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Renal Apolipoproteins in Nephrotic Rats

Harry van Goor,* Marian L. C. van der Horst,* Jane Atmosoerodjo,* Jaap A. Joles,† Arie van Tol,‡ and Joris Grond*

From the Department of Pathology, University of Groningen, Department of Nephrology,† University Hospital, Utrecht, and Department of Biochemistry,‡ Erasmus University, Rotterdam, The Netherlands*

Recent experimental data suggest a role for lipids in the pathogenesis of glomerulosclerosis. In this study, we examined the main apolipoproteins (apo) of high density lipoproteins (A-I, A-IV, E), low density lipoproteins (B), and very low density lipoproteins (B, E) in plasma and kidney tissue of rats with puromycin aminonucleoside or adriamycin nephrosis. In full-blown nephrosis, plasma concentrations of apo A-I and apo B were significantly elevated, apo A-IV and apo E levels did not change. Immunohistological studies in plastic sections revealed increased apo A-I, apo A-IV, and apo E immunoreactivity in glomerular visceral epithelial cells both in puromycin aminonucleoside and adriamycin nephrosis. This was confirmed by immunoelectronmicroscopy. In addition, apo B and apo E were encountered in increased amounts in the mesangium and colocalized with Oil Red O-positive lipid deposits, particularly in puromycin aminonucleoside nephrosis rats. Double-staining showed a preferential localization of apo B and apo E at sites of increased mesangial matrix in close proximity to ED1-positive foam cells, i.e., the mesangial macrophages. The close topographic association between apo B and apo E, lipid deposits, and macrophages in the mesangium lend further support to the concept of lipid-mediated glomerular injury in nephrosis. (Am J Pathol 1993, 142:1804–1812)

During the last decade, experimental evidence has accumulated suggesting that hyperlipidemia may play a role in the progression of renal disease.[1–4] Dietary cholesterol supplementation induced glomerulosclerosis in guinea pigs[5,6] and rats,[7] and aggravated glomerular injury in puromycin aminonucleoside (PAN) nephrosis[8] and uninephrectomized rats.[7,9] Pharmacological treatment of hyperlipidemia reduced glomerular injury in rats with 5/6 nephrectomy,[10] obese Zucker rats,[11] and in rats with PAN nephrosis.[12] Recent multiple linear regression analysis selected hypercholesterolemia and glomerular lipid deposits as major independent risk factors of glomerular injury in rats after renal ablation.[13,14] On the basis of experimental observations, selected patients with unremitting nephrotic syndrome have recently started treatment with a variety of lipid-lowering agents.[15–18]

Plasma lipids are transported in large complexes of physically combined lipid and protein, the lipoproteins. Five major different lipoprotein classes can be distinguished: chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), intermediate density lipoproteins, and high density lipoproteins (HDL). In contrast to humans, in which cholesterol is mainly transported in LDL, in the rat the bulk of plasma cholesterol and its esters are transported in the HDL fraction.[19,20] The major apolipoproteins (apo) of HDL are A-I, A-IV, E, and C; of LDL, apo B; and of VLDL, apo B and apo E. Apo A-I and apo A-IV activate lecithin, and apo B and apo E bind to the LDL receptor system.[21] In this report we examined apo A-I, apo A-IV, apo B, and apo E in plasma and in kidney tissue of rats made nephrotic by injection of PAN or adriamycin (ADR).

Materials and Methods

Experimental Animal Groups

Fifteen male Wistar rats aged 3 months were used. In this strain of rats, both PAN and ADR induce the

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Address reprint requests to Dr. Harry van Goor, Department of Pathology, University of Groningen, Oostersingel 63, 9713 EZ Groningen, The Netherlands.

Present address of Dr. Joris Grond: Department of Pathology, Laboratory of Public Health, Leeuwarden, The Netherlands.

nephrotic syndrome. The first model is also associated withmesangial injury at an early stage.[22] The nephrotic syndrome was induced by a single intravenous injection of PAN (Sigma Chemical Co., St. Louis, MO) in a dose of 6 mg/100 gm body weight, dissolved in 3 ml saline for 3 minutes. ADR nephrosis was induced by a single intravenous injection of Adriablastine RTU (Farmitalia Carlo Erba S.A., Nivelles, Belgium) in a dose of 0.8 mg/100gm body weight, dissolved in 1 ml of saline for 3 minutes. Control rats received saline intravenously. The rats were fed standard rat chow (22% protein and 0.2% NaCl) and tap water *ad libitum*. Body weight was determined at day 1 and 7.

