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Apolipoproteins and lipoprotein receptors in glomeruli in human kidney diseases

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Apolipoproteins and lipoprotein receptors in glomeruli in human kidney diseases. This study offers morphological evidence of the involvement of lipid abnormalities in human glomerular injury. Renal biopsy tissues from patients with several types of glomerular diseases were immunocytochemically examined using antibodies to apolipoproteins (apo) A-I, B-100, and E, and antibodies to low density lipoprotein (LDL) receptors and scavenger receptors. Immunofluorescent staining showed the predominant deposition of apo B and apo E in the mesangial area in mesangial proliferative types of glomerulonephritis; the distribution and staining intensity of these apolipoproteins correlated with the grade of mesangial proliferation and proteinuria, but were independent of plasma lipid levels. Immunoelectron microscopy revealed that apo B and apo E were distributed in droplets within glomerular epithelial and mesangial cells or in a granular pattern in the expanded mesangial matrix. Apo A-I was mainly localized in the visceral epithelial cells of normal human kidneys. Staining for apo A-I was increased in the glomerular epithelial cells of nephritic kidneys, compared to the pattern in normal human kidneys, and was decreased in the sclerosed areas of glomeruli. An immunogold technique revealed the expression of LDL receptors on the surface membranes of glomerular mesangial and epithelial cells. Dual immunofluorescent staining showed that apo B and LDL receptors were occasionally co-localized in nephritic glomeruli. Scavenger receptor was detected on the plasma membranes of mesangial and visceral epithelial cells. The glomerular expression of scavenger receptor was increased in glomeruli with marked mesangial proliferation. In addition, the expression of this receptor was intense in monocytes/macrophages occasionally infiltrating the glomeruli. Our present findings indicate that in human nephritic kidneys, glomerular epithelial and mesangial cells express both LDL receptors and scavenger receptors. The accumulation of apolipoproteins, whether receptor-mediated or mediated by other mechanisms, can occur independently of plasma lipid levels, and may be associated with mesangial expansion and proteinuria.

Accumulating evidence indicates that abnormalities of lipid metabolism are responsible for the progression of glomerular damage in both immunological and non-immunological types of renal diseases [1–3]. A hereditary form of lecithin cholesterol acyltransferase deficiency has been reported to cause glomerular lipid deposition and progressive renal insufficiency [4]. Abnormal lipid accumulation is found within glomeruli and tubular cells in various types of human nephritis [5, 6]. Foam cells, believed to be lipid-laden macrophages, have been identified in kidney vessels and in the interstitium as well as in glomeruli [7]. Mesangial cells and glomerular epithelial cells express surface receptors for low density lipoprotein (LDL) [8, 9], and could, therefore, be affected by abnormal lipid metabolism. Experimentally, glomerular injury, including increased mesangial matrix and cellularity, followed by the appearance of albuminuria occurs with diet-induced hyperlipidemia [10] and with endogenous abnormalities in lipid metabolism [11].

The mechanisms of lipid-induced glomerular injury are still incompletely understood. The apparently infrequent occurrence of renal disease in patients with the most common forms of hyperlipidemia suggests that lipids may cause clinically important renal injury only when additional predisposing factors, such as immune-mediated glomerular cell damage, are present. Based on certain histologic similarities, analogous pathobiologic mechanisms have been suggested for glomerulosclerosis and atherosclerosis [12]. Modified LDL, which is taken up by tissue macrophages via scavenger receptors and is an important pathogen in atherosclerosis [13, 14], has been shown to be cytotoxic to cultured mesangial cells [15, 16], and could contribute to glomerular damage in nephritis, although the precise mechanism by which this could occur is unknown. To further delineate the role of lipid abnormalities in human glomerular injury, we immunocytochemically evaluated the glomerular expression of LDL and scavenger receptors, as well as evaluated the renal deposition of the predominant plasma lipoproteins A-I, B-100, and E, which are essential structural components of high density lipoprotein (HDL), LDL, and very low-density lipoprotein (VLDL), respectively.

Methods

Patients and tissues

The kidney tissues used in this study were obtained by percutaneous needle biopsy or surgical biopsy from 74 patients with various types of kidney diseases: 30 patients with IgA

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Table 1. Antibodies used in this study

Specificity	Antibody	Produced by	Source
Apo A-I	MoAb (ABAI-1)	mouse IgG_1	Canadian Bioclinical
Apo B-100	MoAb (AB B-3)	mouse IgG_{2a}	Canadian Bioclinical
	TRIC-labeled	goat	Binding site
Apo E	MoAb (AB E-1)	mouse IgG_1	Canadian Bioclinical
	TRIC-labeled	goat	Binding site
LDL receptor	MoAb	mouse IgG_{2b}	Amersham
Human scavenger receptor	hSRI-2	rabbit	Matsumoto et al [14]
Human monocyte/macrophages	FMC 32 (CD14)	mouse IgG	AMD
	Anti-Leu-M3 (CD14)	mouse IgG_{2b}	Becton-Dickinson
Type IV collagen (7S domain)	MoAB (MAb IV-1)	mouse IgG	Scheinman and Tsai [19, 34]

Abbreviations are: MoAb, monoclonal antibody; TRIC, tetramethylrhodamin; AMD, Austrarian Monoclonal Development.

