Kidney International, Vol. 37 (1990), pp. 1449–1459

Receptor mediated uptake of apo B and apo E rich lipoproteins by human glomerular epithelial cells

HERMANN-JOSEF GRÖNE, AUTAR K. WALLI, ELISABETH GRÖNE, ANNETTE KRÄMER, MICHAEL R. CLEMENS, and DIETRICH SEIDEL

Department of Pathology, University Hospital, Göttingen, Department of Internal Medicine, University Hospital, Tübingen, and Department of Clinical Chemistry, University of Munich, Munich, Federal Republic of Germany

Receptor mediated uptake of apo B and apo E rich lipoproteins by glomerular epithelial cells. Various pathological disorders are accompanied by the deposition of lipids into glomerular cells. To gain insight into these disorders, it is essential to know if glomerular cells possess lipoprotein receptors. We therefore characterized the activity of lipoprotein receptors in cultured epithelial cells of the human glomerulus. Podocytes were chosen as they are directly exposed to lipoproteins in pathological states like in glomerular proteinuria (such as, nephrotic syndrome). Isolated human glomeruli (purity >95%) were incubated in buffered RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum at 37°C and 5% CO_2. Outgrowing cells were vimentin and keratin positive. Monolayer cultures of human glomerular epithelial cells upon incubation in lipoprotein deficient serum for 48 hours expressed a receptor-dependent uptake of lipoproteins. These cells showed about 10% of the maximal capacity for LDL uptake as compared to fibroblasts; however, the K_m values for binding, internalization and degradation were similar in the cultures of glomerular epithelial cells and fibroblasts. The K_m values for degradation of LDL, chylomicron remnants, β-VLDL from cholesterol-fed rabbits and VLDL from familial LCAT-deficiency patients were 14.2, 4.9, 2.9, 4.5 μg protein/ml medium, respectively, for glomerular epithelial cells. The avid uptake of ^{125}I-labeled apo E-containing lipoproteins was further substantiated by their poor displacement by a 25-fold excess of unlabeled LDL and their ability to down regulate the apo B,E receptor activity. LDL as well as β-VLDL were able to suppress the incorporation of ^{14}C acetate into sterols and to stimulate ^{3}H-cholesterylester formation. These experiments show that cultured glomerular epithelial cells express lipoprotein receptor activity. Plasma concentrations of apo E-containing lipoproteins are increased in certain renal diseases (such as, nephrotic syndrome); these lipoproteins could be rapidly removed by glomerular epithelial cells and lead to lipid deposition in glomeruli.

Deposition of lipids in renal glomeruli may occur in advanced glomerular lesions due to immunologic or metabolic insults [1]. Analogous to the role of lipids in atherosclerosis, the glomerular lipid accumulation has been implicated in the progression of glomerular sclerosis [2].

In minimal change glomerulopathy with focal and segmental sclerosis—a disease encountered in young patients with nephrotic syndrome—foam cells can be observed in sclerotic areas [3].

One of the most widely studied diseases of renal lipoidosis is a rare genetic disorder of lipoprotein metabolism, "Familial lecithin:cholesterol acyl transferase" (LCAT) deficiency. Patients with LCAT deficiency show an abnormal plasma lipoprotein profile. Many LCAT deficiency patients develop proteinuria. In some of these patients renal biopsies have been performed and accumulation of lipids in glomeruli and glomerulosclerosis have been observed [4]. Lipid droplets were found in the endothelial, mesangial and visceral epithelial cells, as well as in the subendothelial and subepithelial parts of the glomerular basement membrane [5]. In addition, increased concentrations of phospholipids and free cholesterol were measured in isolated glomeruli. It is thought that glomerular lipids play an important pathogenic role in the glomerulosclerotic process of familial LCAT deficiency patients [6].

To gain insight into the pathogenesis of glomerular lipoidosis it is desirable to investigate the lipoprotein receptor activity of human glomerular cells in culture. Mesangial cells exhibit phagocytic functions and may thus be expected to take up lipoproteins by a non-saturable, receptor independent mechanism and may under pathological conditions accumulate lipids like macrophages in atherosclerotic plaques [7, 8]. However, it has recently been reported that these cells take up lipoproteins in a manner similar to smooth muscle cells by receptor mediated pathway [9, 10]. Visceral epithelial cells, also called podocytes, are the major cell types of the glomerulus in terms of volume [11]. We therefore investigated the uptake of apo B-100 containing lipoproteins such as LDL and apo B, E containing lipoproteins such as postprandial chylomicron remnants and unusual VLDL particles found in the plasma of rabbits fed high cholesterol diet, and VLDL from the plasma of a patient with familial LCAT deficiency.

Our data show that human glomerular epithelial cells take up apo B-100 containing lipoprotein, that is, LDL as well as apo B, E containing lipoproteins such as chylomicron remnants, β-VLDL from cholesterol-fed rabbits and VLDL from the plasma of a familial LCAT-deficiency patient. However, the affinity for uptake of apo B, E containing lipoproteins is much higher than that for LDL. The receptor mediated uptake of lipoproteins suppresses the cellular sterol synthesis and cholesterylester formation.

