Alterations in plasma lipoproteins and apolipoproteins in experimental allergic encephalomyelitis

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Abstract Plasma lipoproteins were investigated during the active clinical phase of experimental allergic encephalomyelitis (EAE), a demyelinating disease of the central nervous system. Three groups of Lewis rats were compared: untreated controls, Freund's adjuvant-treated controls (FAC), and rats receiving one injection of myelin in Freund's adjuvant. After onset of clinical symptoms, 12 and 16 days after injection, there were higher concentrations of cholesterol and low and high density lipoproteins (LDL and HDL) in EAE plasma. The increase was due to apoE-containing HDL_1 and HDL, according to density, particle size, and apolipoprotein compositions of isolated lipoproteins and immunoblots of whole plasmas after gradient gel electrophoresis. In EAE, the cholesterol-to-apoprotein ratio was increased and the low density lipoprotein distribution profile was shifted toward lower density. The Freund's adjuvant-treated control rats showed some changes qualitatively similar to those of EAE, albeit far smaller in magnitude. Changes in LDL in EAE might be related in part to lowered plasma very low density lipoproteins (VLDL); however, weight loss in control animals did not increase plasma cholesterol or apoE relative to apoA-I. Lesions in the central nervous system and/or activation of macrophages might be causally related to the large increase in plasma apoE. The major changes in apoE-containing lipoproteins are undoubtedly significant for the altered immune function in EAE. — **Shore, V. G., M. E. Smith, V. Perret, and M. A. Laskaris.** Alterations in plasma lipoproteins and apolipoproteins in experimental allergic encephalomyelitis. *J. Lipid Res.* 1987. **28:** 119–129.

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Experimental allergic encephalomyelitis (EAE), an autoimmune disease of the central nervous system (CNS), can be induced in many animal species by a single injection of white matter, purified CNS myelin, or myelin basic protein in complete Freund's adjuvant (1, 2). The disease is characterized by a massive infiltration of inflammatory cells through the blood vessels of the CNS, deposition of fibrin, demyelination, and paralysis (1–3). Symptoms are evident about 11–12 days after immunization. The sequence of events for lesion formation in EAE resembles, in some respects, the temporal profile for plaque development in multiple sclerosis (4). Long before onset of clinical signs, T lymphocytes attach to and then cross the venular walls in the CNS, where they accumulate in the CNS white matter; B cells and macrophages appear later, mainly in the meninges and perivascular cuffs (5). Monocytes differentiate into macrophages, which become the predominant cell of the lesion and are believed to be the main agent of myelin destruction (2). Macrophages peel off the myelin lamellae and become engorged with the membrane fragments (6).

Susceptibility to EAE in a given species (or to MS in humans) is linked to the major histocompatibility locus and non-MHC genes (7–9). Other modulators of immune functions include plasma lipoproteins (10, 11) and lipids, which by exchange with cell membranes may effect changes in fluidity and other membrane properties (12). The plasma apoB- and apoE-containing lipoproteins and/or modified lipoproteins (e.g., malondialdehyde-treated LDL or acetyl-LDL), by interaction with specific receptors in cell membranes, can depress cell-mediated immunity and regulate cholesterol and cholesteryl ester metabolism of lymphocytes, monocytes, and macrophages (13–15).

Abnormalities in or shifts in the homeostatic regulation of plasma lipoprotein profiles and composition could be a

Abbreviations: EAE, experimental allergic encephalomyelitis; CNS, central nervous system; apo, apolipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; VIL, total low density lipoproteins; NC, normal control; FAC, Freund's adjuvant control; SDS, sodium dodecyl sulfate; GGE, gradient gel electrophoresis; PAGE, polyacrylamide gel electrophoresis; PAGIF, polyacrylamide gel isoelectric focusing.

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contributing factor to altered cell-mediated immune function in EAE and multiple sclerosis. Conversely, the activation of macrophages and altered T lymphocyte profiles and neurological functions in these diseases could effect changes in the lipoproteins. Lipemia with large increases in neutral fat that correlated with severity of EAE in guinea pigs during the terminal phase (30 days after induction) was reported in 1957 (16). However, the possible role and abnormalities of plasma lipoproteins in the development of the disease state have not been investigated previously. This investigation focused on the abnormalities of plasma lipoproteins and apolipoproteins in acute EAE of Lewis rats, a susceptible strain (7), at 12 and 16 days after induction.

EXPERIMENTAL

Animals

Male Lewis rats, about 250 g, were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). They were divided into three groups: normal control (NC) that received no treatment before bleeding; Freund's adjuvant control (FAC) that received one injection of 0.25 ml Freund's incomplete adjuvant to which was added 3 mg/ml *Mycobacterium tuberculosis* H37RA (Difco Laboratories, Detroit, MI) on day 0; and EAE that received one injection of 1 mg of guinea pig myelin, prepared according to Smith et al. (17), in 0.25 ml of Freund's adjuvant containing *M. tuberculosis* as above on day 0. Injections were administered intradermally divided between the two hind feet. In Exp. 1, six NC, five FAC, and eight EAE animals, unfasted, were bled on day 12. EAE, as judged by motor impairment and histological examination of spinal cord, was severe in six, moderate in one, and mild in one. About 20% of the body weight was lost in the acute EAE episode. In a second experiment, two FAC animals (one fasted overnight to simulate the reduced VLDL and body weight in EAE and one fed) and two EAE animals were bled on day 16. Impairment was severe in one EAE rat and moderate in the other. The effects of weight loss (11–20%) were examined subsequently in Exps. 3 and 4, with six each NC and FAC rats, divided equally for control and weight loss, and four EAE rats. Blood was collected from the heart into plastic syringes and transferred to 6-ml polyethylene tubes containing 0.05 ml 15% K_3EDTA. Garamycin and ϵ-aminocaproic acid were added to plasma as preservatives.