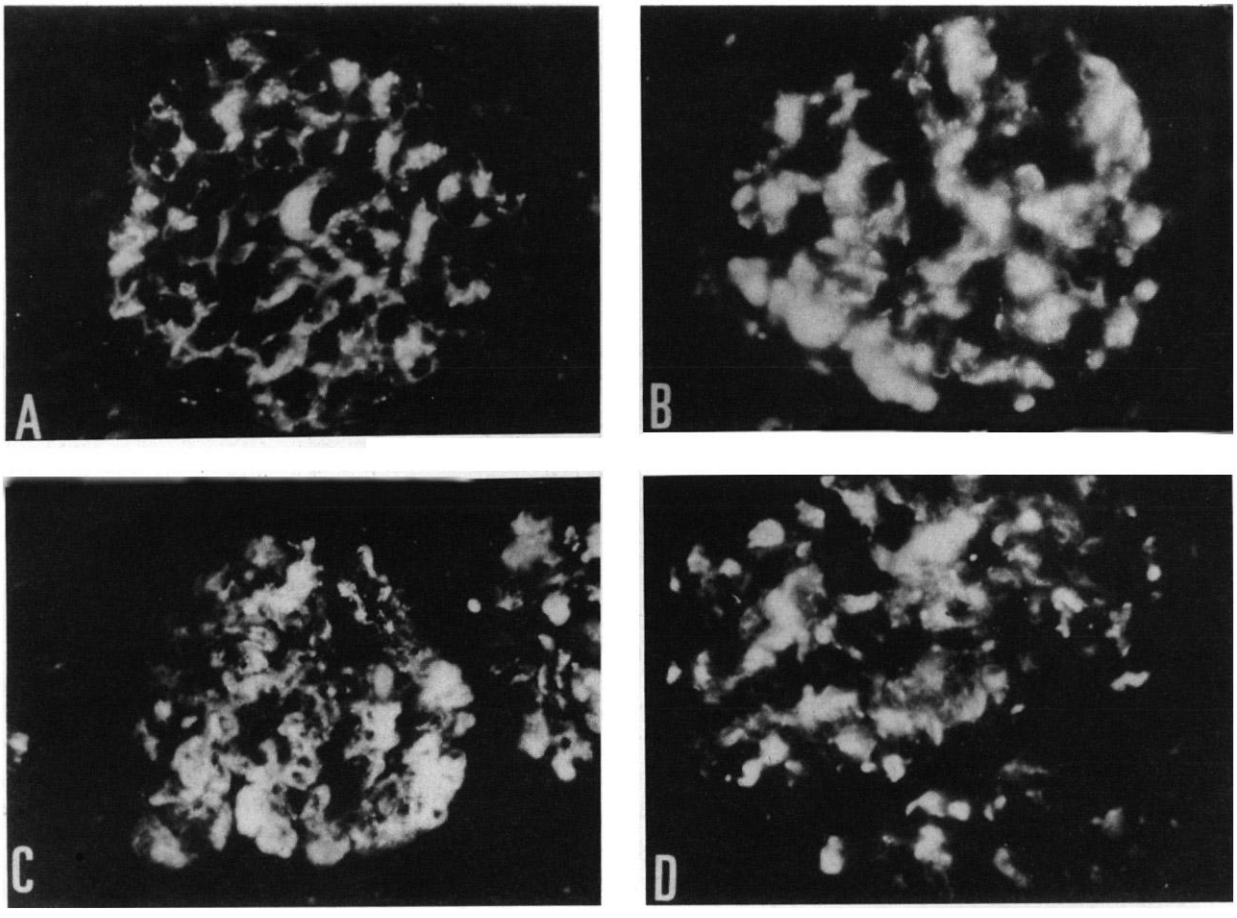


Fig. 1. *Indirect immunofluorescent staining with monoclonal antibodies to apo B-100 (A–C) and apo E (D) in patients with IgA nephritis with mild (A) or marked (B and D) mesangial proliferation, and in a patient with focal glomerular sclerosis (C).* **A.** Apo B is visible in the mesangium, and staining is weak along the glomerular capillary walls (immunofluorescence score 1). **B.** Mesangial deposition of apo B is prominent (immunofluorescence score 2). **C.** Intense staining for apo B is seen in an almost globally sclerotic glomerulus (immunofluorescence score 2). **D.** Positive staining for apo E is prominent in the mesangial area. Original magnification: A–D ×400.

nephritis, 15 with mesangial proliferative (non-IgA) glomerulonephritis, 10 with Henoch-Schönlein purpura nephritis (HSPN), 10 with minimal change nephrotic syndrome, 4 with lupus nephritis (WHO Class IV), and 5 with nephrotic syndrome with focal glomerular sclerosis. Laboratory data including urinalysis, 24-hour urinary protein excretion and creatinine clearance were collected for each patients. Plasma levels of lipids and apoproteins were determined using fasting serum samples obtained from 28 patients with mesangial proliferative glomerulonephritis, including 18 patients with IgA nephritis. Lipids and apoproteins were measured by standard procedures currently used in Japan; total cholesterol and triglyceride were assayed with an autoanalyzer, utilizing enzymatic methods. HDL cholesterol was measured after precipitation of LDL and VLDL cholesterols by a heparin-manganase reagent. Apo A-I, apo B and apo E were immunoturbidometrically determined using the reagent kits (ApoAuto "Daiichi", Daiichi Pure Chemical, Tokyo, Japan) [17].

Table 2. Results of apo B, apo E, LDL receptor and scavenger receptor immunofluorescent staining in glomeruli of patients with various types of glomerular diseases

Disease/Mesangial proliferation[a]	Total no. of patients	Staining in glomeruli			
		Apo B[b]	Apo E[b]	LDL receptor[b]	Scavenger receptor[c]
IgA nephritis	30	18/30	15/30	7/30	3/15
1+	10	3	2	1	0
2+	14	9	7	3	1
3+	6	6	6	3	2
Mesangial proliferative (non-IgA) nephritis	15	10/15	7/15	5/15	2/7
1+	5	2	1	1	0
2+	6	4	3	2	0
3+	4	4	3	2	2
Henoch-Schönlein purpura nephritis	10	3/10	2/10	1/10	1/5
1+	3	0	0	0	0
2+	6	2	1	0	0
3+	1	1	1	1	1
Nephrotic syndrome					
Minimal change	10	1/10	0/10	1/10	1/6
Focal glomerulosclerosis	5	4/5	4/5	3/5	2/5
Lupus nephritis (WHO Class IV)	4	3/4	3/4	2/4	2/4
Total	74	39/74	31/74	19/74	11/42

[a] Mesangial proliferation was graded as mild (1+), moderate (2+) or marked (3+)
[b] Number of patients with positive staining/number of patients examined by immunofluorescence
[c] Number of patients whose kidney tissues showed increased intensity and distribution of staining compared with normal human kidneys/number of patients examined by immunofluorescence

Three other kidney specimens were obtained; these were from patients with renal calculi or renal trauma, and histologically normal portions were used as normal human kidney tissue. For light microscopic observation, the tissues were fixed in 10% buffered formalin and embedded in paraffin. Three μm-thick sections were stained with hematoxylin and eosin, periodic acid-Schiff, and methenamine silver. Mesangial proliferation was graded as described previously [18]. Cryostat sections were stained for neutral lipids with oil red 0.