Clinical and Glomerular Structural Determinations

Urine was collected from day 7 to 8 by housing the rats for 24 hours in metabolic cages with access to water only. Urinary protein excretion was measured using the pyrogallol method. Blood samples for determination of plasma cholesterol, triglycerides, creatinine, and total protein were obtained under ether anesthesia by orbita plexus puncture on day 8 after a 24-hour fast. All measurements were performed according to standard methods.

Plasma concentrations of apo A-I, apo A-IV, and apo E were measured by electroimmunoassay as described previously.[23] Plasma apo B was determined by radial immunodiffusion,[24] using a specific antiserum raised in rabbits against purified rat LDL.[25] Plasma apo B concentrations, expressed in arbitrary units because of the insolubility of purified apo B and the lack of sufficient quantities of pure apo B for use as an absolute standard, were calculated as percentages of a rat serum standard pool (obtained from 50 rats) run simultaneously on the plates with the plasma samples. Six different dilutions of the serum standard pool were run on each plate. All samples were run in triplicate. The assay does not discriminate between apo B_{100} and apo B_{48}. Glomerular size was measured as described previously[13] in periodic acid-Schiff-stained sections. These sections were also used for light microscopical analysis.

Tissue Processing

At autopsy, renal tissue was perfused for 1 minute with phosphate-buffered saline (PBS), pH 7.4, containing 6% sucrose. The left kidney of two PAN and ADR animals was subsequently perfused with 0.05% glutaraldehyde and 4% paraformaldehyde in 0.1 mol/L phosphate buffer. The fixative was removed by reperfusion with PBS, containing 6% sucrose for 1 minute.

After perfusion, coronal tissue slices of 1 mm thickness were cut from the midportion of the kidney and placed in 2% paraformaldehyde in PBS at 4 C and fixed for 3 hours as described previously.[14] Following immersion fixation, tissues were washed overnight in PBS containing 6% sucrose. The next morning the specimens were dehydrated in 100% acetone for 30 minutes at 4 C and infiltrated in Technovit 8100 solution A (Kulzer, GmbH, Wehrheim, Germany) for 6 to 8 hours at 4 C. During the entire procedure, the tissue specimens were gently agitated in a constant rotary motion. Subsequently, 1 part of the accelerator Technovit solution B was added to 30 parts of the tissue containing solution A, followed by another minute of agitation at 4 C. After embedding, paraffin was poured around the block holders to prevent inhibition of the polymerization by atmospheric oxygen. Polymerization was accomplished overnight at 4 C on crushed ice. Two-μ sections were cut on a Reichert-Jung Supercut plastic microtome using HD knives. Tissue blocks were stored at −20 C; sections at 4 C. Additional pieces of tissue were quickly frozen in liquid freon (−80 C) to obtain frozen sections for Oil Red O (ORO) staining.

Staining Procedures for Light Microscopy

For the immunohistochemical localization of apolipoproteins, plastic sections were processed as described previously.[13,14] In short, sections were dried at 37 C for 2 hours and subsequently incubated in appropriate dilutions of rabbit anti-rat polyclonal antibodies directed to apo A-I, apo A-IV, apo E,[23] and apo B.[24,25] Glomerular macrophages were assessed by immunostaining with a monoclonal mouse anti-rat antibody (ED1,[26] kindly provided by Dr. C. Dijkstra, Free University, Amsterdam). Following a wash in PBS for 7 minutes, endogenous peroxidase was blocked in PBS, containing 0.06% H_2O_2 for 30 minutes at room temperature. After another wash in PBS, the second step antibodies, peroxidase-conjugated swine anti-rabbit in the case of the apolipoprotein antibodies, and peroxidase-conjugated rabbit anti-mouse antibody in the case of the ED1 antibody, were applied for 1 hour at room temperature in a dilution of 1 to 20 in PBS, containing 5% normal rat serum. Peroxidase activity was developed according to standard procedures in diaminobenzidine + H_2O_2 for 12 minutes at room

temperature. Counterstaining was performed using periodic acid-Schiff and Mayers hematoxylin.