Received for publication March 27, 1989
and in revised form November 20, 1989
Accepted for publication January 19, 1990

Methods

Biochemicals

All chemicals and biochemicals were obtained from Sigma Chemie GmbH (München, FRG), E. Merck GmbH (Darmstadt, FRG) or Boehringer (Mannheim, FRG). Bovine and human serum albumin, fatty acid free, were obtained from Behring (Marburg, FRG); cell culture medium and fetal calf serum were from Gibco Europe Ltd. (Eggenstein, FRG). Sterile cell culture plastic and glassware were purchased from Becton Dickinson (Heidelberg, FRG). ^{125}I-sodium iodide, 2-[^{14}C] sodium acetate and 9, 10-[^{3}H] oleic acid were purchased from Amersham Buchler GmbH & Co., KG (Braunschweig, FRG). The mouse monoclonal vimentin antibody (V9) and mouse monoclonal antibody against keratin intermediate filaments (LU 5) were from Dr. M. Osborn, Max Planck Institute of Biophysical Chemistry (Göttingen, FRG). Goat antimouse IgG was labeled with fluorescein isothiocynate (FITC) purchased from Cappel Chemical Laboratory (Cochraneville, Pennsylvania, USA).

Isolation of human glomeruli

Human glomeruli. Human kidneys were obtained from renal carcinoma patients. Immediately after surgical removal, carcinoma free parts of kidneys were excised and stored in sterile, ice-cold calcium-free phosphate buffered saline, PBS (136.9 mM NaCl, 2.68 mM KCl, 1.47 mM KH_2PO_4 and 10.44 mM Na_2HPO_4, pH 7.4). All subsequent steps were carried out in the same buffer under sterile conditions. Cortices were freed from capsule and medulla, minced to a fine paste and passed through a stainless steel sieve (mesh size 250 μm). This was followed by several washes on a metal sieve of pore size 106 μm. Glomeruli retained on this sieve were collected in PBS, carefully pipetted in and out for one to two minutes through a pasteur pipette and centrifuged for five minutes at 120 g. The pelleted glomeruli were resuspended in RPMI 1640, (14.1 mM Hepes, 23 mM glutamine, pH 7.4) supplemented with 26 mg/liter bovine pancreas insulin, 100 mg/liter streptomycin, 100.000 U/liter penicillin and 20% fetal calf serum.

Light and scanning electron microsocpy showed that pellets were isolated, decapsulated glomeruli of 95% purity with an intact layer of podocytes.

Cultured glomerular cells. Glomeruli suspended in supplemented RPMI 1640 medium were seeded at a density of approximately 2000 glomeruli in 30 × 10 mm culture dishes and incubated at 37°C in humidified 5% CO_2. After about one week, cells growing out of glomeruli adherent to culture dishes could be observed, and thereafter the medium was changed at an interval of two days. After about 28 days, primary cultures outgrowing from glomerular cells were trypsinized and separated from the glomeruli and glomerular debris by filtration through a nylon sieve (75 μm pore size). The filtrate was subcultured and monolayers were confluent within nine days. Monolayer cultures in first passage were used throughout this study.

Cultured human fibroblasts

Skin biopsies obtained from normal adult donors were cultured in plastic dishes in Dulbecco's minimal essential medium which contained 25 mM $NaHCO_3$, 20 mM Hepes buffer, pH 7.4, and 10% fetal calf serum. Culture medium was also supplemented with 100 mg streptomycin/liter and 100.000 U penicillin/liter [12]. Outgrowing fibroblasts were maintained in a humidified incubator (5% CO_2 at 37°C).

Isolation of lipoproteins

Blood from normal donors and a patient suffering from familial LCAT deficiency was drawn into tubes containing EDTA (1 mg/ml) after an overnight fast. After separation of chylomicrons by centrifugation at 100,000 g ($d < 1.006$ g/ml) for 40 minutes, the infranate was brought to $d = 1.019$ g/ml by addition of solid NaCl and centrifuged for 24 hours at 150,000 g and 10°C [13]. The floating lipoproteins (VLDL + IDL) were collected by tube slicing technique. The infranate was then adjusted to $d = 1.060$ g/ml by addition of solid NaCl and centrifuged at 150,000 g (24 hr at 10°C). The floating LDL was collected by tube slicing technique.

The LCAT deficiency patient was a 42-year old female born in Southern Germany. Both the clinical as well as biochemical investigations established the diagnosis of familial LCAT deficiency [14]. After separation of chylomicrons by centrifugation at 100,000 g ($d < 1.006$ g/ml) for 40 minutes, infranate was brought to $d = 1.006$ g/ml by addition of solid NaCl and centrifuged for 24 hours at 150,000 g at 10°C for isolation of VLDL.

β-VLDL from cholesterol-fed rabbits was isolated as described elsewhere [15].

Chylomicron remnants were prepared from chylomicrons that were obtained from two-hour postprandial plasma of normal human subjects. Chylomicrons were incubated with post-heparin rat plasma (2 mg chylomicron cholesterol and 4.5 ml post-heparin plasma in 0.1 M Tris HCl buffer, pH 8.5, that contained 5% albumin) for one to two hours at 37°C. The remnants were then isolated by centrifugation at 100,000 g for 100 minutes at 10°C at a solvent density of 1.019 g/ml.

Lipoproteins were labeled with ^{125}I by the iodine monochloride method as modified for lipoproteins [16]. All iodinated and non-iodinated lipoproteins were sterilized by a passage through a 0.45 μm millipore filter, kept sterile, tightly closed at 4°C and used within one to two weeks.