Antibodies

The monoclonal and polyclonal antibodies used in this study are listed in Table 1. Antibody to human scavenger receptor was produced by immunizing rabbits with bovine serum albumin-coupled synthetic peptide (hSRI-2) corresponding to the C-terminus of the collagen-like domain [14]. The following antibodies were purchased from Cappel (Malvern, Pennsylvania, USA): F(ab')2 fragments of goat anti-mouse IgG labeled with fluorescein-isothiocyanate (FITC) and tetramethylrhodamin (TRIC), F(ab')2 fragments of goat anti-rabbit IgG labeled with FITC, and goat anti-mouse IgG labeled with peroxidase. Goat anti-mouse IgG conjugated with colloidal gold (particle size: 15 nm) was obtained from E-Y Laboratories (San Mateo, California, USA).

Immunocytochemistry

Indirect immunofluorescence and dual staining were performed as described previously [20–23]. In the dual staining, FITC-labeled goat anti-mouse IgG and TRIC-labeled goat anti-rabbit IgG were used. The number of glomeruli evaluated ranged 1 to 10 (mean, 3.4). The intensity and distribution of fluorescence in each glomerulus were scored as negative (score 0), and 1+ positive (score 1) or 2+ positive (score 2) (Fig. 1, A, B and C). The mean score (the sum of scores/the number of glomeruli evaluated) was calculated for each patient. To analyze the relationship between apoprotein deposition and histologic changes, plasma lipid profile and clinical findings, the patients were divided into three groups: Group 1, negative immunofluorescence (score 0); Group 2, weak immunofluorescence (mean score $0< \leq 1.5$); and Group 3, strong immunofluorescence (mean score $1.5< \leq 2$).

As controls, kidney sections were incubated with nonimmune sera or with unrelated mouse IgG monoclonal antibody, followed by incubation with FITC- or TRIC-labeled goat anti-mouse IgG, or with secondary antibody alone. These controls were negative.

Immunoelectron microscopy was performed using pre-embedding and post-embedding method as described previously [21, 23]. For pre-embedding staining [21], tissues were fixed in paraformaldehyde-lysine-periodate fixative and then embedded in OCT compound. Then they were incubated with the primary antibodies, followed by reaction with the appropriate secondary antibody labeled with peroxidase. The sections were then treated with diaminobenzidine/hydrogen peroxide solution, post-fixed with osmic acid, and embedded in Epon 812. Ultra-thin sections prepared by microtome were observed under an electron microscope. For the post-embedding method [23], specimens were embedded in Lowicryl K4M (Chemische Werke Lowi GmbH, Waldkreiburg, Germany). Ultra-thin sections, placed on 200-mesh nickel grids, were incubated with primary antibodies and then reacted with secondary antibodies labeled with gold particles. The sections were then fixed in 0.2% glutaraldehyde/0.2 M sodium cacodylate buffer, stained with uranyl acetate, and viewed under an electron microscope.

Statistical analysis

The correlation between the grade of apoprotein immunofluorescence and that of mesangial proliferation was assessed using the chi-square test. The relationship between apoprotein immunofluorescence and plasma levels of total cholesterol,

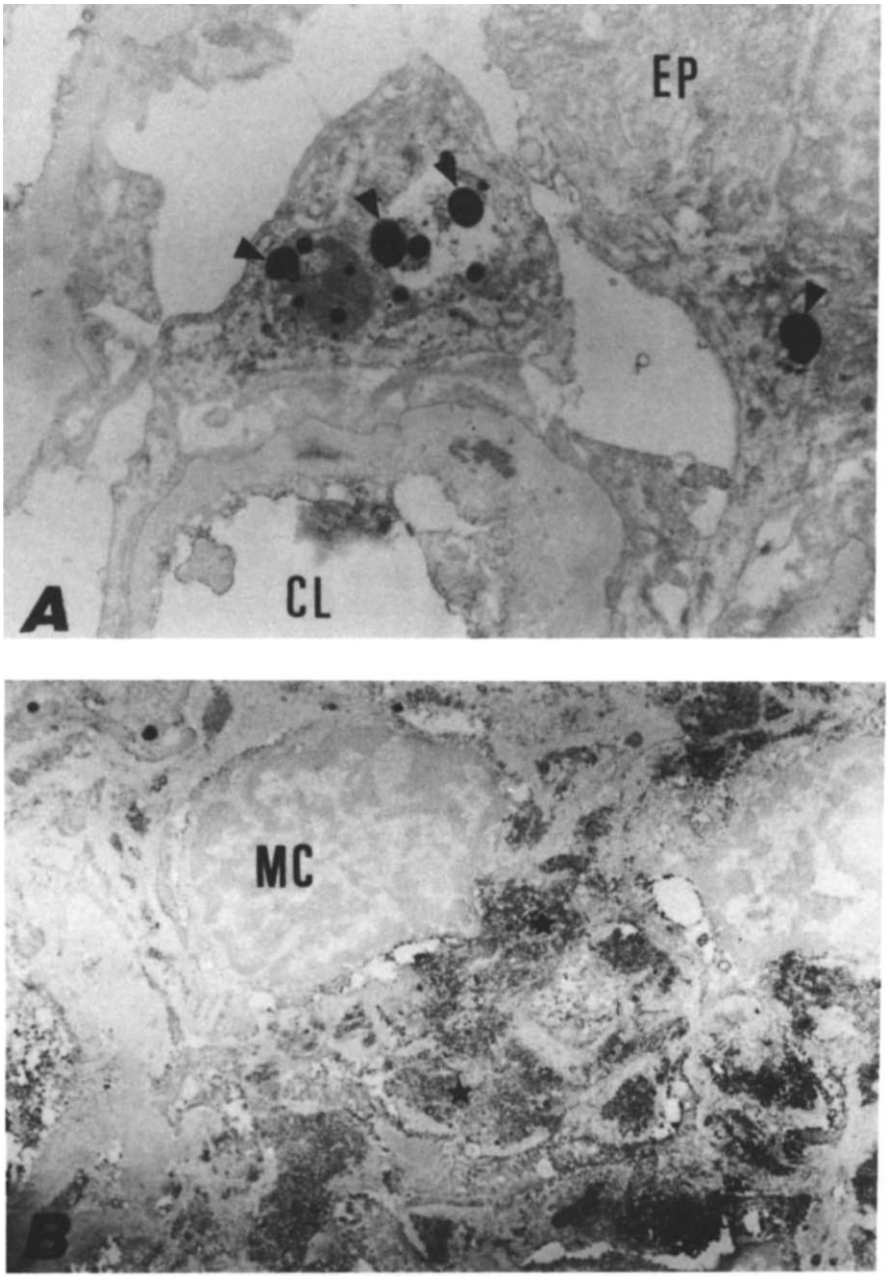


Fig. 2. *Ultrastructural observation of apo B in glomeruli of patients with focal glomerular sclerosis (A) and IgA nephritis (B), stained with monoclonal antibody to apo B-100 and peroxidase-labeled secondary antibody.* A. Apo B-positive droplets are seen within the cytoplasm of visceral epithelial cells (arrowheads). B. Granular reaction products (asterisks) accumulated in the mesangial matrix. Original magnification: A ×8,000, B ×4,000. Abbreviations are: CL, glomerular capillary lumen; EP, glomerular epithelial cell; MC, mesangial cell.

triglyceride, HDL cholesterol, apo A-I, apo B, and apo E, and 24-hour urinary protein excretion and creatinine clearance was evaluated using the Kruskal-Wallis test. A value less than 0.05 was considered statistically significant.