Control experiments were performed to test the specificity of the antibodies. Previously, double immunodiffusion against purified antigen ruled out reaction of the polyclonal anti-apolipoproteins (anti-apo) A-I, A-IV, E,[23] and B (Van Tol, unpublished observations) sera with unrelated antigens in plasma.[23] Rabbit anti-rat antibodies to apo A-I, apo A-IV, apo B, and apo E were absorbed with normal rat serum (containing all apolipoproteins) in a concentration of 5% for 15 to 30 minutes at room temperature. Subsequently, staining results of the absorbed antibodies were compared with the unabsorbed. Absorbance completely removed specific glomerular immune reactivity for apolipoproteins. Routine control experiments, i.e., replacement of the first antibody by nonimmune rabbit serum or PBS were consistently negative.

To improve morphological detail, sections were dried at 60 C for 30 minutes, and then covered with two drops of complete Technovit 8100 medium (solution A + B, 30:1) as used for embedding. Subsequently, cover glasses were placed on the sections. Polymerization of the plastic was performed at 4 C. Sections were carefully examined under a light microscope to determine the exact localization of apolipoproteins and the number of glomerular macrophages.

Double-staining procedures were performed to study the spatial relationship between apolipoproteins and macrophages. For that purpose, plastic sections were incubated with a mixture of an anti-apolipoprotein antibody and the ED1 monoclonal antibody for 2 hours at 37 C. Following a wash in PBS for 7 minutes, endogenous peroxidase was blocked in PBS, containing 0.06% H_2O_2 for 30 minutes at room temperature. After another wash in PBS, a mixture of two second step antibodies, peroxidase-conjugated swine anti-rabbit and alkaline phosphatase–conjugated goat anti-mouse IgG_1, was applied for 1 hour at room temperature in a dilution of 1 to 20 in PBS, containing 5% normal rat serum. After washing in PBS, the alkaline phosphatase activity was developed using the method of Burstone.[27] Sections were again washed in PBS for 10 minutes, and subsequently the peroxidase activity was developed according to standard procedures in diaminobenzidine + H_2O_2 for 12 minutes at room temperature. Sections were covered with Imsol/Mount (Klinipath, Zevenaar, The Netherlands), dried at 60 C for 15 minutes, and covered with glycol methacrylate mixture (see above) and coverslips.

Lipid Histochemistry

Glomerular lipid deposits were scored in 4-μ frozen sections stained by the ORO method. To determine the spatial relationship between ORO-positive lipid material and apolipoproteins, frozen sections were fixed in 2% paraformaldehyde in PBS for 3 minutes at 4 C, washed in distilled water, and stained with ORO according to standard procedures. After the ORO staining, the sections were immunostained for apolipoproteins according to standard procedures for frozen sections. ORO-stained sections were scored semiquantitatively on a scale of 1 to 4+.[13] Briefly, if 25% of the glomerulus was affected, a score of 1+ was adjudged, 50% was scored as 2+, 75% as 3+, and 100% as 4+. The ultimate score was then obtained by multiplying the degree of change by the percentage of glomeruli with the same degree of injury and additions of these scores. A total number of 50 glomeruli per animal was scored moving from cortex to medulla.

Staining Procedures for Immunoelectronmicroscopy

Small pieces of tissue (1 mm^3) were cut out of the cortex of kidneys perfused with 0.05% glutaraldehyde/4% paraformaldehyde for 5 minutes and postfixed by immersion in the same fixative for 30 minutes at 4 C in a constant rotary motion. Following fixation, tissues were washed overnight in PBS containing 6% sucrose. The next morning the specimens were dehydrated in graded ethanol concentrations: 50% ethanol for 15 minutes, 70% ethanol for 15 minutes, and 100% ethanol for 60 minutes (with 3 changes). Dehydration took place at 4 C in a constant rotary motion. Subsequently, the tissue specimens were infiltrated in a new embedding mixture (RES G33: hydroxypropylmethacrylate, 35 ml; isobornyl methacrylate, 5 ml; polypropyleneglycol monomethacrylate 5 ml; butanediol dimethacrylate, 5 ml, lucidol CH 50 [benzoylperoxide], 0.3 g; kindly provided by Dr. P.O. Gerrits, Department of Anatomy, University of Groningen, Oostersingel 65, 9713 EZ Groningen, The Netherlands). After infiltration for 5 hours at 4 C in a constant rotary motion, the tissue pieces were embedded in closed beem capsules. The embedding mixture consisted of 1 part of the hardener (Technovit solution B) added to 30 parts of the infiltration solution. Polymerization took place overnight at 4 C on crushed ice. Blocks were stored at −20 C and grids at 4 C.