Assay for binding, internalization and proteolytic degradation of lipoproteins

Cultures of glomerular epithelial cells in first passage and human skin fibroblasts in third to fifth passage were trypsinized and seeded into 20 mm petri dishes. After seven days the cultures were washed with Dulbecco's phosphate buffer saline (PBS buffer) and fresh medium that contained 10% lipoprotein deficient serum (LDS, 4 mg protein/ml) was used instead of fetal calf serum to induce maximal apo B, E receptor activity. After a 48 hour incubation, monolayers were washed with PBS and fresh LDS containing medium was added together with ^{125}I-labeled lipoproteins with or without a 25-fold excess of unlabeled lipoproteins. The cultures were incubated for five hours at 37°C [17]. The medium was removed and the cells were placed on ice. The culture dishes were then washed five times with a cold buffer containing 0.15 M NaCl, 50 mM Tris (pH 7.4) and 2 mg/ml bovine fatty acid free serum albumin as described by Goldstein, Basu and Brown [18]. Assays for binding, internalization and degradation were performed by standard methods

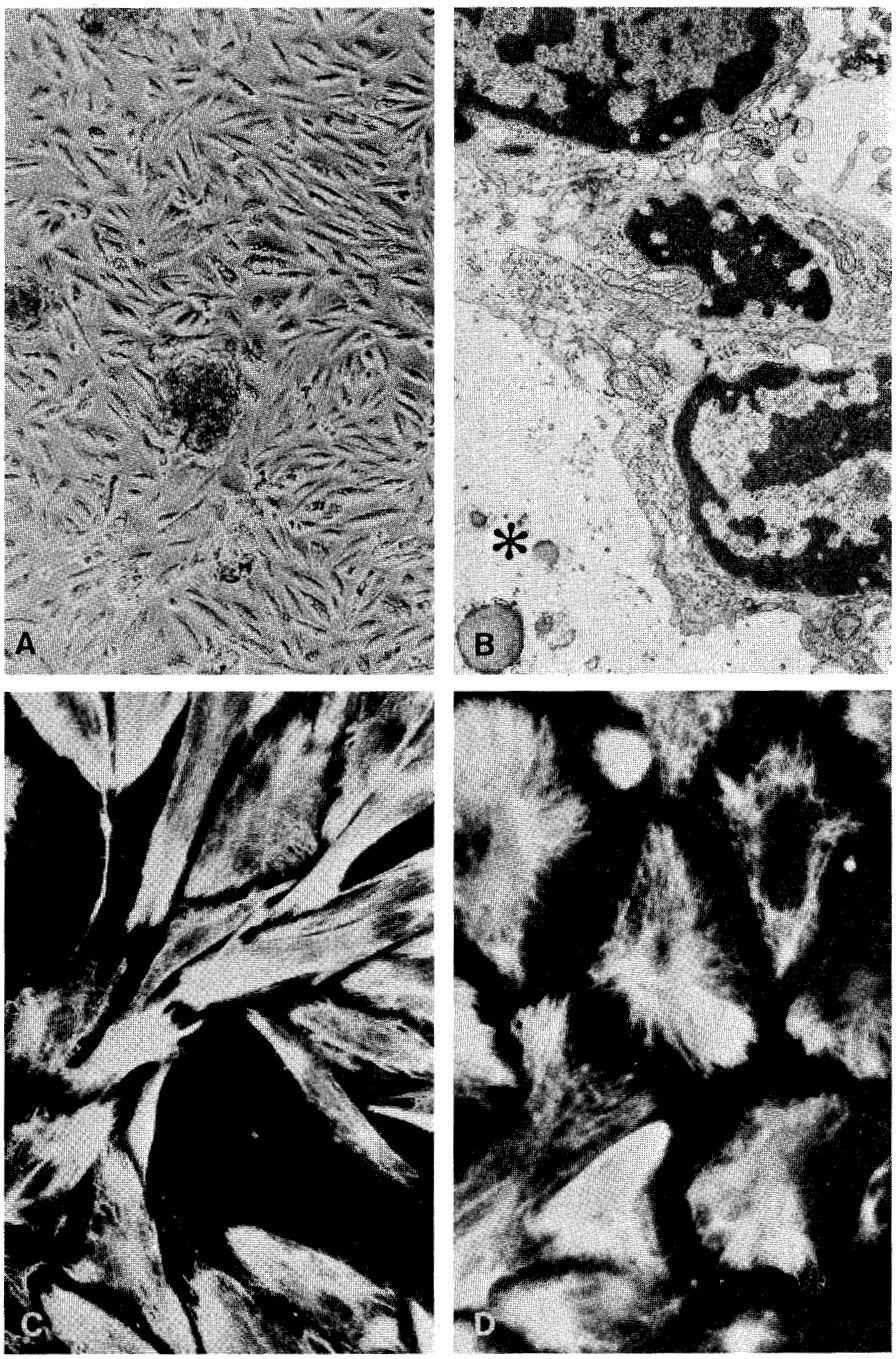

Fig. 1A. *Primary culture of human glomerular cells.* Cells with epithelial appearance have formed a confluent layer around necrobiotic glomeruli (24th day of culture, × 100). **B.** Cells growing out of a glomerulus (asterisk) show rudimentary foot processes (Transmission electron microscopy, × 3629). **C** and **D.** Immunofluorescence of human glomerular cells in primary subculture (7th day after subculture); cells react with antibodies to keratin (C) and vimentin (D).

[18]. The heparin releasable activity represents binding at 37°C. After the release of cell surface bound activity by heparin, monolayers were dissolved in 1 ml of 0.1 N NaOH and radioactivity and protein was measured. The radioactivity in the pellet was taken as a measure for internalization. Non-iodide tricholoroacetic acid (TCA)-soluble radioactivity served as a measure for lipoprotein degradation. Non-specific binding, internalization and degradation was defined as amount of the lipoprotein taken up in the presence of 25-fold excess of unlabeled ligand.