Results

Apolipoproteins

Apo B and apo E immunofluorescence were not detected in the glomeruli of normal human kidneys, but were detected focally in the media of arterioles and peritubular capillaries. These apolipoproteins were frequently observed in the glomeruli of patients with IgA nephritis, mesangial proliferative (non-IgA) nephritis, Henoch-Schönlein purpura nephritis, focal glomerular sclerosis and lupus nephritis, but were rarely observed in minimal change nephrotic syndrome (Table 2). Apo B and apo E were localized predominantly in the mesangium (Fig. 1), and the distribution and intensity of their fluorescence correlated significantly with the grade of mesangial proliferation ($P < 0.05$). Apo B (Fig. 1C) and apo E were also stained in sclerosed areas of glomeruli. Immunoelectron microscopy revealed that apo B- and apo E-positive droplets were present in the cytoplasm of glomerular epithelial cells in focal glomerular sclerosis (Fig. 2A), and in the cytoplasm of mesangial cells in mesangial proliferative types of glomerulonephritis. In addition, apo B (Fig. 2B) and apo E were found as amorphous aggregations in the mesangial area.

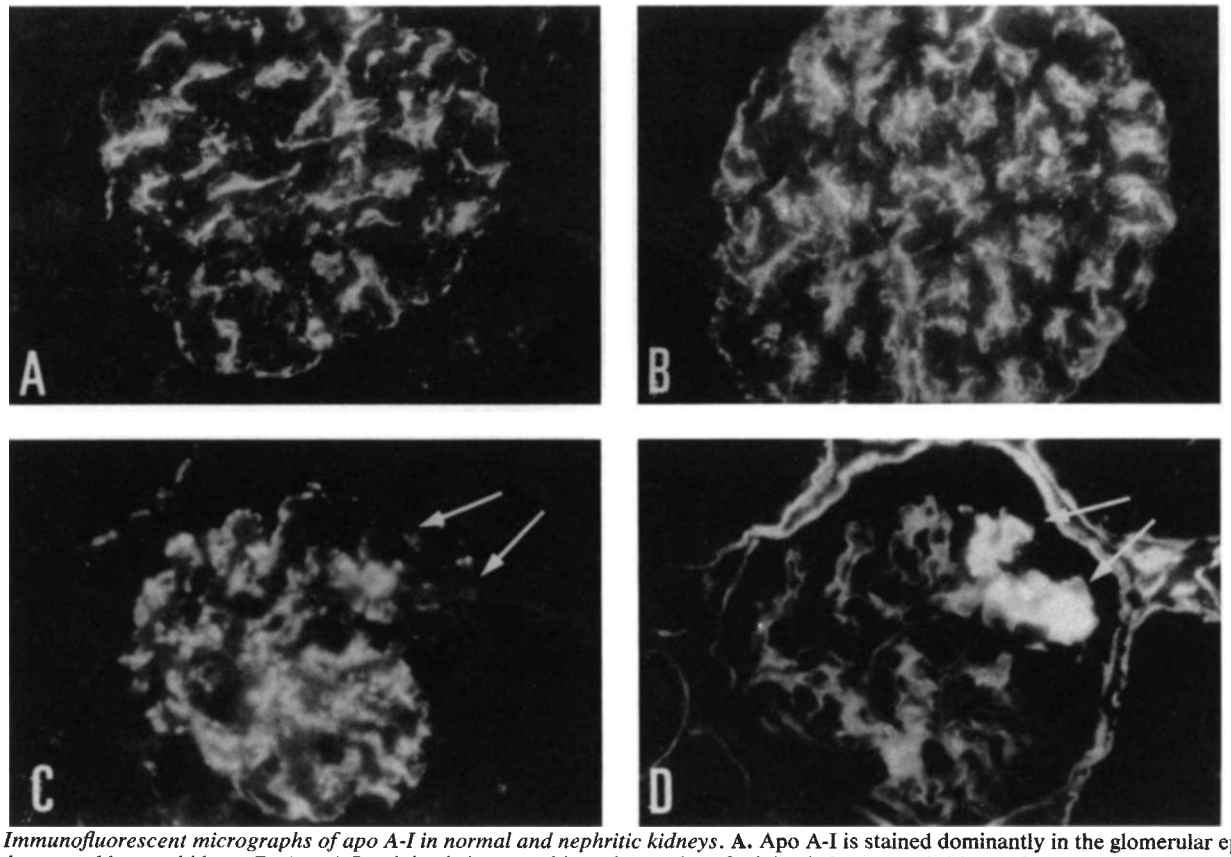


Fig. 3. *Immunofluorescent micrographs of apo A-I in normal and nephritic kidneys.* **A.** Apo A-I is stained dominantly in the glomerular epithelial cells of the normal human kidney. **B.** Apo A-I staining is increased in a glomerulus of minimal change nephritic syndrome. **C and D.** Dual staining of apo A-I (C) and laminin (D) in a glomerulus of focal glomerular sclerosis. Apo A-I staining is decreased in the sclerosed area (arrows) where laminin staining is intense. Original magnification: A–D ×400.