One-μ sections were cut on a LKB ultramicrotome using glass knives and stained with toluidine blue to

select areas with glomeruli. The blocks were trimmed, and 60-nm sections were cut using a diamond knive and mounted on formvar-coated nickel grids. All incubations and washing steps were performed at room temperature on 50 µl drops. Grids were pretreated with 0.1% BSA-C (Aurion, Wageningen, The Netherlands) in 1% bovine serum albumin (BSA, Serva Feinbiochemica, Heidelberg, Germany) in Tris-buffered saline, pH 7.6 (TBS), for 10 minutes to inhibit aspecific binding of the first antibody. After washing in TBS three times for 5 minutes, and in 1% BSA in TBS (1% BSA/TBS) three times for 5 minutes, they were incubated in appropriate concentrations of anti-apo A-I, apo A-IV, apo B, or apo E antibody. Subsequently, the grids were washed six times for 5 minutes in 1% BSA/TBS and then incubated with 15 nm gold-conjugated goat anti-rabbit antibody (Jansen Biotech N.V., Olen, Belgium, 1:15 in 1% BSA/TBS, containing 0.01% BSA-C) for 1 hour. After three 5 minute washings in 1% BSA/TBS and three in TBS, the grids were postfixed for 10 minutes in 2% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.8, and washed three times for 5 minutes in distilled water. Counterstaining was performed using 5% uranyl acetate in water for 5 minutes, and Reynolds lead citrate for 2 minutes. Grids were examined on a Akashi OO2A electronmicroscope.

Statistical Analysis

Reported values are the group median and range. Differences between groups were tested for significance using Wilcoxon's rank sum test. The null hypothesis of no differences between two groups was rejected when $P < 0.05$.

Results

All 15 rats survived the experimental period of 8 days. Plasma and urine data are listed in Table 1. Compared to PAN rats and control rats, body weights of ADR rats were significantly lower at day 8. Administration of PAN and ADR caused full-blown nephrotic syndrome with severe proteinuria and elevation of plasma cholesterol and triglyceride levels. In addition, serum protein level was decreased in ADR rats. Apo A-I and apo B were significantly elevated in PAN- and ADR-injected rats. Serum apo B in ADR rats was significantly higher than in PAN rats ($P < 0.01$). Although plasma concentrations for apo E tended to be higher in nephrotic animals, this did not reach statistical significance. No differences were observed for plasma concentrations of apo A-IV between nephrotic and control rats.

At the light microscopical level, some glomeruli of PAN-treated rats showed modest mesangial matrix expansion and mesangial cellularity, confirming observations by Diamond et al,[28] who found increased numbers of macrophages and proliferating cell nuclear antigen-positive cells 2 weeks after PAN injections. In ADR rats and control rats, no mesangial matrix expansion nor hypercellularity were observed. Focal and segmental glomerulosclerosis as a total lesion was not present in both experimental groups. Glomerular size did not vary significantly comparing PAN rats 166 (153 to 174) µ or ADR rats 155 (147 to 164) µ, with control rats 151 (148 to 157) µ, although there was a tendency in PAN rats to have larger glomeruli.

Glomerular lipid deposits were significantly increased in nephrotic rats, particularly in PAN nephrosis. These lipid deposits were localized in mesangial areas. In control rats, the lipid score was 4 (0 to

Table 1. *Plasma and Urine Data 8 Days after a Single Intravenous Injection of ADR or PAN*

	Control (n = 5)	ADR (n = 5)	PAN (n = 5)
BW day 1 (g)	238(210–243)	237(231–249)	240(223–246)
BW day 7 (g)	275(240–277)	210(198–216)*	266(244–278)
UprotV (mg/24h)	16(13–20)	249(177–257)*	239(101–254)*
Screat (µmol/l)	39(37–40)	39(36–81)	40(34–44)
Sprot (g/l)	52(48–55)	41(39–43)*	42(38–51)
Schol (mmol/l)	2.1(2.0–2.5)	7.7(4.2–9.2)†	4.7(3.0–5.3)†
TG (mmol/l)	0.7(0.6–0.7)	1.9(1.8–3.4)†	1.8(0.9–2.2)†
Apo A-I (mg/dl)	35(28–41)	161(104–187)*	121(64–129)*
Apo A-IV (mg/dl)	8(6–9)	4(2–10)	10(4–11)
Apo B (A.U.)	108(89–135)	441(255–692)*	215(141–244)*
Apo E (mg/dl)	16(13–22)	23(16–34)	22(18–34)