Measurement of cellular sterol synthesis and cholesterol esterification

Cellular sterol synthesis was measured by the incorporation of [^{14}C] acetate into sterols: Cultures of glomerular epithelial cells were incubated with unlabeled lipoproteins (after 48 hr in LDS) in 1 ml of fresh medium containing LDS. After six hours, the medium was removed and fresh 1 ml of medium containing 0.8 μCi of [^{14}C] acetate and unlabeled carrier acetate (0.2 μmol/ml, specific activity 4 μCi/μmol) was added to each dish

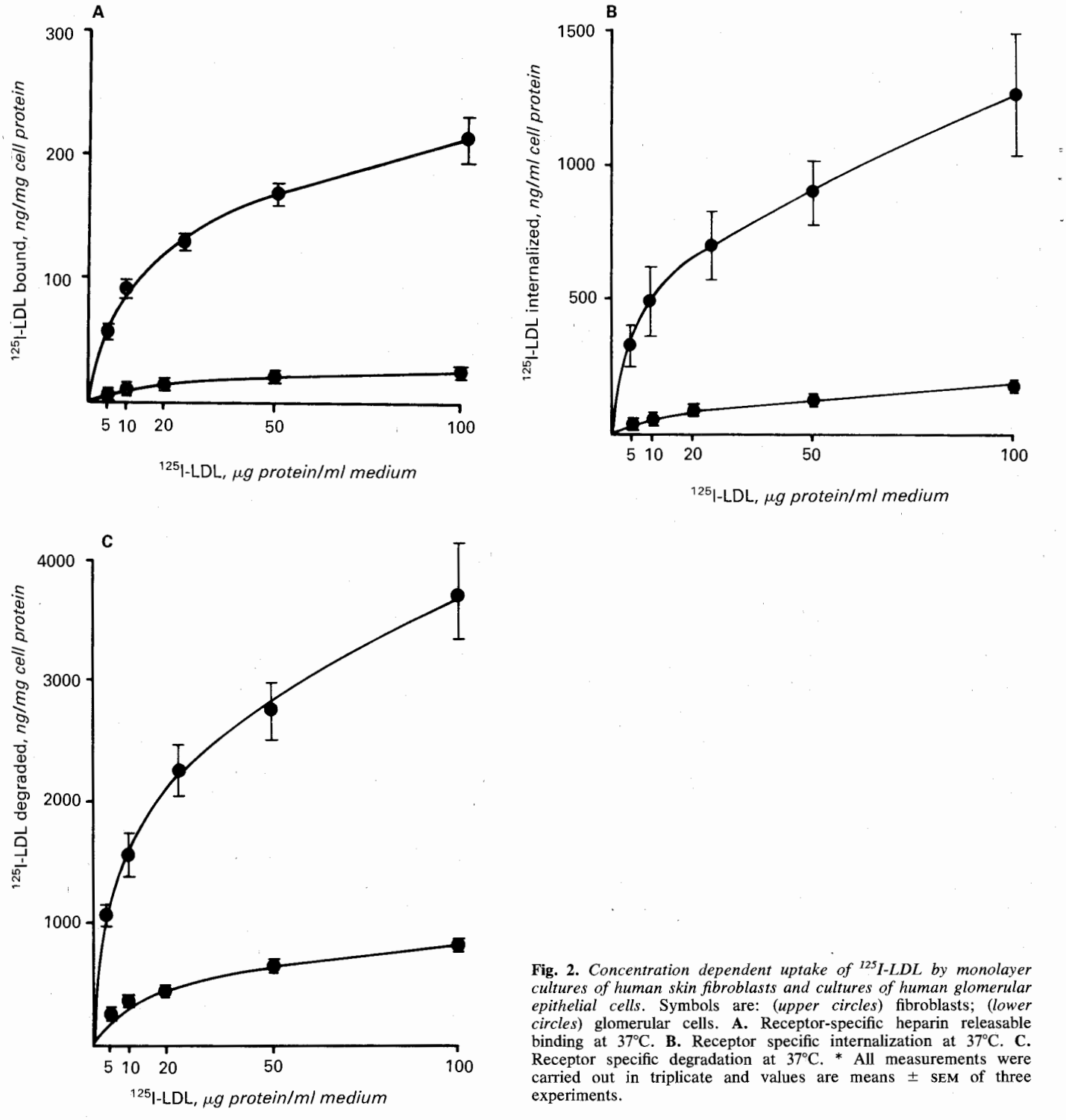

Fig. 2. *Concentration dependent uptake of ^{125}I-LDL by monolayer cultures of human skin fibroblasts and cultures of human glomerular epithelial cells.* Symbols are: (*upper circles*) fibroblasts; (*lower circles*) glomerular cells. **A.** Receptor-specific heparin releasable binding at 37°C. **B.** Receptor specific internalization at 37°C. **C.** Receptor specific degradation at 37°C. * All measurements were carried out in triplicate and values are means ± SEM of three experiments.

and incubations continued for two hours at 37°C. The cells were washed twice with PBS/BSA medium and three times with PBS alone. They were dissolved in 2 ml of 0.1 mM NaOH, ultrasonified and a portion of it was removed for measurement of [^{14}C] activity and protein [17–19].

Cell lysate (1 ml) was saponified by addition of 1 ml of 100% ethanol and 0.2 ml of 90% KOH at 80°C, for three hours. Non-saponifiable lipids were extracted into 2.5 ml of hexane. The hexane layer was washed once with 2.5 ml of 0.1 mM sodium acetate. The hexane phase was collected, evaporated and [^{14}C] activity was measured. Incorporation of [^{14}C] acetate into sterols is expressed in picomoles of acetate found in non-saponifiable lipids.