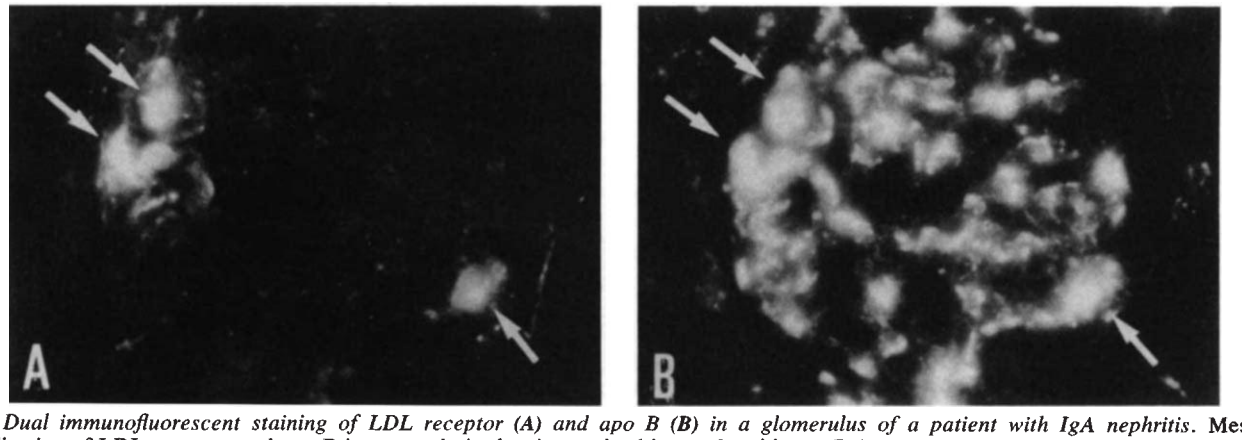
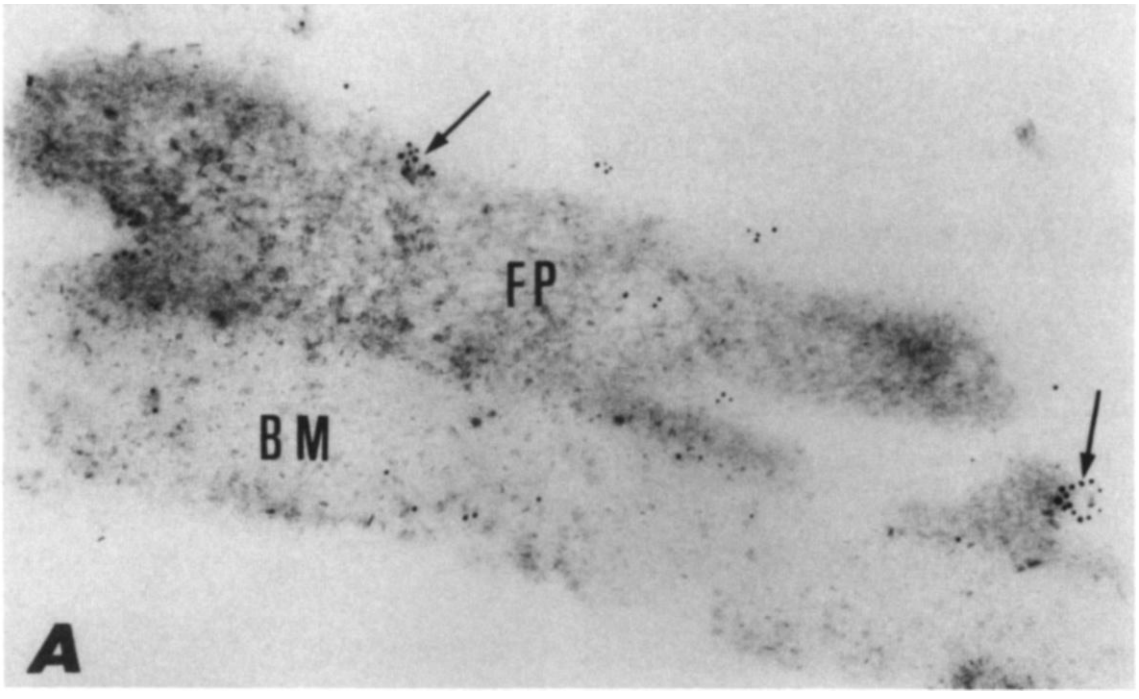


Fig. 4. *Dual immunofluorescent staining of LDL receptor (A) and apo B (B) in a glomerulus of a patient with IgA nephritis.* Mesangial co-localization of LDL receptor and apo B is seen only in the sites stained intensely with apo B (arrows).

Monoclonal antibody to apo A-I stained glomerular epithelial cells and weakly stained the cytoplasm of some tubular epithelial cells in normal and nephritic human kidneys. The intensity of the immunofluorescence was increased in the glomeruli of patients with minimal change nephrotic syndrome, focal glomerular sclerosis, and mesangial proliferative types of glomerulonephritis compared with normal human kidneys, but the immunofluorescence was reduced or lost in sclerosed areas (Fig. 3). Post-embedding immunoelectron microscopy revealed that apo A-I was localized in the cytoplasm and foot processes of visceral epithelial cells.

Oil red 0 staining was performed in 20 sections, and the results were compared with the immunofluorescent staining for apo B. The dye was distributed in a scattered fashion, particularly in the mesangial areas of glomeruli and in the renal interstitium of nephritic kidneys. The distribution of oil red 0



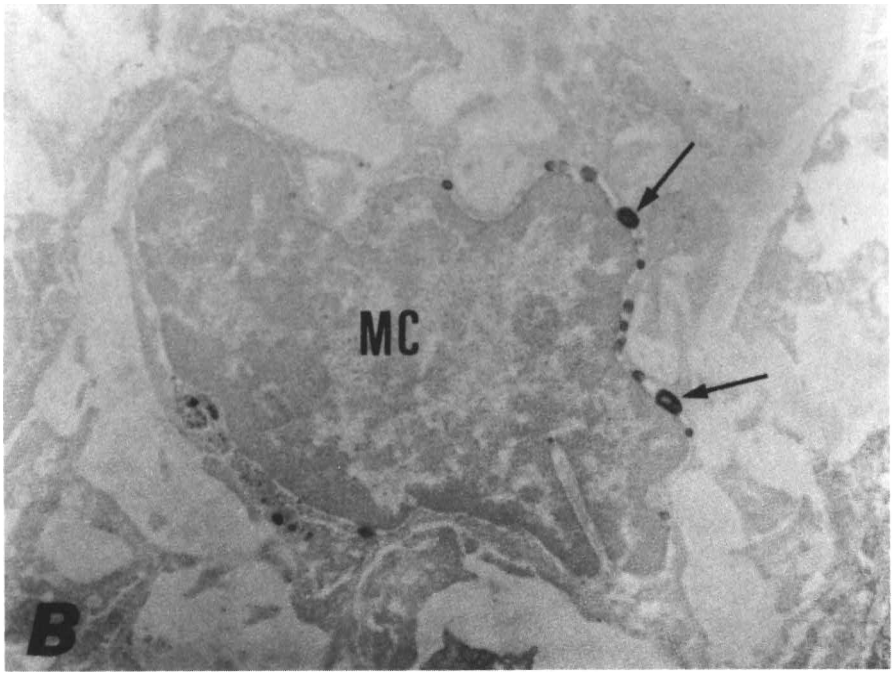


Fig. 5. *Immunoelectron microscopic photographs of LDL receptor in a patient with IgA nephritis.* **A.** Colloidal gold staining. Gold particles are distributed on the surface of foot processes of visceral epithelial cells. There are small clusters of these particles (arrows) in some parts (coated pits ?). **B.** Peroxidase staining. Reaction products are seen on the mesangial cell surface (arrows). Original magnification: A ×5,000, B ×3,500. Abbreviations are: BM, glomerular basement membrane; FP, foot process of glomerular epithelial cell; MC, mesangial cell.

staining in the mesangial area was similar to that of apo B staining, but was weaker in intensity and less diffuse than the latter.