BW: body weight; UprotV: urinary protein excretion; Screat: serum creatinine; Sprot: total serum protein; Schol: fasting serum cholesterol; TG: triglycerides; Apo: apolipoprotein; A.U.: arbitrary units.
* $P < 0.01$, † $P < 0.05$ versus control, Wilcoxon rank sum test.

8, median score and ranges). In ADR rats, this score was 32 (8 to 58), and in PAN rats 108 (4 to 170, both $P < 0.05$ versus control rats).

Glomerular macrophage influx was found to be increased in PAN-injected rats. The number of ED1+ cells per glomerular profile in control rats was 1.9 (1.1 to 3.3) (median and ranges) and in PAN rats 4.5 (2.2 to 7.1; $P < 0.01$). Glomeruli of ADR-injected rats were almost devoid of macrophages with 0.3 (0.1 to 0.8) ED1+ cells per glomerular profile ($P < 0.01$ versus controls). An example of an ED1 staining on a kidney section from a PAN-treated rat is shown in Figure 1.

Glycol methacrylate-embedded sections were used to precisely localize apolipoproteins A-I, A-IV, B, and E in kidney tissue. Apo A-I, apo A-IV, and apo E were remarkably enhanced in the glomerular epithelium of nephrotic rats. Figures 2 and 3 show