Lipoprotein isolation

Total low density lipoproteins (VIL), including very low, intermediate, and low density lipoproteins (VLDL, IDL, and LDL, respectively) and total high density lipoproteins (HDL) were isolated by sequential ultracentrifugation according to Lindgren, Jensen, and Hatch (18). For VIL isolation 4 ml of plasma + 2 ml of VIL solution (ϱ = 1.1745 g/ml; NaBr, NaCl, EDTA, pH 7.4) was centrifuged in a Beckman 40.2 rotor (Beckman Instruments, Palo Alto, CA) at 40,000 rpm for 24 hr. The top 1 ml contained the d > 1.063 g/ml lipoproteins (VIL_{0-1}). The second ml (VIL_{1-2}), usually discarded in human lipoprotein isolations, was also analyzed. For HDL isolation, the bottom 4 ml was added to 2 ml of VLH solution (ϱ = 1.4743 g/ml; NaBr, NaCl, EDTA, pH 7.4) and centrifuged in a 40.2 rotor at 40,000 rpm for 40 hr. The top 1 ml contained the d > 1.20 g/ml lipoproteins (HDL_{0-1}). The next 0.5 ml ($HDL_{1-1.5}$) was also removed for analysis. The bottom 4.5 ml was analyzed by a modified Lowry method (19) for total protein. In Exp. 1 pools of two plasma samples were used in seven of eleven samples taken for isolation of lipoproteins because of insufficient volume for individual analysis. EAE pools included only severely affected rats. The VIL_{0-1} of NC, FAC, and EAE (pools of three lipoprotein samples per group) were subfractionated by density gradient ultracentrifugation. The gradient was formed by sequentially layering in a $^9/_{16}$" × 3½" tube 0.5 ml of 50% sucrose, 2 ml of undialyzed VIL_{0-1}, 3 ml of a ϱ = 1.040 g/ml solution (NaBr, NaCl, EDTA) and finally 7 ml of a ϱ = 1.0063 g/ml solution (NaCl, EDTA, pH 7.4). After 65 hr in a swinging bucket rotor at 40,000 rpm, twelve 1-ml fractions and finally 0.5 ml were pipetted sequentially down the tube. In Exp. 3, the d < 1.21 g/ml lipoproteins were isolated in a single 24-hr centrifuge run at 40,000 rpm (to minimize possible loss of apolipoproteins). Appropriate salt background solutions (18) were centrifuged under the same conditions for subsequent measurement of the background densities. Solution and subfraction densities were measured in a Paar-Mettler calculating density meter (Anton Paar K.G., Graz, Austria).

Lipoprotein analyses

Gradient gel electrophoresis (GGE) (20) separated lipoproteins according to particle size. Aliquots of isolated, undialyzed lipoproteins were electrophoresed on 2.5–16% polyacrylamide slab gels (7 × 8 cm; 3 mm thick) in boric acid (0.08 M)-Tris (0.09 M)-Na_2EDTA (0.003 M), pH 8.35 for 18 hr at 10 ma/gel. The HDL were also electrophoresed on 2.5–27% slabs (7 × 12 cm) (Isolab Inc., Akron, OH) for 27 hr. The gels were stained with Coomassie blue R250 unless immunoblotting was to be done. For identification of apoB-containing lipoproteins in the VIL_{1-2}, aliquots containing ~15 μg of lipoprotein protein were separated on 2.5–16% gels and then transferred to nitrocellulose sheets (21). An apoB marker, i.e., an LDL-containing subfraction from density gradient separation of VIL_{0-1},

was added to one of the NC VIL_{1-2} samples, which was also electrophoresed without added LDL. Detection was with rabbit anti-human apoB serum that cross-reacted with rat apoB. In Exp. 4, 15-μl aliquots of whole plasma in alternate lanes on 2–16% and 4–30% slab gels (Pharmacia Inc., Piscataway, NJ) were electrophoresed, stained for lipid with oil red O, and scanned densitometrically (20) by Dr. F. T. Lindren and Ms. Laura Glines of the Donner Laboratory, University of California at Berkeley. We also electrophoresed whole plasma (3 μl) and immunoblotted the gels with goat anti-rat apoE and anti-rat apoA-I. These antisera were provided by Dr. Paul Roheim, Dept. of Physiology, Louisiana State University Medical Center, New Orleans. Protein standards, i.e., molecular weight calibration kits (Pharmacia Inc.), were run in alternate lanes on 12-lane slabs. These marker mixtures included thyroglobulin (669,000), apoferritin (440,000), catalase (232,000), lactate dehydrogenase (140,000), bovine serum albumin (67,000), and carboxylated latex beads of diameter 0.038 ± 0.0075 μm (Dow Chemical Co., Indianapolis, IN). Particle diameters of thyroglobulin, apoferritin, lactic dehydrogenase, and serum albumin are 170 Å, 122 Å, 81.6 Å, and 71 Å, respectively (20).

Delipidated, desalted apoproteins and molecular weight markers (the above mixture and/or a mixture containing phosphorylase B (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400)) in 1 or 2% sodium dodecyl sulfate (SDS) were electrophoresed in the presence of 0.1% SDS in 2.5–27% acrylamide slab gels (22) or in cylindrical gels (5 × 95 mm; 3.75% or 10% acrylamide) (23). Samples, with and without mercaptoethanol (23), for SDS-polyacrylamide gel electrophoresis (PAGE) contained 15 μg apoprotein for the slab gels and 20, 30, or 40 μg for the cylindrical gels. Polyacrylamide gel isolectric focusing (PAGIF) (pH 4–6) of apoHDL was carried out in 6% polyacrylamide gels (5 × 100 mm) containing 6 M urea as described before (23). The samples contained 100 μg of apoprotein. Gels were stained with Coomassie blue R250 and scanned at 595 nm in a Zeineh soft laser scanning densitometer (Biomedical Instruments, Inc., Chicago, IL).

