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ISOLATION AND CHARACTERIZATION OF AN APOPROTEIN FROM THE d<1.006 LIPOPROTEINS OF HUMAN AND CANINE LYMPH HOMOLOGOUS WITH THE RAT A-IV APOPROTEIN

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SUMMARY: A comparative study of the apoproteins of the d<1.006 lipoproteins from human, canine, and rat lymph revealed striking similarities including the presence of an apoprotein apparently homologous to the 46,000 molecular weight A-IV apoprotein of the rat. Homology was based on similarities in molecular weight and amino acid composition and on immunochemical cross-reactivity of the human and canine apoproteins. In contrast to the dog and rat, the human A-IV equivalent was not a major plasma lipoprotein apoprotein. Another 46,000 molecular weight apoprotein, apo(E--A-II) complex, was present in certain human plasma lipoproteins but was easily distinguished from apo-A-IV by its reducibility to the E and A-II subunits following mercapto-ethanol treatment. Apo-A-IV was not affected by this treatment.

Dietary fat is transported by chylomicrons from its absorption site in the small intestine into the plasma via secretion of these triglyceride-rich lipoproteins into the intestinal lymph by intestinal mucosal cells. Human thoracic duct chylomicrons and rat intestinal lymph chylomicrons have been reported to contain the B, A-I, and C apoproteins (1-4). In addition, the rat chylomicrons contain the A-IV apoprotein (2,4), a 46,000 MW apoprotein which was first described in plasma high density lipoproteins (HDL)[2] (5). Because of our interest in human and animal lipoproteins and their respective apoprotein constituents, a comparative study of human, dog, and rat lymph apoproteins was initiated. We wish to report the preliminary results of this study

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[2]Abbreviations: HDL, high density lipoproteins; SDS, sodium dodecyl sulfate; VLDL, very low density lipoproteins.

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MATERIALS AND METHODS

Human thoracic duct lymph was obtained via an indwelling cannula from renal failure patients undergoing chronic lymph drainage for the purpose of pretransplantation lymphocyte depletion. Canine and rat lymph were obtained by cannulation of the thoracic and mesenteric ducts, respectively. The d<1.006 lipoproteins (a mixture of large and small chylomicrons) were prepared by ultracentrifugation of the lymph for 14 h at 50,000 rpm in a Beckman Type 60 ti rotor. The lipoproteins were purified by recentrifugation at d=1.006. The HDL_C from a dog fed cholesterol and cottonseed oil and the HDL_1 from normal dogs were obtained by Geon-Pevikon block electrophoresis as previously described (6,7). Subfractionation of normal human plasma HDL (d=1.063-1.125) to obtain HDL-I was performed as described elsewhere (8).

Apoproteins from column chromatography were prepared by modification of the tetramethylurea method of Kane (9) as previously described (10). Apoproteins were fractionated on Sephadex G-200 (2.5x290 cm) in 4M guanidine, 0.2 M Tris, pH 8.2. Analytical and preparative SDS gels were run on 11% polyacrylamide in a Tris-glycine buffer (10). The procedure of Stephens was used for the preparative SDS gels (11). Ouchterlony immunodiffusion was performed in 1% agarose gel as described previously (12). Amino acid compositions were determined on a Beckman model 121M amino acid analyzer following hydrolysis of the apoproteins with 6N HCl in a sealed N_2 atmosphere for 22 h at 110°.

RESULTS AND DISCUSSION

A comparison of the apoprotein patterns of the d<1.006 lymph lipoproteins from rat, man, and dog revealed several similarities (Fig. 1). The lymph lipoproteins all contained the following apoproteins: apo-B (at the top of the gel), the C apoproteins (the low molecular weight proteins), apo-E, and apo-A-I. The homology of the canine, rat, and human E and A-I apoproteins of plasma lipoproteins had been established previously (10,13), and these authentic apoproteins were used to establish their identity in the lymph lipoproteins. In addition, as will be discussed, the protein band labeled A-IV appeared to represent a homologous protein in common in the d<1.006 lymph lipoproteins of rat, man, and dog. The A-IV apoprotein was isolated, as shown in Fig. 1, and characterized. Rat and human apo-A-IV coelectrophoresed on SDS polyacrylamide gel electrophoresis and had an apparent molecular weight of 46,000 daltons, as previously reported for rat A-IV (5). The canine apo-A-IV, although very similar, had a slightly lower apparent molecular weight of 44,000 to 45,000. The apoprotein patterns were unchanged by mercaptoethanol treatment of the samples.