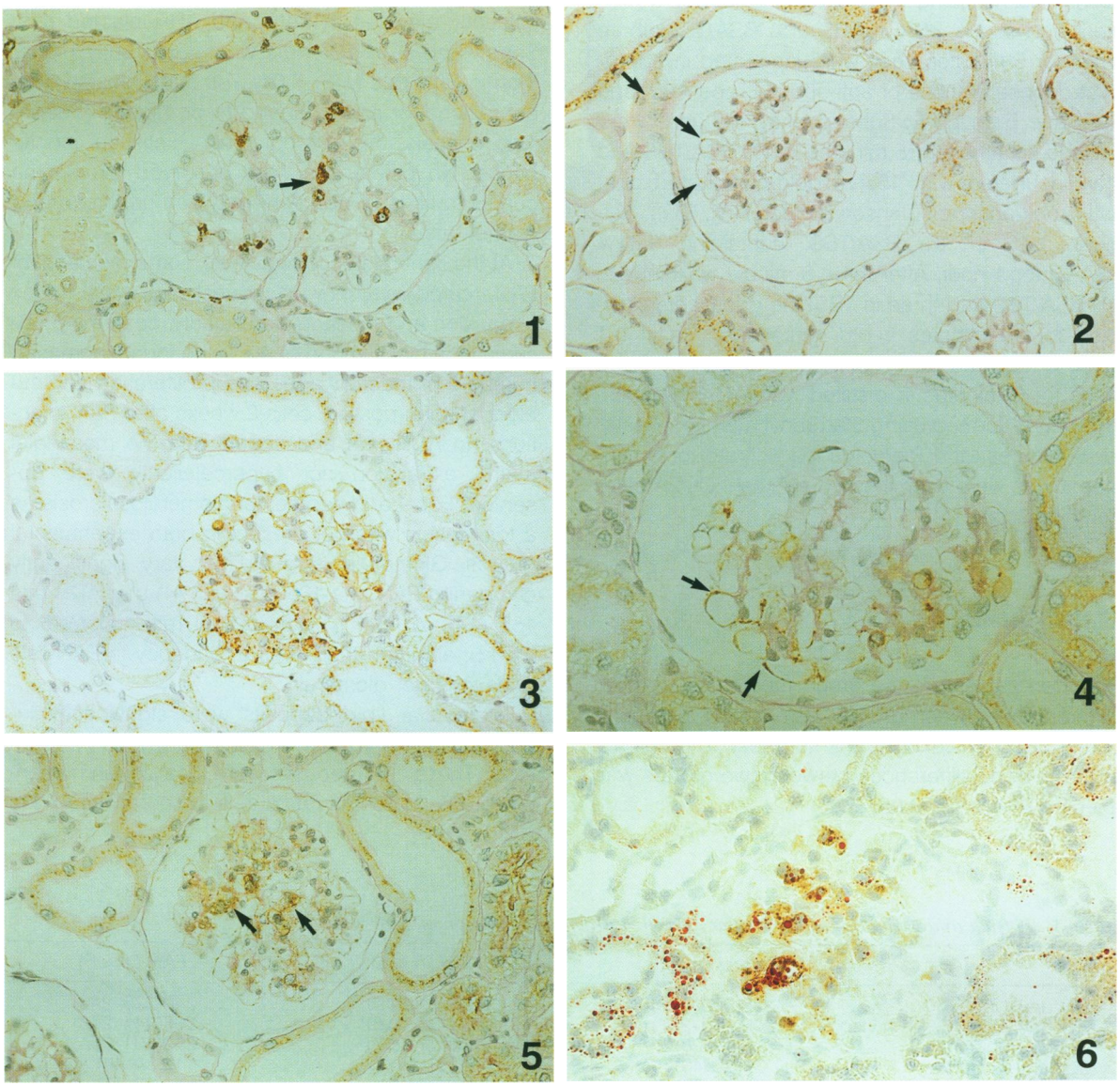

Figure 1. *Immunoreactivity for macrophages (ED1) in a PAN nephrotic rat. A number of these cells exhibit a foamy appearance (arrow); ×500.*
Figure 2. *Immunoreactivity for apo A-I in a control rat. Occasionally reaction product is seen in the glomerular visceral epithelium (arrow); ×500.*
Figure 3. *Immunoreactivity for apo A-I in a PAN nephrotic rat. Reaction product is abundantly present throughout the glomerular visceral epithelium; ×500.*
Figure 4. *Immunoreactivity for apo E in an ADR nephrotic rat. Reaction product is predominantly present in the glomerular visceral epithelium (arrow); ×800.*
Figure 5. *Immunoreactivity for apo E in a PAN nephrotic rat. Strong reactivity is seen in the glomerular mesangium (arrow); ×500.*
Figure 6. *Double-staining for apo E (brown) and ORO (red) in a PAN nephrotic rat. Within the glomerular mesangium apo E co-localizes with lipid deposits; ×500.*

representative micrographs of apo A-I immunostaining in renal tissue from a control (Figure 2) and a PAN rat (Figure 3). Figures 4 and 5 are representative examples of apo E immunostaining in ADR and PAN rats, respectively. Note the increased accumulation of apo E in mesangial areas of PAN rats. The same mesangial deposition was observed for apo B. Table 2 summarizes the results of apolipoprotein immunostaining. Double-staining of apo B or apo E with ORO demonstrated the co-localization of the majority of lipid deposits with these apolipoproteins (Figure 6). At the ultrastructural level, glomeruli of both PAN and ADR rats displayed a replacement of epithelial foot processes by expanses of flattened epithelial cytoplasma with multiple cytoplasmatic vacuoles, containing reabsorbed protein and blebbing. By immunoelectronmicroscopy, apo A-I, apo A-IV, and apo E were localized in small amounts in the cytoplasma of glomerular visceral epithelial cells of normal rats. Gold particles were observed in endocytotic vesicles. In PAN and ADR rats, apo A-I, apo A-IV, and apo E were found in increased amounts in protein resorption droplets in the glomerular visceral epithelial cells. An example of apo A-I electroimmunoreactivity is shown in Figure 7.

In the glomerular mesangium of PAN rats, large amounts of apo B and apo E were found as documented (see Table 2 and Figure 5). Using double-staining procedures, both apo B and apo E were located in the mesangium, often around glomerular foam cells expressing ED1, i.e., cells of the monocyte/macrophage lineage. By double immunoelectronmicroscopy, apo E was found in large amounts in the mesangial matrix surrounding ED1-positive cells (Figures 8 and 9).