For immunodetection of apoA-I, apoB, and apoE distribution in GGE, the lipoproteins were electrophoretically transferred to nitrocellulose sheets (21). Transfer was carried out in Tris-glycine buffer, pH 8.4, at 250–500 ma for 36 hr. Transfer was essentially complete, since little Coomassie blue-staining material remained in the polyacrylamide slab gel. After blocking unreacted sites with a mixture containing 0.05% Tween 20 and 1% bovine serum albumin in phosphate-buffered saline (30 min at 37°C on a platform rocker) and subsequent washing with the Tween 20 solution without albumin (3 ×), the sheets were reacted with the first antibody (anti-apolipoprotein) for 3 hr at room temperature. After washing again, the sheets were reacted 1 hr with biotinylated second antibody (anti-immunoglobulin) and then coupled to avidin–horseradish peroxidase complex (24). After washing with phosphate-buffered saline, pH 7.5, at room temperature, the NC sheet was added to peroxidase substrate (4-chloro-1-naphthol) solution for color development (15 min at room temperature) and finally washed with distilled water.

Protein concentration of all isolated lipoproteins was determined by a modified Lowry method (19). Cholesterol (free plus esterified) concentration of plasma and isolated lipoproteins was determined enzymatically (25).

RESULTS

Cholesterol concentrations in plasma and isolated lipoproteins

Total cholesterol concentration was measured in 11 of 19 plasmas of Exp. 1 (**Table 1**) and in all the plasma samples of Exps. 3 and 4. The values were fairly uniform in the FAC and NC groups and more variable but consistently higher in EAE animals. Weight loss had no significant effect on plasma cholesterol concentration in NC and FAC rats. The plasma cholesterol values in 12-day FAC were lower than in NC mainly because of the lower HDL (Table 1 and **Table 2**). In FAC, the cholesterol (mg/dl plasma) associated with isolated HDL was lower than in NC at 12 days and normal at 16 days, but the VIL_{0-1}-cholesterol was lower in only one 12-day sample and normal in the others. The VIL_{1-2}-cholesterol concentration was somewhat higher in FAC than in NC, but in both groups it was much lower than in EAE. In all EAE samples, the VIL_{0-1}- and HDL_{0-1}-cholesterol values were also higher than in FAC or NC samples (Table 1).

Cholesterol-to-protein ratios in isolated lipoproteins

In all EAE VIL_{0-1}, the cholesterol-to-protein ratio was higher than in the corresponding lipoprotein fractions of NC and FAC (Table 1). Fasting did not significantly change the cholesterol-to-protein ratio in FAC VIL_{0-1} (Table 1, Exp. 2). The average ratio for VIL_{0-1} in EAE was 22–25% higher than in controls. In the HDL, differences in the ratio among the three groups were small, but the EAE values were generally higher and the 16-day FAC values were lowest (Table 1).

Apolipoprotein concentration in isolated lipoproteins

Plasma low (VIL_{0-1} and VIL_{1-2}) and high (HDL_{0-1}) density lipoprotein apoproteins were significantly more concentrated during the EAE active phase than in FA or

TABLE 1. Total cholesterol concentrations (mg/dl plasma) in plasma and isolated plasma lipoproteins and ratio (wt/wt) of cholesterol to protein in the lipoproteins

Rats	Plasma		VIL_{0-1}		HDL_{0-1}	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Exp. 1 (12-day)						
NC						
Total cholesterol	58 ± 1.5	(56–59)	13.9 ± 0.4	(13.5–14.2)	37.3 ± 1.8	(35.3–38.8)
Ratio			0.80 ± 0.03	(0.77–0.84)	0.57 ± 0.02	(0.55–0.59)
FAC						
Total cholesterol	50 ± 1.5	(48–51)	14.0 ± 2.67	(10.9–15.8)	30.2 ± 4.4	(25.3–33.7)
Ratio			0.82 ± 0.11	(0.70–0.90)	0.60 ± 0.02	(0.57–0.61)
EAE						
Total cholesterol	82 ± 10.3	(70–95)	23.8 ± 4.4	(20.6–28.8)	53.0 ± 6.6	(47.5–60.3)
Ratio			1.00 ± 0.04	(0.96–1.03)	0.64 ± 0.05	(0.60–0.70)
			Total Cholesterol	Cholesterol/ Protein Ratio	Total Cholesterol	Cholesterol/ Protein Ratio
Exp. 2 (16-day)						
FAC fasted			13.5	(0.85)	34.2	(0.48)
FAC fed			15.0	(0.88)	35.7	(0.52)
EAE 1			23.0	(1.14)	52.0	(0.63)
EAE 2			19.6	(0.98)	60.4	(0.59)

P values for FAC versus EAE plasma cholesterol, VIL_{0-1}-cholesterol, and HDL_{0-1}-cholesterol were <0.005, <0.05, and <0.01, respectively. The corresponding values for NC versus EAE were <0.02, <0.02, and <0.02, respectively. Abbreviations: NC, normal control; FAC, Freund's adjuvant control; EAE, experimental allergic encephalomyelitis.

normal controls at 12 and 16 days (Exps. 1 and 2, respectively) (Table 2). These increases were not the result of hemoconcentration since the protein concentrations of the $d < 1.21$ g/ml fractions of plasma did not differ significantly. In both experiments, total VIL, i.e., VIL_{0-1} plus VIL_{1-2}, apoprotein increased 45% on the average (27% for the least symptomatic EAE to 80% for the moderately affected one) above the FAC level, which was essentially