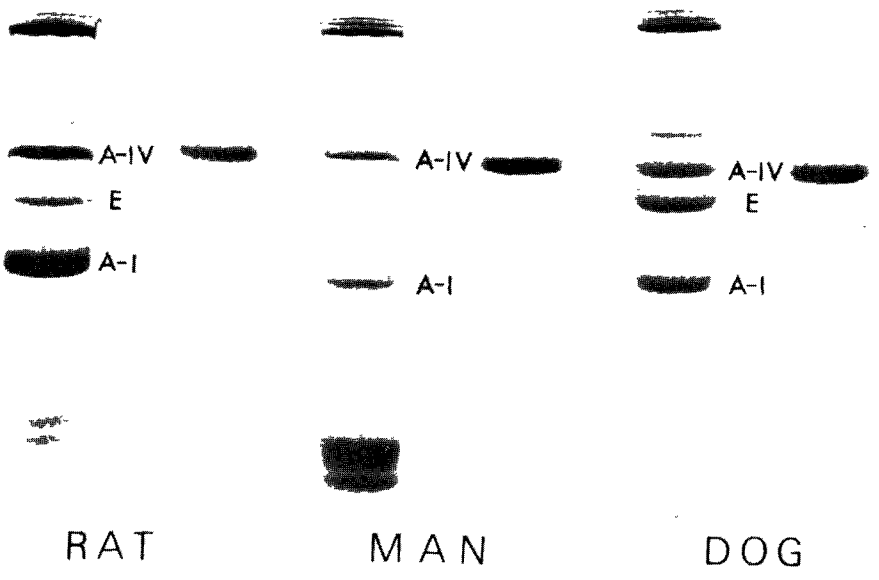

Figure 1. SDS-polyacrylamide gels of the d<1.006 lipoproteins from rat, human, and dog lymph and the A-IV apoproteins from each species.

The rat apo-A-IV has been identified as a constituent of plasma HDL (5) as well as lymph chylomicrons (2,4). Likewise, in the dog this apoprotein was present in the d<1.006 lymph lipoproteins and in the plasma HDL and HDL_C of normal and cholesterol-fed animals, respectively (see below). Identity of the canine apo-A-IV of plasma HDL with the protein in the d<1.006 lymph lipoproteins was established by coelectrophoresis and immunochemical cross-reactivity using an antiserum prepared to the purified apo-A-IV of plasma HDL_C (data not shown). The amino acid analyses of rat, human, and canine apo-A-IV revealed similarities which supported the apparent homology of these proteins among the species (Table I). Furthermore, strong support for the homology of canine and human A-IV was obtained immunochemically by the reaction of partial identity when human A-IV reacted against antiserum prepared to canine apo-A-IV (Fig. 2). The apo-A-IV from the rat did not react with this antiserum, an observation which is consistent with the previously

TABLE I. Amino Acid Composition of the Various Apo-A-IV Preparations[a]

	Chylo[b,c] (Rat)	Chylo[b,d] (Human)	HDL_c[c] (Dog)	HDL[d] (Rat)	Chylo[e] (Rat)
Asp	12.5	10.0	11.0	12.7	13.3
Thr	4.4	3.6	4.1	4.9	5.3
Ser	3.9	5.1	6.4	4.9	5.6
Glu	24.6	23.0	22.1	22.2	21.6
Pro	3.7	3.7	3.4	4.2	4.4
Gly	2.2	4.7	3.5	4.0	4.2
Ala	4.0	8.2	8.1	6.2	6.3
Val	5.6	4.6	6.0	6.2	6.0
Met	2.6	0.7	1.3	2.1	2.0
Ileu	1.8	1.5	1.3	1.9	1.8
Leu	13.2	13.6	13.7	13.1	12.6
Tyr	1.6	2.2	2.2	1.2	1.1
Phe	4.2	3.0	3.3	3.2	3.4
Lys	9.4	6.8	8.0	7.9	7.3
His	1.6	2.0	2.9	1.3	1.5
Arg	4.6	6.2	6.1	3.9	3.7

[a]Expressed as mol%; rat and human chylomicron apo-A-IV isolated by SDS gels; dog HDL_c and rat HDL apo-A-IV isolated by Sephadex G-200.

[b]Isolated from d<1.006 lymph lipoproteins.

[c]Average of duplicate determinations on a single preparation.

[d]Average of duplicate determinations on two preparations.

[e]Reference 4.