Discussion

The acute nephrotic syndrome induced by ADR or PAN is known to be associated with elevated plasma concentrations of VLDL, LDL, and HDL.[29–31] The alterations in plasma apolipoprotein concentration in nephrotic rats observed in the present study reported confirm data by other groups.[29–31] In PAN nephrosis, total plasma apo A-IV was found to be decreased by about 60%, whereas total apo B and apo E were increased fivefold and twofold respectively due to increases of VLDL and LDL.[31] The most remarkable change in the HDL fraction was the almost complete absence of apo E and apo A-IV, accompanied by a massive increase of apo A-I.[29–31]

Glomerular macrophage number was significantly increased in PAN rats. This observation is in concordance with data reported by Diamond and coworkers in this model.[32] Part of the glomerular macrophages exhibited a foamy appearance with fine isometric vacuolization of the cytoplasm. The transformation of bone marrow–derived macrophages to foam cells within the mesangium takes place by internalization of plasma lipoproteins and accumulation of cholesterol esters.[4] Various modifications[33] may convert native LDL into oxidized LDL, which is a specific ligand for the scavenger receptor on macrophages.[33] The uptake of oxidized LDL by macrophages may further stimulate these immune effector cells to produce growth factors, cytokines, and other mediators capable of stimulating matrix synthesis by mesangial cells and glomerular cell proliferation.[34] In ADR, glomerular macrophages were significantly decreased. Although peripheral blood monocytes were not counted, this effect is probably due to bone marrow depression.[14,35] Because glomerular macrophage depletion has been shown to prevent glomerular damage in PAN nephrosis,[36] or in remnant glomeruli after renal ablation,[14] the low incidence of glomerulosclerosis in ADR nephrosis as observed in a previous

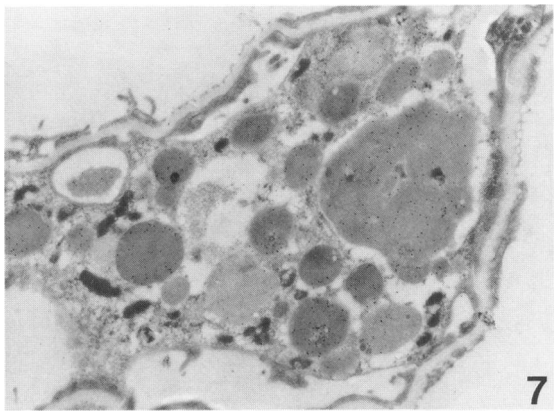

Figure 7. *Immunoelectronmiscroscopical apo A-I staining in an ADR nephrotic rat. Gold particles are present in visceral epithelial protein resorption droplets;* ×17,000.

Table 2. *Apolipoprotein Immunoreactivity in Control and Nephrotic Rats*

	GVE			Mesangium		
	Control	PAN	ADR	Control	PAN	ADR
Apo A-I	±	+++	+++	±	±	±
Apo A-IV	±	++	++	±	+	±
Apo B	−	±	±	±	+++	+
Apo E	+	++	++	±	+++	±/+

Immunoreactivity in glomerular visceral epithelium (GVE) and mesangium of control and nephrotic rats. −: negative; ±: trace; +: weak staining; ++: moderate staining; +++: strong staining.

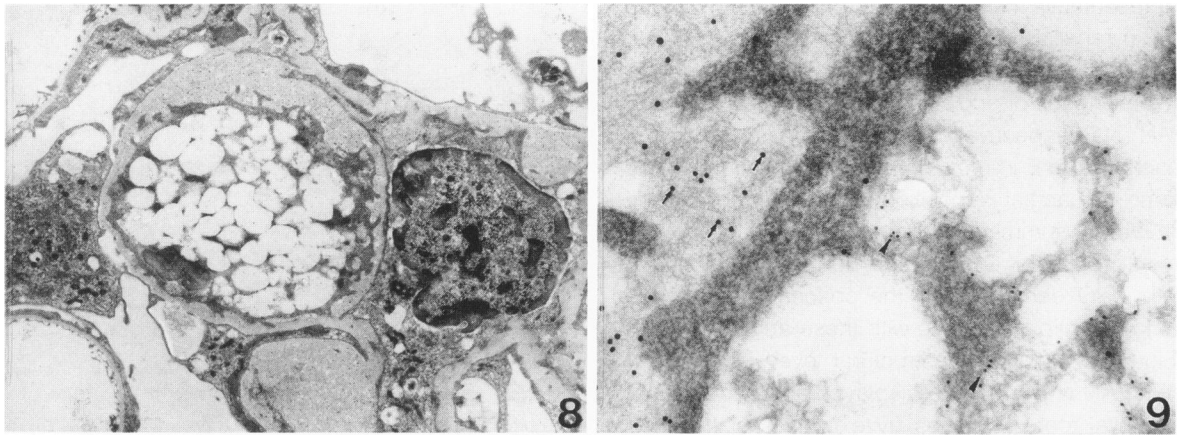

Figure 8/9. *Immunoelectronmicroscopical double-labeling for ED1 (10 nmol/L, macrophages) and apo E (15 nmol/L) in a PAN nephrotic rat. In Figure 8, large amounts of apo E can be seen in the increased extracellular matrix surrounding a foam cell; ×10,200. At high magnification (Figure 9), the foam cell appears to be macrophage-derived; ×85,000.*