TABLE 2. Protein distribution among plasma subfractions

Rats	VIL_{0-1}	VIL_{1-2}	HDL_{0-1}	$HDL_{1-1.5}$	$d > 1.21$
	mg apoprotein/dl plasma				*g/dl*
Exp. 1 (12-day)					
NC (n = 6)[a]	17.4 ± 0.5	6.7 ± 0.4	65.0 ± 4.0	9.2 ± 2.5	7.0 ± 0.5
Range	16.8–17.8	6.3–7.0	60.8–68.8	7.4–12.0	6.5–7.4
FAC (n = 5)[a]	16.8 ± 1.1	6.4 ± 1.0	50.5 ± 5.6	7.4 ± 0.8	7.0 ± 0.4
Range	15.5–17.5	5.8–7.6	44.3–55.3	6.5–8.2	6.5–7.3
EAE (n = 8)[a]	24.0 ± 4.5	9.7 ± 1.7	82.6 ± 4.5	9.1 ± 2.1	7.0 ± 0.2
Range	18.7–30.0	7.5–11.7	76.8–86.2	7.6–11.7	6.7–7.2
EAE[b] (n = 7)[a]	25.3 ± 4.5	9.4 ± 1.8	83.5 ± 4.5	8.6 ± 2.1	7.0 ± 0.2
Range	20.3–30.0	7.5–11.7	76.8–86.2	7.6–11.7	6.7–7.2
Exp. 2 (16-day)					
FAC fasted	16.0	7.4	71.7	9.4	7.6
FAC fed	17.0	7.0	69.0	8.8	7.3
EAE 1	28.5	10.0	82.5	7.3	7.1
EAE 2	20.0	9.2	102.5	6.9	7.4

P values for FAC versus EAE VIL_{0-1}, VIL_{0-1} + VIL_{1-2}, HDL_{0-1}, and HDL_{0-1} + $HDL_{1-1.5}$ apoproteins were <0.01, <0.002, <0.001, and <0.001, respectively.

[a] Mean ± SD.

[b] Excludes rat with mild symptoms.

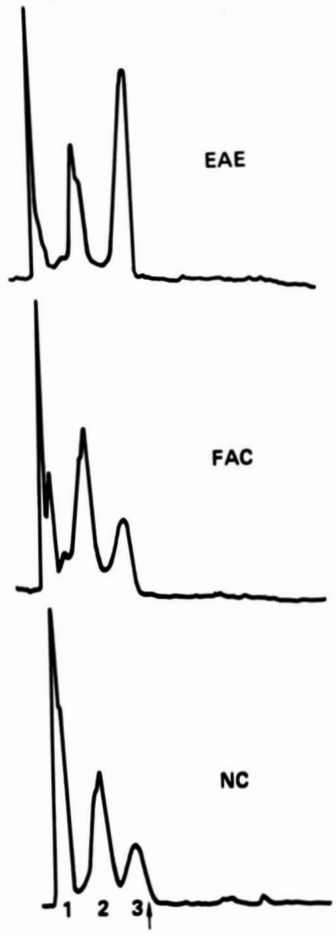

Fig. 1. Densitometric scans of 2.5–16% polyacrylamide gradient gel electrophoretograms of plasma VIL_{0-1} from NC, FAC, and EAE rats. Gels were stained with Coomassie blue R250. Peaks 1, 2, and 3 correspond to VLDL, LDL, and HDL_1, respectively; the arrow marks the position of the thyroglobulin standard peak maximum.

the same as for NC. The VIL_{1-2}, which in humans contains ~5% of the total VIL apoprotein ((26) and our unpublished observations), contained relatively much more apoprotein in the Lewis rat. Plasma concentrations of VIL_{1-2} were highest in the moderately and mildly affected animals (11.7 and 10.7 mg of apoprotein/dl, respectively) at 12 days.

ApoHDL concentration in EAE averaged 64% and 28% higher than in FAC and NC, respectively, in Exp. 1. The HDL concentration in the mildly affected animal was within the range for those severely affected. The highest concentration (102.5 mg of apoprotein/dl) occurred in a moderately affected rat at 16 days. At 16 days (Exp. 2), apoHDL concentration of FAC, which was lower than in NC at 12 days, had returned to normal; in EAE apoHDL remained high. The total protein concentration in VIL and HDL in FAC was unaffected by an 18-hr fast just before bleeding (Table 2).

Lipoprotein gradient gel electrophoretic (GGE) profiles

The increase in plasma VIL_{0-1} in EAE was due to an increase in a normally minor lipoprotein constituent with particle diameter ~180–200 Å (i.e., somewhat larger than the thyroglobulin standard and smaller than LDL),

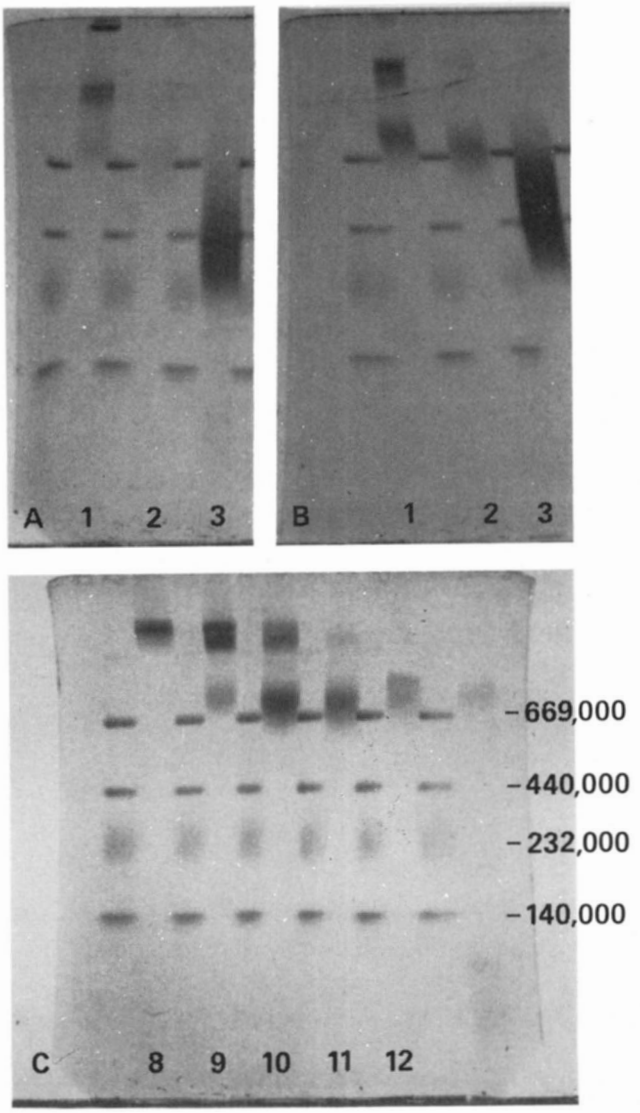

Fig. 2. Electrophoretograms of isolated lipoproteins in 2.5–16% polyacrylamide gradient gels after staining with Coomassie blue R250. Patterns A 1,2,3 correspond to NC VIL_{0-1}, VIL_{1-2}, and HDL_{0-1}, respectively; patterns B 1,2,3 are the comparable EAE fractions. Gel C contains EAE VIL_{0-1} subfractions 8 to 12 from density gradient ultracentrifugation (see Methods and Fig. 3). Alternate lanes contain protein standards.