Lipoprotein receptors

In normal human kidneys, LDL receptor immunofluorescence was visible in the cytoplasm of tubular epithelial cells, but was negative in the glomeruli. LDL receptor was positive, however, in the glomeruli of some patients with glomerular disease (Table 2). It was distributed predominantly in the mesangium and weakly along the glomerular capillary walls. Dual staining showed that LDL receptor was occasionally co-localized with apo B in the glomeruli, especially in the sites where apo B staining was intense (Fig. 4). Immunoelectron microscopy showed that LDL receptor was expressed on the membrane surface of visceral epithelial cells, as well as on mesangial cells (Fig. 5).

Weak immunofluorescence for scavenger receptor was detected along the glomerular capillary walls and mesangium of normal human kidneys. In some of nephritic kidney tissues, the

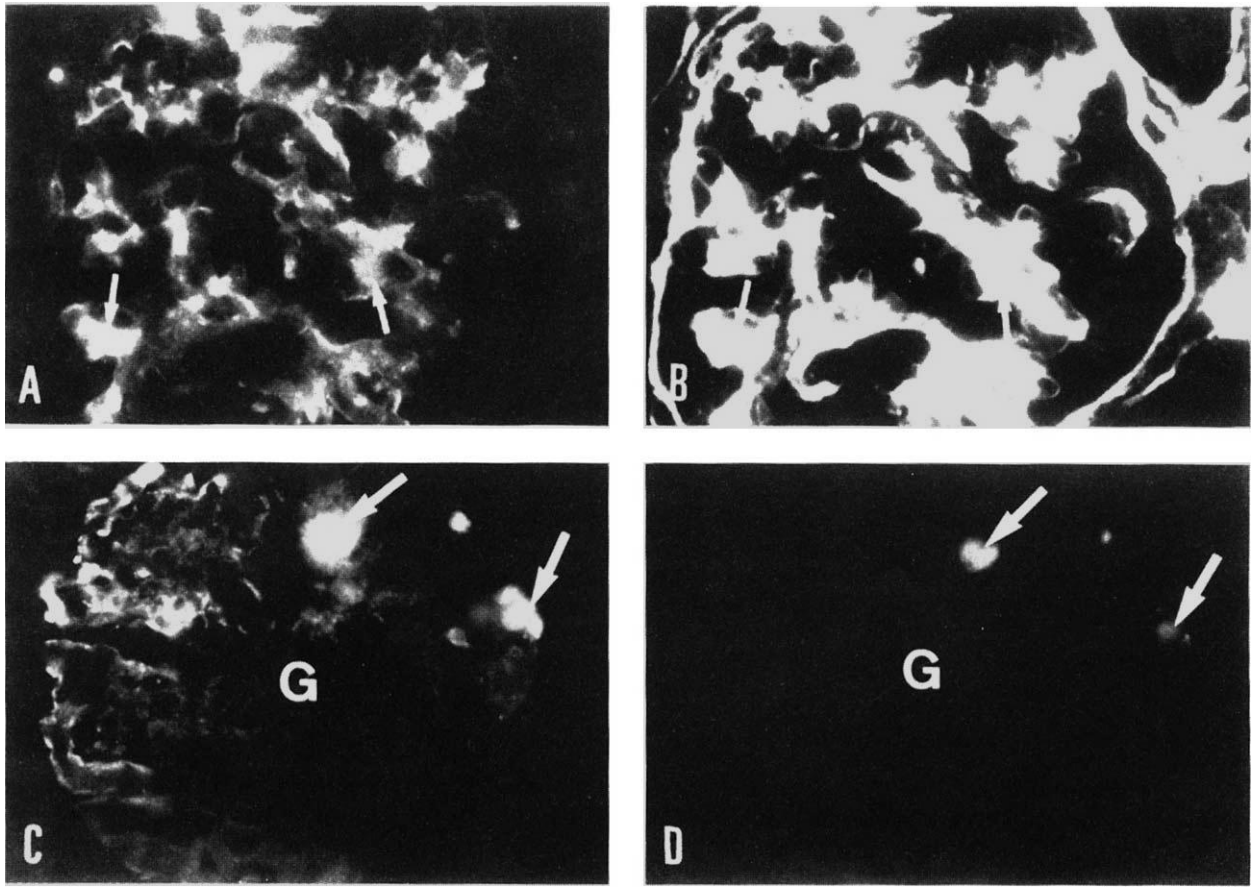


Fig. 6. *Immunofluorescent photographs of scavenger receptor in patients with IgA nephritis.* Dual staining for scavenger receptor (**A**) and type IV collagen 7S domain (**B**) in the same tissue section exhibit distribution of the receptor in the mesangium (arrows) and along the glomerular capillary walls. Monocyte/macrophage staining in the serial section containing the same glomerulus was negative. Dual staining for scavenger receptor (**C**) and monocytes/macrophages (**D**). The scavenger receptor is intensely expressed by monocytes/macrophages within the glomerulus (arrows), and is weakly expressed in other parts of the glomerulus. Original magnification: A–D ×400. Abbreviation is: G, glomerulus.