study[22] may be related to this initial glomerular macrophage depletion.

The kidneys are known to be involved in the catabolism of apo A-I,[37] apo A-IV,[38] and apo E.[39] In the present study, we observed immunoreactivity for these three apolipoproteins in the tubules. In the nephrotic state, tubular immunoreactivity was increased and more distal tubular segments became involved in the resorption process. We suggest that the increased glomerular visceral epithelial immunoreactivity in the nephrotic state merely reflects mechanisms involved in the proteinuric state.[40] The presence of ORO positivity in tubular areas from PAN and ADR nephrotic rats suggest that at least some of the apo A-I, apo A-IV, and apo E immunoreactivity originates from HDL or HDL-like particles that have passed the damaged filtration barrier.

In contrast to apo A-I, apo B and apo E were found in large amounts in the glomerular mesangium of PAN rats in close association with ORO-positive deposits, which contain predominantly cholesterol and cholesterol ester.[41] Because apo A-I is abundantly present in nephrotic HDL[29–31] and apo B is the major apolipoprotein in LDL, these data suggest that glomerular cholesterol deposits observed in PAN are mainly derived from circulating LDL and VLDL, but not from HDL. Interestingly, in a previous study, glomerulosclerosis-prone nephrotic Wistar rats were found to have higher plasma levels of LDL compared to nephrotic glomerulosclerosis-resistant rats of the PvG/c strain.[42]

Double-staining with ED1 monoclonal antibody revealed the presence of apo B and apo E around large ED1-positive foam cells. Similar observations are reported for intimal macrophages in atherosclerosis.[43,44] Immunoelectromicroscopical observations revealed that although apo E was present in small amounts inside foam cells, the major part was found in the increased extracellular matrix surrounding these cells. Macrophage products are known to be involved in mesangial matrix overproduction.[34] Glomerular macrophage depletion prevents matrix expansion in several models.[14,36] In addition, Klein et al[45] reported an increased total synthesis of heparan sulfate proteoglycan and little alteration in the chondroitin sulfate and dermatan sulfate content of isolated glomeruli from PAN nephrotic rats. Heparin sulfate is known to have binding sites for LDLs.[46,47] The presence of LDL in the mesangial matrix may therefore be caused by specific binding to matrix proteins instead of merely reflecting increased mesangial trafficking of macromolecules. Additionally, macrophages are known to be capable of secreting apo E,[48] thus part of the observed apo E may be produced by mesangial foam cells. Apo E produced by macrophages is proposed to function in cholesterol efflux from cholesterol-loaded cells collaborating with HDL in the interstitial fluid.[48,49] However, resident macrophages recovered from the ascites fluid of nephrotic PAN rats retain normal cholesterol concentrations, whereas simultaneously secreting protein that is enriched in apo E, implicating that other factors are involved as well.[50] Recently, Yamada et al[51] decreased hypercholesterolemia and prevented the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbits by intravenous injections of apo E. Also, Shimano et al[52] reported that overexpression of apo E in transgenic mice causes a marked reduction in VLDL and LDL, as well as resistance to diet-induced hypercholesterolemia. The interesting observations mentioned above suggest promising

new possibilities for treatment of hyperlipidemia. It is important to elucidate whether apo E treatment in experimental nephrotic syndrome prevents or ameliorates glomerulosclerosis and whether the formation of foam cells in established glomerular injury is reversible.

In summary, the nephrotic syndrome in rats is associated with increased loss of apolipoproteins through the glomerular basement membrane with localization in glomerular epithelial cells. The preferential localization of apo B in the glomerular mesangium in parallel with increased lipid accumulation and influx of macrophages suggests a major contribution of LDL-derived cholesterol in lipid-mediated glomerular injury in PAN nephrosis.

Acknowledgments

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