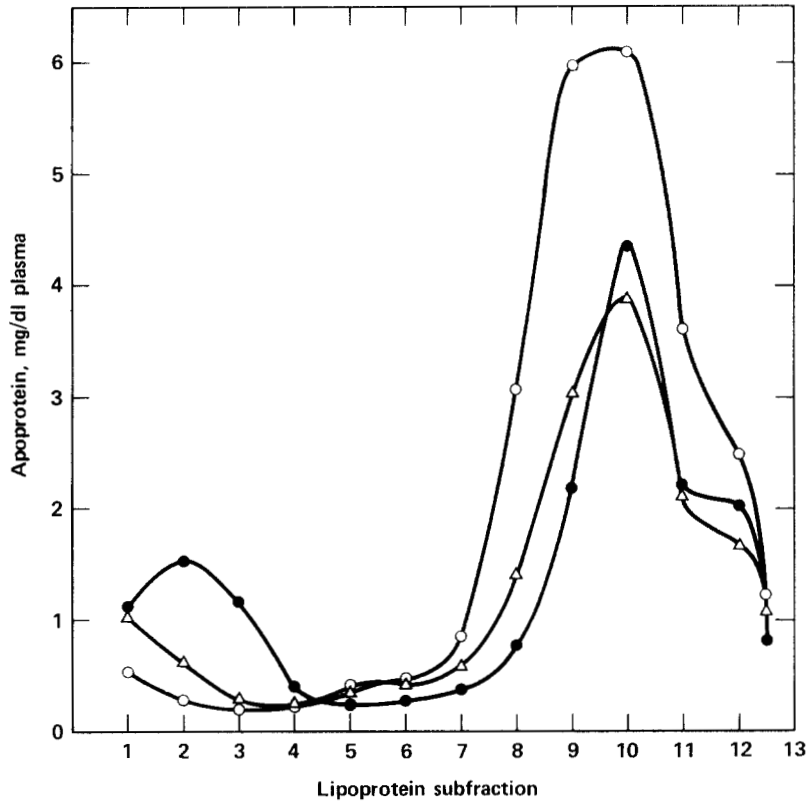

Fig. 3. Protein distribution after density gradient ultracentrifugation of pooled VIL_{0-1} from NC (●), FAC (△), and EAE (○) plasmas from Exp. 1 (see Methods). The density range for fractions 1 to 12 is 1.006 g/ml to 1.080 g/ml. The pools of NC-VIL and FAC-VIL contained approximately equal volumes of all the appropriate VIL_{0-1} samples in Table 1 and 17.4 and 16.8 mg of apoVIL/dl plasma, respectively. The EAE pool contained 23.8 mg of apoVIL/dl and represented only the severely affected animals.

according to the Coomassie blue-stained GGE patterns of isolated lipoproteins and their densitometric scans (**Fig. 1** and **Fig. 2,** patterns A1 and B1) from Exp. 1 and 2 and oil red O-stained GGE patterns of whole plasma (not shown) from Exp. 3 and 4. This constituent, HDL_1 or HDL_c (27), was much higher in all the EAE rats, except for the mildly affected rat, than in controls with or without weight loss. Similar but somewhat smaller particles (~170 Å in diameter) were the major constituent of the VIL_{1-2} (Fig. 2, patterns A2 and B2) and were also increased in concentration in EAE, including the moderately and mildly affected rats. The densitometric scans of the GGE patterns indicated no major differences in LDL concentration among the NC, FAC, and EAE rats. However, the ratio of lower to upper bands, i.e., lower to higher molecular weight particles, in the double-banded LDL (Fig. 1, peak 2) decreased in EAE and in the fasted FAC. The VLDL concentrations were: NC > FAC > fasted FAC and NC ~EAE. Low levels of VLDL undoubtedly resulted from reduced food consumption and weight loss with onset of EAE symptoms. However, fasting of the FAC (Table 2, Exp. 2) or weight loss in NC and FAC did not alter the differences between EAE and control lipoprotein concentrations or cause a large increase in HDL_1. The HDL are larger on the average than human HDL, which were electrophoresed under the same conditions (patterns not shown). The increased HDL in EAE appears disproportionately in the larger particles (Fig. 2, patterns A3 and B3).

Lipoprotein density profiles

The distribution of VIL_{0-1} protein across the density range for the three groups of rats is compared in **Fig. 3.** Fraction 1, which contained VLDL, was similar in FAC and NC and lower in EAE. Fractions 2 (also VLDL) and 3 were more concentrated in NC than in FAC or EAE.

TABLE 3. Ratio of cholesterol to protein in VIL_{0-1} subfractions and VIL_{1-2}

Subfraction[a]	NC	FAC	EAE
8			1.09
9	0.82	1.00	1.12
10	0.82	0.97	1.11
11	0.45	0.61	0.77
12	0.13	0.14	0.29
VIL_{1-2}[b]	0.34 ± 0.03	0.46 ± 0.06	0.81 ± 0.05

[a] From density gradient ultracentrifugation of pooled VIL_{0-1} samples (three per group).
[b] Mean ± SD; n = 3 for NC and FAC; n = 5 for EAE.