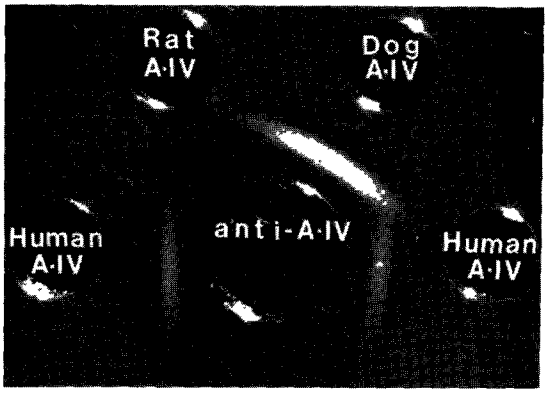

Figure 2. Double immunodiffusion of purified human, rat, and dog A-IV apoproteins reacted against antisera to the dog apo-A-IV.

noted lack of cross-reactivity of other rat apoproteins (apo-E and A-I) with the homologous proteins of the dog and man (unpublished data).

Unlike the plasma lipoproteins of the rat and dog, apo-A-IV of man appeared to be a minor constituent of the lipoproteins isolated from plasma. However, there was a distinctly different 46,000 MW apoprotein in plasma

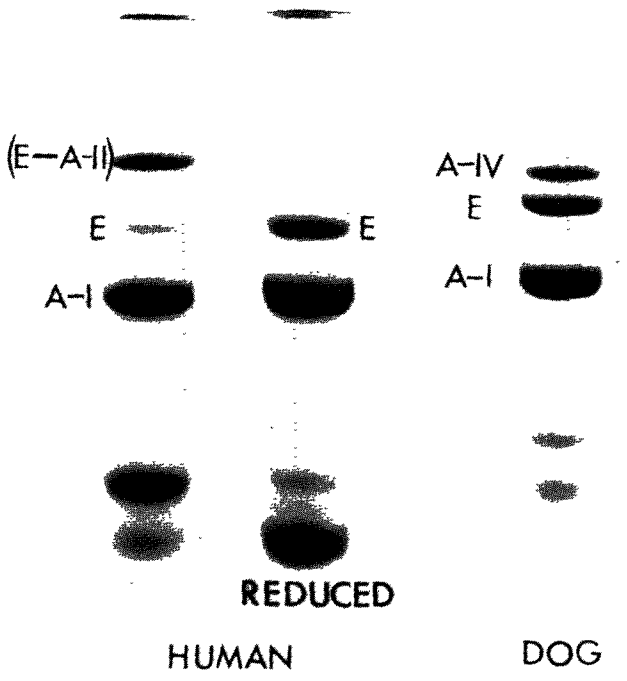

Figure 3. SDS-polyacrylamide gels of untreated human HDL-I, mercaptoethanol-reduced human HDL-I, and canine HDL_1.

VLDL and HDL of normal human subjects, as previously reported (8). Although this apoprotein, referred to as the apo(E—A-II) complex (8), coelectrophoresed with the apo-A-IV of human lymph lipoproteins and rat apo-A-IV, it was clearly a different protein. The apo(E—A-II) complex has been shown to be composed of apo-E and apo-A-II subunits linked by a disulfide bond which could be easily reduced by mercaptoethanol treatment. It was a major apoprotein constituent of the HDL-I subfraction of the d=1.063-1.125 lipoproteins of man (Fig. 3). This subfraction was isolated by Geon-Pevikon electrophoresis of this ultracentrifugal fraction (Fig. 3). Mercaptoethanol treatment of the human HDL-I resulted in the conversion of the apo(E—A-II) complex to the E and A-II subunits (Fig. 3). Dogs possessed a lipoprotein subclass which has been shown to be equivalent in most physical and chemical properties to the human HDL-I (14) except that the dog lipoprotein contained

the apoprotein now identified as apo-A-IV instead of the apo(E--A-II) complex (Fig. 3). The apoprotein pattern of the canine HDL_1, as it has been called, was unaltered by treatment with reducing agents and a reducible apo(E--A-II) complex equivalent has not been identified in the lipoproteins of either the dog or rat.

In summary, the d<1.006 lymph lipoproteins of the dog and man contain an apoprotein which appears to be homologous to the 46,000 molecular weight rat apo-A-IV. The apo-A-IV is a major constituent of certain rat and canine plasma lipoproteins and human lymph d<1.006 but not a major constituent of human plasma lipoproteins. However, another 46,000 MW apoprotein, the apo(E--A-II) complex, is present in the plasma VLDL and HDL. It remains to be determined if there are functional similarities between apo(E--A-II) and apo-A-IV.

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