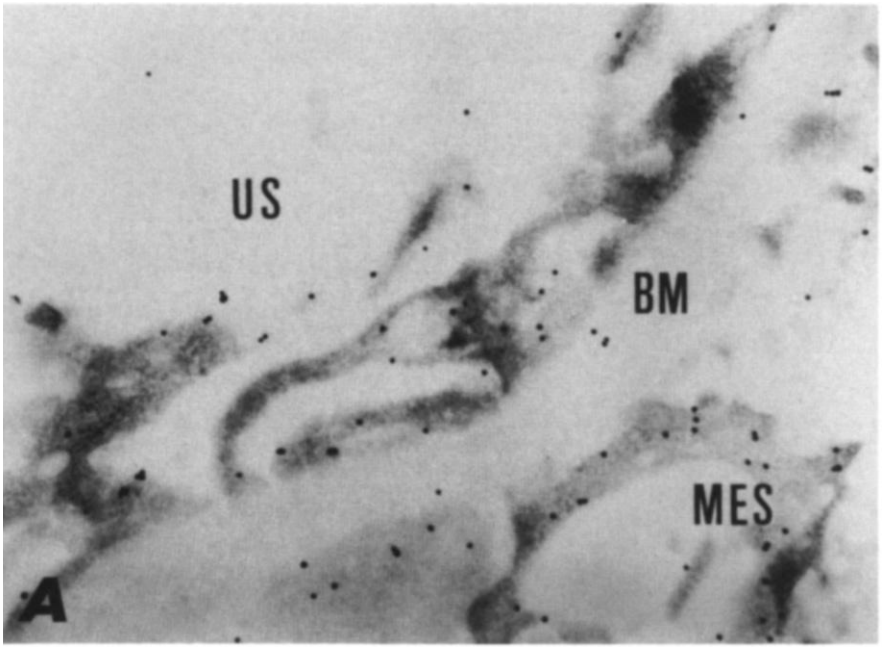
expression of scavenger receptor was enhanced in these loci (Table 2, Fig. 6 A and C). Dual immunofluorescent staining with anti-scavenger receptor antibody and anti-type IV collagen (7S domain) antibody confirmed mesangial distribution of the receptor (Fig. 6 A and B). The immunofluorescence for scavenger receptor tended to be intense in the glomeruli of patients with moderate or marked mesangial proliferation. Immunoelectron microscopic studies further demonstrated that the receptor was present on the surface of glomerular epithelial and mesangial cells (Fig. 7 A and B). Dual immunofluorescence for scavenger receptor and monocytes/macrophages revealed strong expression of the receptor in monocytes/macrophages which are occasionally infiltrating the glomeruli of lupus nephritis and IgA nephritis (Fig. 6 C and D). The localization of scavenger receptor was generally discordant with that of apo B.

Apolipoprotein deposition and laboratory data

The relationship between apoprotein deposition and laboratory findings in patients with mesangial proliferative types of glomerulonephritis, including IgA nephritis and mesangial proliferative (non-IgA) glomerulonephritis, was evaluated (Table 3). There was no significant correlation between the grade of glomerular apo B or apo E deposition and plasma levels of total cholesterol, triglyceride, HDL-cholesterol, apo A-I, apo B, or apo E. Deposition of apo B and apo E was significantly ($P < 0.05$) correlated with the patients' 24-hour protein excretion but not with their creatinine clearance. Since apo A-I immunofluorescence was detected in the glomeruli of normal human kidneys and was reduced or lost in the sclerosed glomeruli in nephritis tissues, we did not evaluate the relationship between apo A-I deposition and patients' laboratory data.

Discussion

LDL receptors have been demonstrated on most cell types. Culture studies have indicated that rat mesangial cells have specific LDL receptors [7, 9]. Mesangial cells show receptor-mediated endocytosis of receptor-bound LDL, and high concentrations of LDL are toxic to these cells [15, 16]. Cultured human glomerular epithelial cells also have LDL receptors which mediate the binding, internalization, and degradation of LDL [8]. Scavenger receptors [13, 14], different from LDL receptors in both their binding characteristics and protein structure, are expressed in a restricted number of cell types. Whether glomerular mesangial and epithelial cells possess



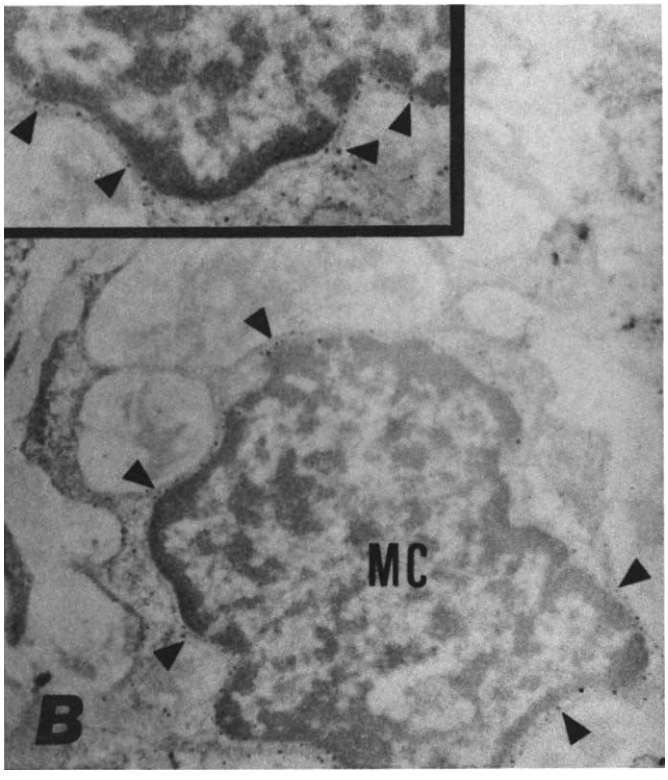


Fig. 7. *Immunoelectron microscopy of scavenger receptor in a patient with IgA nephritis.* **A.** Colloidal gold staining for scavenger receptor, displaying labeling of the foot processes of glomerular epithelial cells. **B.** Peroxidase staining for scavenger receptor, showing labeling of mesangial cells (arrows). Original magnification: A ×12,000, B ×6,000 (inset ×8,000). Abbreviations are: BM, basement membrane in the paramesangial zone; MES, mesangium; US, urinary space; MC, mesangial cell.

scavenger receptors is unclear, since they apparently did not bind and internalize acetylated LDL [8]. However, recent studies by Coritsidis et al [15] have shown that rat mesangial cells in culture avidly bind and take up oxidized LDL to a greater extent than native LDL.