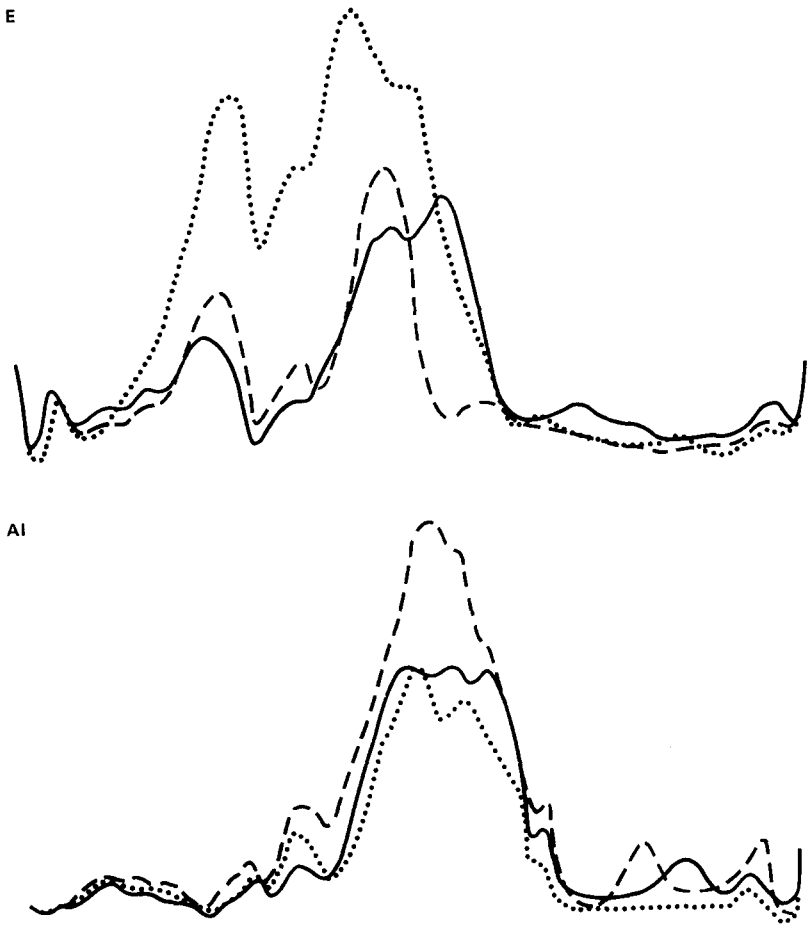

Fig. 4. Densitometric scans of Western blots of plasmas after GGE. E and A-I were developed with anti rat apoE and apoA-I, respectively. EAE, dotted lines (....); FAC, solid lines (—); FAC with weight loss, broken lines (– – –).

The IDL fractions 3 to 6 were similar in EAE and FAC. In EAE, the major increases were in the LDL density range, i.e., in subfractions 8, 9, 10, and 11 (d 1.024–1.060 g/ml), and the distribution of these fractions was shifted toward the less dense region, i.e., fractions 8 and 9 were increased most. FA alone appeared to cause increases in the subfractions 8, 9, and 10, but to a lesser extent than EAE. The GGE patterns of the VIL_{0-1} subfractions (Fig. 2C) indicated that the HDL_1 was partially purified in the density gradient and was higher in density than the major LDL. The HDL_1 was most concentrated in fraction 10 (d 1.045 g/ml) in EAE and both controls with concentrations in the order EAE > > FAC > NC. The decreasing concentration of HDL_1 with increasing density in subfractions 10, 11, and 12 is inconsistent with overlapping of HDL into the LDL density range or HDL contamination in the isolated VIL_{0-1}.

Consistent with differences in cholesterol-to-protein ratio (Table 1), the density distribution of the LDL was different in the three groups of rats, according to the GGE patterns of the VIL_{0-1} subfractions (not shown for NC and FAC). In NC, LDL concentration was greatest in subfraction 10 (d 1.045 g/ml) and considerably less in subfraction 9 (d 1.031 g/ml). (Normal male human LDL typically yielded a major subfraction of d 1.031 g/ml when separated under the same conditions.) Subfraction 9 contained the major LDL in FAC and EAE, but subfraction 8 contained more LDL in EAE than in FAC and vice versa for subfraction 10. These differences in density distribution of the LDL were associated with the difference in double banding of the LDL noted in GGE of whole VIL_{0-1} (Fig. 1). These two LDL subclasses were partially separated by density gradient ultracentrifugation (Fig. 2C). The upper band (larger LDL) was more abundant in EAE and comprised most of VIL_{0-1} subfraction 8. Both bands were present in subfraction 9 and the lower band (smaller LDL) was the major LDL species of subfractions 10 and 11.

The cholesterol enrichment of EAE lipoproteins was greatest in VIL_{1-2} and VIL_{0-1} subfractions 11 and 12 (**Table 3**), i.e., for the fractions that contained HDL_1 and very little LDL. However, EAE VIL_{0-1} subfractions 8 and

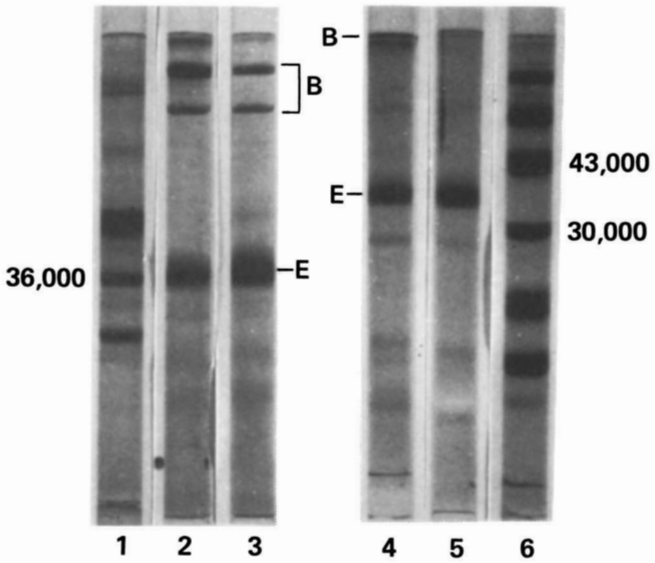

Fig. 5. Electrophoretograms of FAC and EAE apoVIL$_{0-1}$ (40 μg each; gels 2,3, respectively) and apoVIL$_{1-2}$ (20 μg each; gels 4,5, respectively) and standards (gels 1,6) in SDS-PAGE. Gels 1 to 3 contained 3.75% acrylamide and gels 4 to 6, 10%. All were stained with Coomassie blue R250.