Cellular cholesterol esterification: After a 48 hour incubation

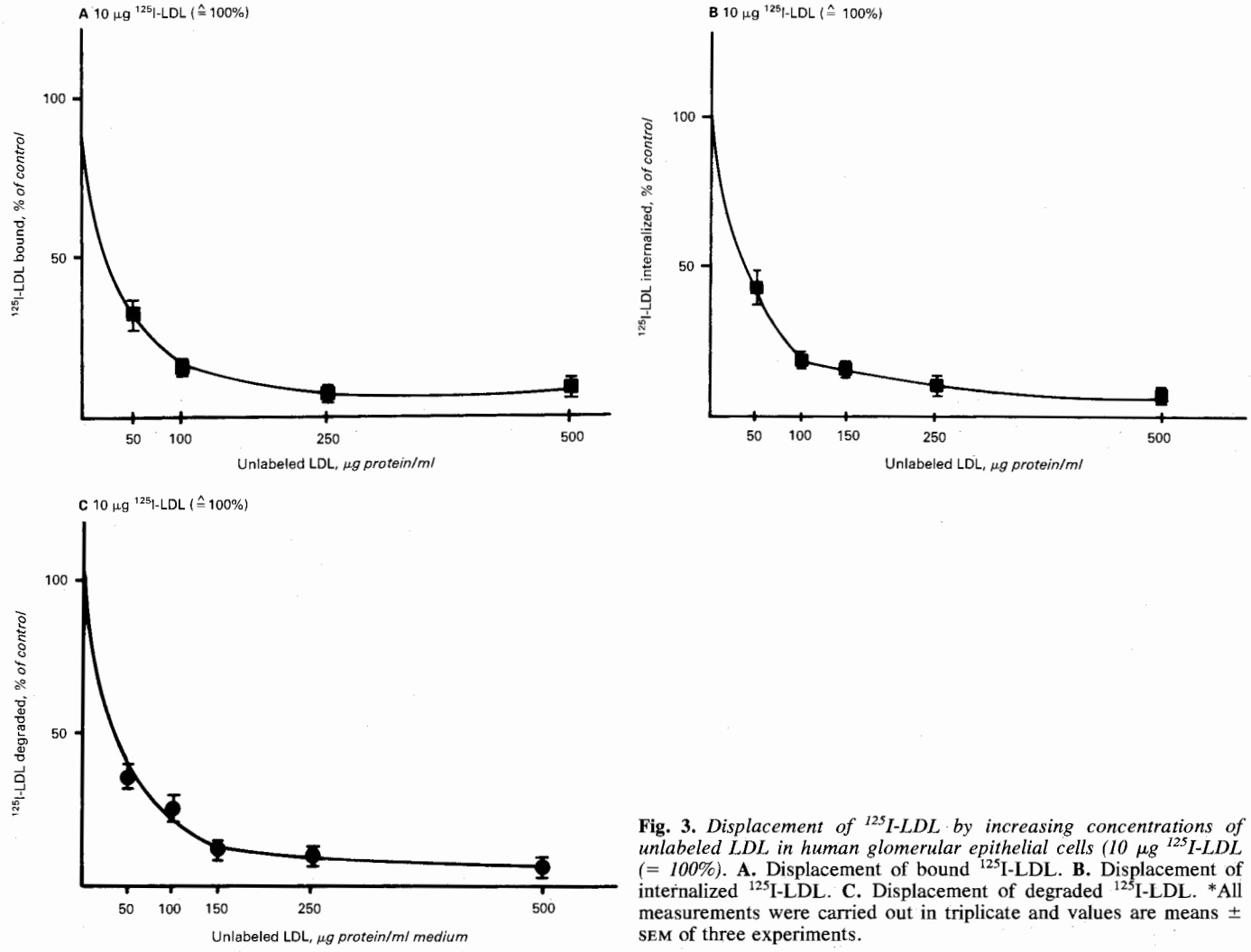

Fig. 3. *Displacement of ^{125}I-LDL by increasing concentrations of unlabeled LDL in human glomerular epithelial cells (10 μg ^{125}I-LDL (= 100%).* **A.** Displacement of bound ^{125}I-LDL. **B.** Displacement of internalized ^{125}I-LDL. **C.** Displacement of degraded ^{125}I-LDL. *All measurements were carried out in triplicate and values are means ± SEM of three experiments.

Table 1. K_m and maximal capacities for uptake of various lipoproteins in glomerular epithelial cells

Lipoprotein	K_m *μg protein/ml medium*	Maximal capacities *ng/mg protein per 5 hr*
LDL		
Surface bound	17.1 (21.7)	29.9 (471)
Internalized	21.7 (21.7)	209.2 (2306)
Degraded	14.2 (20.3)	971.6 (9331)
Chylomicron remnants		
Cell associated	2.8 (12)	642.3 (6020)
Degraded	4.9 (8.8)	67.1 (536)
β-VLDL		
Cell associated	7.3 (6.6)	798.4 (1845)
Degraded	2.9 (5.6)	376.4 (2238)
VLDL-LCAT		
Cell associated	6.5 (11.2)	98.9 (1145)
Degraded	4.5 (13.4)	362.1 (4209)

The data for Scatchard plots are derived from Figures 2, 4, 5 and 6. Values in brackets denote the data from cultures of human fibroblasts.

