corresponding bidimensional EIA with anti A-I + B (3).

these lipoproteins, demonstrate with high sensitivity the distribution of the major apoproteins in the serum. These procedures applied at the same time to normal and Tangier serum clearly reveal numerous anomalies in the distribution of Tangier apoproteins. Some of these have not been described previously.

As described in sections 2.4–2.6, apo A-I of Tangier serum is present in the pre-LDL region and as a trace in the HDL₃ region of the lipidogram. On the other hand, the apo A-II appears in the HDL₃, pre-LDL and LDL regions of the lipidogram. These data corroborate those in the literature [2,5,6,9] and are in accordance with the results obtained with different techniques: immunoelectrophoresis [5,6,10], and ultracentrifugation [6,9].

The 2D electrophoresis of apoproteins from delipidated lipoproteins that have already been electrophoretically fractionated reveals a small quantity of apo A-I in the normal HDL₃ region of the lipidogram: the main proportion of this component is in the abnormal pre-LDL region (HDL_T region). The test in section 2.5 reveals the

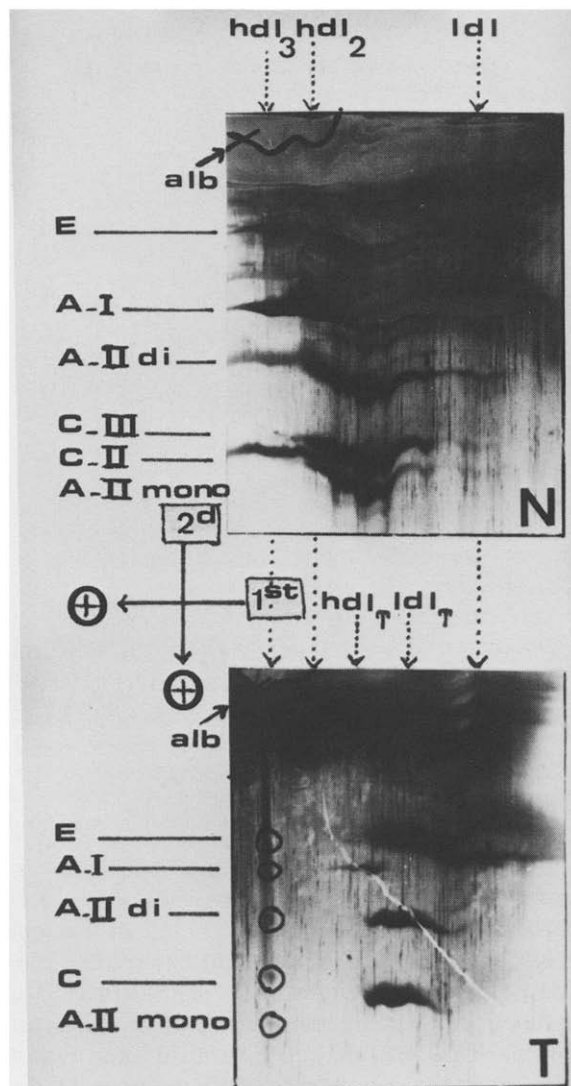


Fig.3. 2D electrophoresis of lipoprotein apoproteins (see section 2.6). These 2nd dimension plates show great differences in the distribution of major apoproteins in normal (N) and Tangier (T) serum. They reveal the apoproteic components of the lipoproteins separated in the 1st dimension. Thus, HDL_T appear mainly composed of apo A-I_T particles. On the other hand, LDL_T contains apo E, A-II, C, but only low quantities of the corresponding particles are present in normal LDL.

heterogeneity of this apo A-I fraction composed of two peaks, probably [4,11] normal apo A-I and pro-apo A-I (A-I₊₂). Similar observations concern the distribution of apo A-II, of which the main

Table 1

Distribution of major apoproteins in HDL and LDL fractions of normal (N) and Tangier (T) serum

	HDL ₃		HDL ₂		HDL _T		LDL	
	N	T	N	T	N	T	N	T
E	+	tr	+	0	0	++	+	++
A-I	+++	tr	++++	0	0	+	+	0
A-II dimer	++	tr	+++	0	0	++	+	++
C	++ (C-II)	tr	+++ (C-II, C-III)	0	0	++	tr	++
A-II monomer	0	tr	+	0	0	0	tr	++

part appears to be distributed, in dimer form, in the LDL_T region (fig.3).

Apo B are greatly disturbed in Tangier serum, as shown by the tests in sections 2.4–2.6. Normal apo B appears as a single large band in the normal LDL region of the immunofixation strip. It appears as a two-humped peak in Laurell's bidimensional EIA; this distribution of apo B is confirmed by 2D analysis (section 2.6) (figs 2,3). In contrast, in Tangier serum this distribution of apo B is low and supplementary forms of apo B may be seen. These forms are dominant and correspond to the fast LDL band (LDL_T) (fig.1), to the three highest peaks of Laurell's EIA and to the three spots in the test in section 2.6 in the LDL_T region (figs 2,3). Furthermore, a small apo B peak which, like the previous ones, has not been previously described, is exhibited in the front of the HDL_T (figs 2,3).

Finally, the anomalies in the distribution of apo A-I and A-II previously described in the literature are clearly visualized by these different immunochemical tests. Other disturbances concerning the apo B we have described correspond to the anomalies in electrophoretic mobility of LDL_T. Moreover, as is demonstrated by the 2D electrophoresis of delipidated lipoproteins, the distribution of major apoproteins in the electrophoretic track of normal or Tangier serum is characteristic of each apoprotein. This is in perfect accordance with the new concept of lipoprotein particles [12,13] which correspond to all the types of associations of apoproteins which are not visible on traditional electrophoretic or immunoelectrophoretic patterns.

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