9 that contained mainly LDL (Fig. 2C) also showed an increase in cholesterol-to-protein ratio (Table 3). In FAC the ratios for the major VIL$_{0-1}$ subfractions were increased to a smaller extent (Table 3) and the ratio for total VIL$_{0-1}$ was only slightly higher than that in NC (mean value = 0.82 vs. 0.80).

ApoB distribution in VIL$_{1-2}$

The VIL$_{1-2}$ from NC, FAC, and EAE reacted strongly with anti-human apoB after GGE and Western blotting. However, HDL$_1$ did not react and the apoB-containing species were somewhat smaller in diameter than the LDL of VIL$_{0-1}$ and marker VIL$_{0-1}$ subfraction. Both LDL bands of the marker VIL$_{0-1}$ subfraction reacted strongly with anti-apoB. The Coomassie blue-stained GGE patterns indicated the apoB-reactive species of VIL$_{1-2}$ to be very minor components.

ApoA-I and apoE distributions in plasma

Densitometric scans of Western blots of GGE of whole plasmas from Exp. 4 are shown in **Fig. 4,** which compares the distributions of apoE and apoA-I in EAE with those in FAC with and without weight loss. The apoA-I distribution reflects mainly HDL since very little apoA-I was present in isolated VIL (PAGE data given below). Differences in apoA-I scans are not striking except possibly for a relative increase in larger HDL in FAC after weight loss. In EAE, apoE was greatly increased in the region between LDL and HDL and in the HDL as well. Controls for weight loss showed a change in apoE distribution with increases in the larger HDL and HDL$_1$ but not the major increase of EAE.

Apolipoproteins of isolated lipoproteins

The SDS-PAGE and PAGIF indicated that in EAE apoE increased relative to other apolipoproteins in all major fractions of plasma lipoproteins. In apoVIL$_{0-1}$, apoB and apoE were the major apoproteins and the ratio of apoE to apoB was much higher in EAE than in FAC (**Fig. 5,** patterns 2 and 3) or in NC (not shown). In Exp. 2 fasting did not increase apoE in FAC VIL$_{0-1}$ or VIL$_{1-2}$. In VIL$_{0-1}$ apoE was present mainly in subfractions 9, 10, 11, 12, and VLDL and was most concentrated in subfractions 10 and 11 (patterns not shown); i.e., except for VLDL, apoE distribution correlated with that of HDL$_1$. The EAE apoVIL$_{1-2}$ also contained a higher proportion of apoE than control VIL$_{1-2}$ (Fig. 5, patterns 4 and 5). Very little apoA-I was present in any of the VIL$_{1-2}$ or VIL$_{0-1}$.

Densitometric scans of the SDS-PAGE patterns of the apoHDL$_{0-1}$ and apoHDL$_{1-1.5}$ (**Fig. 6** and **Fig. 7,** respec-

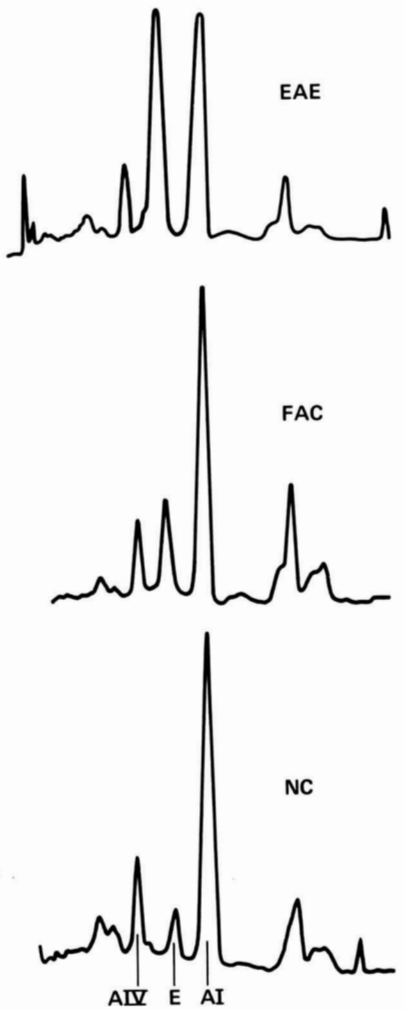

Fig. 6. Densitometric scans of the SDS-10% acrylamide gel electrophoretograms of apoHDL isolated from NC, FAC, and EAE plasmas. Samples contained 30 μg of protein. The stain was Coomassie blue R250.

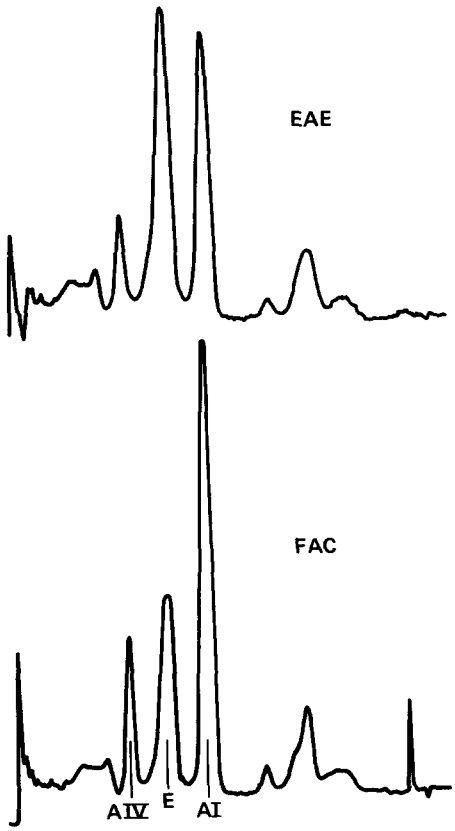

Fig. 7. Densitometric scans of Coomassie blue-stained electrophoretograms of apoHDL$_{1-1.5}$ (see Methods and Table 2) in SDS-10% acrylamide gels. Samples contained 30 μg of protein.