in DME medium containing LDS, the medium was removed and replaced with fresh medium containing test lipoproteins and incubated for six hours. After this incubation fresh 2 ml of DME and LDS medium containing 1 μCi of 9, 10 (n)-[^{3}H] oleic acid bound to fatty acid free albumin in a molar ratio of 1:4 was added to monolayer cultures (specific activity 20 μCi/μmol and final [^{3}H] oleate concentration of 50 nmol/ml). After two hours incubation at 37°C [18–20], cultured cells were then washed as before, scraped into plastic tubes with a rubber policeman and pelleted by centrifugation. Water (0.4 ml) was added and pellets homogenized by ultrasonification. An aliquot (0.2 ml) of homogenate was extracted with 25 volumes of chloroform and methanol mixture (2:1), and the chloroform phase was washed with 0.034% $MgCl_2$ (0.2 ml/ml of chloroform methanol extract). The washed chloroform phase was dried under vacuum and resuspended in 200 μl of chloroform and methanol mixture (2:1). One hundred microliters of the chloroform and methanol solution was chromatographed on silica gel thin layer plates using a solvent system hexane:diethylether:acetic acid (83:16:1, vol/vol/vol). Bands comigrating with cholesteryl oleate standard were scraped and [^{14}C] oleate incorporated in cholesteryl ester quantitated. The water homogenate of cultures was used for measurement of cellular protein as well as direct measurement of [^{3}H] activity. Cholesteryl oleate formed was expressed as picomole cholesteryl oleate formed per mg cell protein.

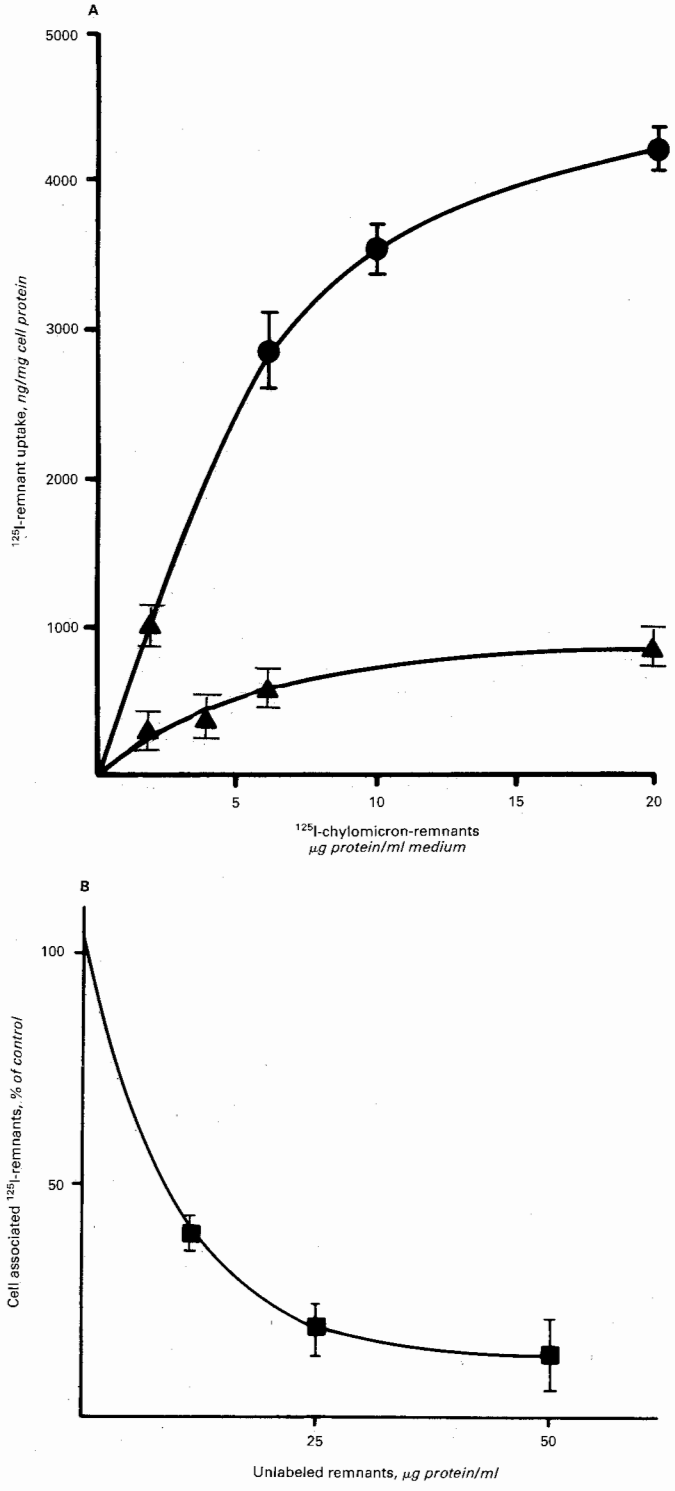

Fig. 4A. *Concentration dependent uptake of ^{125}I-chylomicron remnant uptake by monolayer cultures of human skin fibroblasts (●) and human glomerular epithelial cells (▲).* As a measurement of uptake at 37°C, ^{125}I-cell associated activity was determined. All measurements were carried out in triplicate and values are means ± SEM of three experiments. **B.** Displacement of ^{125}I-chylomicron remnants (2.5 μg) by increasing concentrations of unlabeled chylomicron remnants in cultures of human glomerular epithelial cells. As a measure of uptake at 37°C, ^{125}I-cell associated activity was determined. All measurements were carried out in triplicate and are average of two experiments.