Our present study immunocytochemically demonstrated the expression of both LDL receptor and scavenger receptor on the glomerular epithelial and mesangial cells of human kidneys. Our immunofluorescence study revealed LDL receptor in the glomeruli of some patients with glomerulonephritis, particularly in those with increased mesangial proliferation, although this receptor was not detected in normal glomeruli. The expression of scavenger receptor was observed in normal glomeruli, and was increased in the glomeruli of nephritic kidneys. The glomerular expression of LDL receptors was occasionally colocalized with apo B-100, a ligand for the LDL receptor. These

Table 3. Analysis of the relationship between glomerular apoprotein deposition and plasma lipid level or other laboratory findings in patients with mesangial proliferative type of glomerulonephritis

	Plasma lipid level *mg/dl*						U_{Prot} *g/24 hr*	C_{Cr} *ml/min/ 1.73 m²*
	T-C	Trig	HDL-C	Apo A-I	Apo B	Apo E		
Apoprotein B deposition								
Group 1 (N = 3)	154 ± 37	75 ± 7.8	61 ± 0.7	131 ± 14	75 ± 2.3	4.7 ± 1.6	0.55 ± 0.21	95 ± 3.1
Group 2 (N = 8)	148 ± 42	68 ± 27	67 ± 12	120 ± 23	75 ± 14	3.7 ± 0.5	0.85 ± 0.28	89 ± 12
Group 3 (N = 17)	188 ± 48	91 ± 30	63 ± 9.2	131 ± 20	80 ± 22	4.6 ± 1.4	1.8 ± 1.4	85 ± 11
P	NS	NS	NS	NS	NS	NS	< 0.05[a]	NS
Apoprotein E deposition								
Group 1 (N = 9)	157 ± 41	71 ± 77	65 ± 12	123 ± 23	72 ± 10	3.8 ± 0.9	0.42 ± 0.30	91 ± 11
Group 2 (N = 9)	155 ± 41	78 ± 14	65 ± 6.1	129 ± 25	72 ± 17	4.3 ± 0.8	0.90 ± 0.84	92 ± 10
Group 3 (N= 10)	198 ± 51	96 ± 37	63 ± 11	131 ± 51	89 ± 23	4.8 ± 1.8	2.3 ± 1.5	80 ± 8.6
P	NS	NS	NS	NS	NS	NS	< 0.05[a]	NS

A total of 28 patients with mesangial proliferative type of glomerulonephritis, consisting of 18 patients with IgA nephritis and 10 with mesangial proliferative (non-IgA) nephritis, were divided into three groups: Group 1; negative immunofluorescence (score 0); Group 2; weak immunofluorescence (mean score 0< ≤1.5); Group 3; strong immunofluorescence (mean score 1.5< ≤2). Data are expressed as mean ± SD. Abbreviations are: T-C, total cholesterol; Trig, triglyceride; HDL-C, HDL cholesterol; U_{Prot}, urinary protein; C_{Cr}, creatinine clearance; NS, not statistically significant.

[a] Significant correlation between apoprotein deposition and the laboratory finding (the Kruskal-Wallis test analysis)

observations suggest that *in vivo* glomerular cells in nephritic conditions can take up both native LDL and modified LDL via their respective specific receptors.

The oxidization of LDL has been suggested to be a major contributing factor in the pathogenesis of atherosclerosis [24]. This concept may also be applicable to the glomerular cell damage found in hyperlipidemic conditions [1–3, 12], which is similar to the LDL-induced degeneration of smooth muscle cells. The uptake of oxidized LDL by mesangial cells in culture could alter eicosanoid synthesis and cytokine production [25], influencing local hemodynamics and vascular permeability. Actually, the addition of oxidized LDL to mesangial cells in culture was shown to increase PGE_2 synthesis, while the same dose of LDL did not have such an effect [15]. Monocytes/macrophages have been shown to be the dominant immune cell infiltrating the glomeruli of patients with IgA nephritis, and they are associated with the magnitude of proteinuria [22, 26]. We found here that scavenger receptors were also expressed by monocytes/macrophages identified in the glomeruli of human nephritis.

Our data show that nephritic kidneys frequently have mesangial accumulations of apo B and apo E. At an ultrastructural level, these apolipoproteins were observed as droplets within the cytoplasm of glomerular cells or as amorphous granular deposits within the mesangial matrix. Similar observations were previously reported by Sato et al [27], although the sample size in their study was relatively small and they did not perform immunoelectron microscopy. Other than being taken up by specific receptors, apolipoproteins could be deposited simply by the mechanism of alterations in the mesangial traffic of macromolecules, or by that of charge affinity to glycosaminoglycans in the mesangial matrix [28]. They could also be phagocytosed by mesangial cells [29]. The glomerular deposition of apo B in the present study was associated with mesangial expansion in mesangial proliferative types of glomerulonephritis. LDL has been shown to promote the proliferation of cultured mesangial cells, and, at greater concentrations, to inhibit this proliferation, showing morphological and biochemical toxicity to these cells [7, 16]. These factors might explain this association.

We found that apo B-positive droplets were present in the visceral epithelial cells of patients with focal glomerular sclerosis. Abundant lipid vacuoles in glomeruli have been reported in a patient with idiopathic focal glomerulosclerosis [30]. Verani and Hawkins [31] found that the presence of foam cells and the proliferation of epithelial cells were characteristic of the early lesions of recurrent focal glomerular sclerosis in transplanted kidneys. The apo B-positive lipid droplets we found in the visceral epithelial cells might be harmful to these cells, having an effect similar to that of native or modified LDL on mesangial cells [15, 16], thus resulting in epithelial cell injury followed by sclerosis.

Apo A-I is the main apolipoprotein constituent of HDL cholesterol. Differently from apo B and apo E, we observed apo A-I predominantly in the visceral epithelial cells. The fluorescent intensity of apo A-I was frequently greater in nephritic kidneys than in normal human kidneys, and was decreased in sclerosed areas of the glomeruli. Recent studies suggest that the kidney is the primary site for apo A-I catabolism [32]. As HDL molecules are too large (molecular wt 330,000) to be filtered through normal glomerular basement membrane, it is postulated that the free form of apo A-I (molecular wt 28,000) is so filtered, afterward being reabsorbed predominantly in the proximal tubules. Therefore, apo A-I found in the visceral epithelial cells may reflect apo A-I that is filtered, even greater in diseased state, through glomerular basement membrane barrier and partly absorbed by glomerular epithelial cells. It is not yet known whether glomerular cells themselves synthesize this apolipoprotein [33]. Since there was no significant correlation between the glomerular deposition of apo B and apo E and their plasma levels, it is unlikely that the glomerular deposition of apolipoproteins reflects increased levels of these substances in the circulation. Thus, local factors such as immune-mediated or non-immune mediated mesangial and epithelial cell injury, rather than systemic hyperlipidemia, would seem to be important for lipid deposition in human glomerulonephritis.

Acknowledgments

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