tively) show a relative increase of apoE and decrease in apoA-I in EAE. The HDL$_{1-1.5}$ contained only small amounts of albumin. All EAE HDL samples showed increased apoE. The PAGIF and SDS-PAGE patterns (not shown) for the HDL$_{0-1}$ of the two least symptomatic EAE rats of Exp. 1 showed an even larger reduction in apoA-I and apoA-IV and increase in apoE than in severely affected rats. Densitometric scans of apoHDL patterns show that apoE was somewhat increased in FAC HDL, although always far less than in EAE HDL. The ratio of apoE to apoA-I in SDS-PAGE patterns of HDL and total d < 1.21 g/ml lipoproteins was similar before and after weight loss in NC and FAC. The PAGIF patterns of apoHDL were similar to those reported for apoHDL of Sprague-Dawley rats (28, 29), except for relatively less apoC-III-0 in the Lewis rats. The isoform banding patterns for apoA-I, apoE, and the apoCs were similar in NC, FAC, and EAE. The PAGIF patterns confirmed the identities of apoproteins in SDS-PAGE.

DISCUSSION

The most striking change in plasma lipoproteins in EAE in the Lewis rat is the very large increase in apoE-containing HDL and HDL$_1$. Severely symptomatic rats showed large apoE increases in both lipoproteins, while the least affected showed a large increase in HDL and relatively little in HDL$_1$. The cause of the increase in apoE is obscure, but on the basis of present information increased synthesis seems more plausible than decreased catabolism. While much of the plasma apoE is synthesized by liver, a variety of other tissues including macrophages and brain are known to synthesize apoE (14, 30–33). The main site of apoE synthesis and secretion in the CNS is the astrocyte (34). ApoE synthesis by macrophages might be stimulated by phagocytosis of cholesterol-rich myelin in EAE. Other investigators have found apoE synthesis to be greatly increased in macrophages in vitro after cholesterol loading via acetyl-LDL (14, 33) and in liver after a cholesterol-rich diet (35). ApoE might be one of the proteins whose synthesis was greatly increased in EAE lesions (36, 37). Much of the non-myelin protein increase appeared to occur in metabolically active lymphoid cells. Recent studies have shown that apoE synthesis increases dramatically after crushing the optic and sciatic nerves (38) and that nonneuronal cells are involved (39). The EAE lesion might mimic nerve injury in stimulating apoE synthesis. However, it is difficult to envisage the CNS as the sole source of the large apoE increase in EAE plasma, even though brain tissue contains a considerable amount of mRNA for apoE (32). The increased vascular permeability of the CNS in the EAE animal (6) might facilitate entry of apoE synthesized in brain into the blood. It is even possible that brain apoE has access to the blood normally, since it was one of several apolipoproteins detected in cerebrospinal fluid (40).

Changes in apoB-containing LDL in EAE and to a lesser extent in FAC mimic some of the changes in LDL observed after feeding an atherogenic diet to a variety of nonhuman primates (41, 42). The cholesterol-to-apoB ratio and particle size are increased and density is decreased; however, plasma LDL concentration did not increase. Increased LDL particle size in EAE could have resulted from increases in content of phospholipids (43) derived from VLDL catabolism. The capacity of the LDL for other lipids, e.g., triacylglycerol and cholesteryl esters, is thought to increase with increasing phospholipid-to-apoB ratio, but increased cholesterol loading would depend on subsequent interactions of the LDL. In human plasma, smaller LDL are positively and larger LDL are negatively correlated with VLDL (44).

Plasma cholesterol concentrations in the control Lewis rats were in the ranges for Wistar (parent strain; less susceptible to EAE) (45–47) and Sprague-Dawley (EAE-resistant) (30, 35) male rats. Our recovery of total plasma cholesterol in isolated lipoproteins was >90% in all the Lewis rats. However, our cholesterol-to-protein ratios for VIL$_{0-1}$ and its subfractions, except for the EAE samples, were lower than corresponding values calculated from

published data on other strains of rat (30, 45, 47, 48). Our ratio for HDL was in the range reported for male Wistar (45, 48, 49) and Sprague-Dawley (30) plasma HDL. The variations in cholesterol-to-protein ratio may reflect differences in isolation and analytical procedures as well as differences in strain of rats. The apoprotein profiles in our SDS-PAGE and SDS-GGE patterns of NC HDL and VIL resembled those for Sprague-Dawley lipoproteins (22, 50). The EAE profile differed markedly from that in experimental nephrosis, in which increased plasma cholesterol concentration was accompanied by a large increase in plasma HDL and HDL_1 that were enriched in apoA-I and deficient in apoA-IV and apoE as a consequence of increased hepatic synthesis of apoA-I and probably decreased lipolysis (51, 52). The plasma cholesterol and lipoprotein changes in EAE are not general features of the acute phase in inflammatory response (53). That plasma LDL with low cholesterol-to-apoB ratio are relevant to development of the autoimmune response to myelin in the Lewis rat is an interesting but entirely speculative possibility.

Plasma lipoprotein changes in EAE may be largely the consequence rather than causal in lesion development. Even so, the lipoproteins could have an important modulating effect on development of clinical symptoms. ApoE is a transport protein for cholesterol, cholesteryl esters, and possibly phospholipids. Effects of the apoE-containing lipoproteins include inhibition of mitogen-induced lymphocyte proliferation and cell-mediated immunity (15). These lipoproteins bind and are taken up by the apoB, E receptor of cell membranes (14). Thus they could exert regulation of sterol metabolism in CNS cells. Regulation of cholesteryl ester synthesis by LDL and desmosterol has been reported for cultured C6 glial cells (glioma-derived) (54), which have the characteristics of both astrocytes and oligodendrocytes but will not synthesize myelin.

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