Analytical techniques

The protein content of cell extracts and lipoproteins was measured by the method of Lowry et al [21]. The concentration of unesterified cholesterol, cholesteryl ester, phospholipids and triglycerides was determined by standard enzyme kit methods [22]. Lipoproteins were identified by lipoprotein electrophoresis (Lipidophor Technique, Immuno A.G., Vienna, Austria) and LP-X by agar gel electrophoresis (Rapidophor Technique, Immuno A.G.) and also by chemical and immunochemical analyses [23]. The apolipoprotein composition of various lipoprotein fractions was determined by SDS-PAGE on 4 to 30% polyacrylamide slab gels after lipoproteins were delipidated with ethanol-diethyl ether (3:1) [24]. For ultrastructural examination culture cells were fixed in 2% glutaraldehyde for two hours, embedded in araldite and cut parallel to the bottom of cultured dishes. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM 10 electron microscope.

Results

Characterization of cultured glomerular cells

Primary cultures and first subcultures of cells outgrowing of human glomeruli were characterized as glomerular epithelial cells by light and electron microscopy and immunological reactions with antibodies to intermediate filaments. Glomerular cells appeared as attached polygonal epithelial cells (Fig. 1A). These cells showed a positive reaction for keratin (Fig. 1C) and vimentin (Fig. 1D). Electron microscopic examination of cells growing out of glomeruli revealed rudimentary foot process-like projections which were in direct contact with visceral epithelial cells (Fig. 1B).

Uptake of ^{125}I-LDL by kidney epithelial cells

To establish whether kidney epithelial cells take up LDL by receptor mediated pathway, cultures of glomeruli were incubated for 48 hours in a medium containing LDS to upregulate the LDL receptor activity. After this incubation varying concentrations of ^{125}I-LDL were added to cultures. Similar experiments were also carried out on cultured fibroblasts for comparative purposes. ^{125}I-LDL was bound, internalized and degraded by cultures of glomerular epithelial cells in a concentration dependent manner and the process was saturable (Fig. 2). The specificity of ^{125}I-LDL uptake was over 75%. Analysis of the data according to the method of Scatchard showed the following constants: The K_m for binding, internalization and degradation was 17.1 μg/ml, 21.7 μg/ml and 14.2 μg/ml, respectively. The maximal capacities were 29.9 ng/mg cell protein, 209.2 ng/mg cell protein and 971.6 ng/mg cell protein for binding, internalization and degradation, respectively (Table 1). These values for K_m compare favorably with the values obtained for binding, internalization and degradation in cultured fibroblasts. However, the values for maximal capacities are about 9% of that observed in cultures of skin fibroblasts. These data show that glomerular epithelial cells specifically take up LDL but with low capacity when compared to cultured fibroblasts. The specificity of uptake of ^{125}I-LDL was confirmed by displacement experiments. When glomerular epithelial cells were incubated with ^{125}I-LDL (10 μg/ml) in the presence of

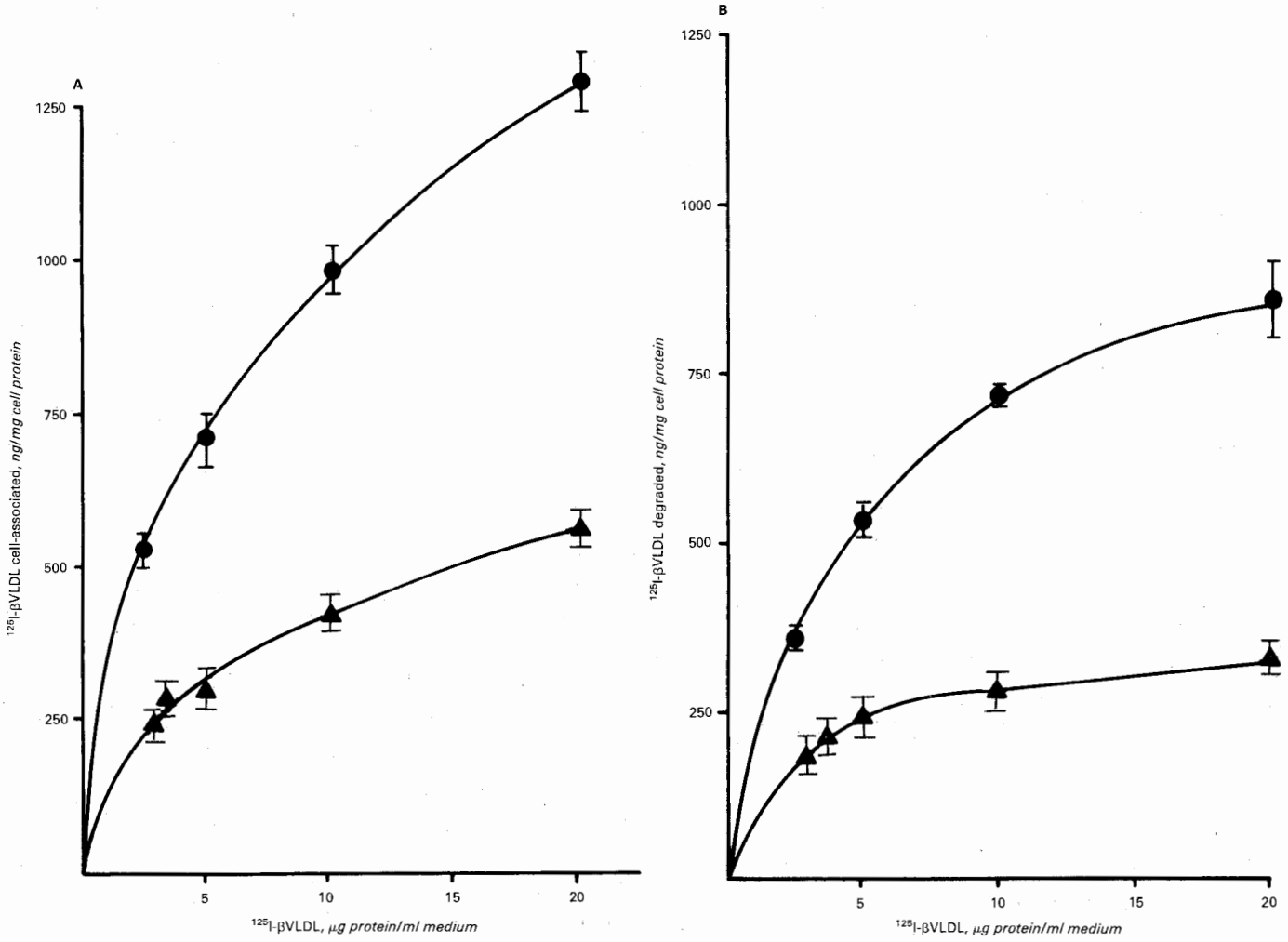

Fig. 5. *Concentration dependent uptake of ^{125}I β-VLDL from cholesterol-fed rabbits by monolayer cultures of human skin fibroblasts (●) and human glomerular epithelial cells (▲).* **A.** Receptor specific cell associated activity at 37°C. **B.** Specific degradation at 37°C. All measurements were carried out in triplicate and are average of two experiments.

unlabeled LDL, 10-fold excess of unlabeled LDL suppressed the degradation of ^{125}I-LDL by about 76% (Fig. 3).

Uptake of chylomicron remnants

Apo E containing lipoproteins show high affinity towards the classical apo B, E receptors in cultured fibroblasts. In order to examine whether the glomerular epithelial cells also show such a high affinity towards apo E rich lipoproteins, chylomicron remnants obtained from postprandial human chylomicrons were used. The chemical composition of these remnants was 63% triglycerides, 14% cholesterol (of which 3% was unesterified), 16% phospholipids and 7% protein. Analysis by electrophoresis (SDS-PAGE) on polyacrylamide gel in the presence of sodium dodecyl sulphate showed the presence of apo B-48, apo B-100 and apo E. The ^{125}I-labeled remnants were taken up in a concentration dependent and saturable manner by glomerular epithelial cells. As a measure of uptake, the cell associated NaOH soluble activity was measured (Fig. 4A). The analysis of data by Scatchard plot yielded the following constants: K_m values for cell associated activity were 2.8 μg/ml for glomerular epithelial cells and 12 μg/ml for cultures of fibroblasts. Thus glomerular epithelial cells and cultured skin fibroblasts show qualitatively similar if not identical high affinity for chylomicron remnant uptake. However, the maximal capacities for uptake differ. Thus maximal capacity for cell associated activity in glomerular epithelial cells was only about 12% of the value obtained in cultured fibroblasts (Table 1). The specificity of chylomicron remnant uptake by glomerular cells was confirmed by displacement experiments. A 12-fold excess of unlabeled chylomicron remnants inhibited the ^{125}I chylomicron remnant uptake by about 80% (Fig. 4B).

Uptake of β-VLDL from cholesterol fed rabbits and VLDL from a patient with familial LCAT deficiency

The plasma of rabbits, which are fed high cholesterol diet, accumulate cholesteryl ester rich VLDL particles which show β-mobility instead of pre-β-mobility in agarose gel electrophoresis. These lipoproteins are rich in apo E and are recognized by LDL receptor in cultured fibroblasts and are taken up avidly

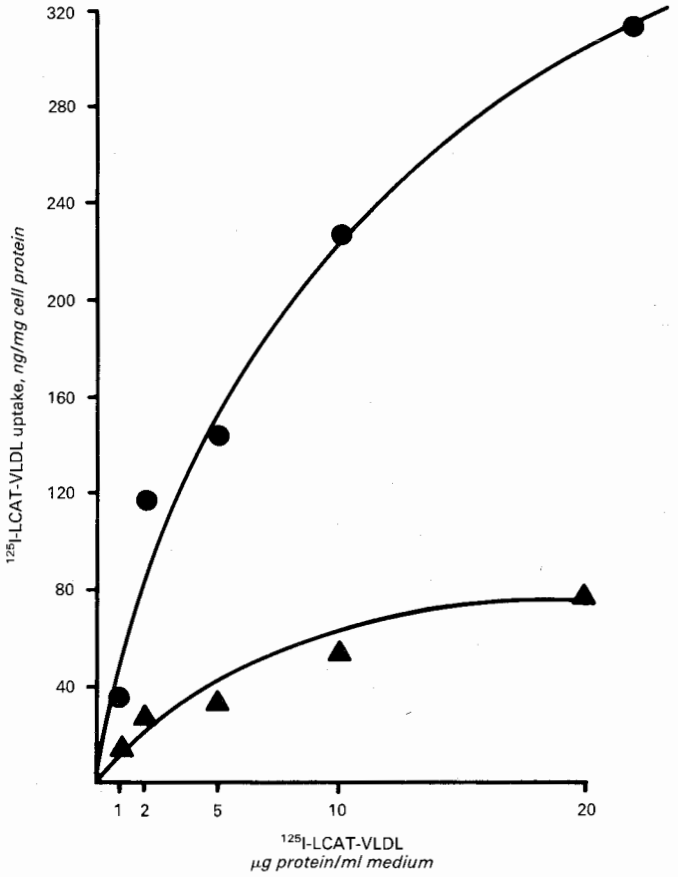

Fig. 6. *Concentration dependent uptake of ^{125}I-VLDL from a patient with familial LCAT-deficiency by cultures of human glomerular epithelial cells.* As a measure of uptake receptor specific degradation (●) and cell associated (▲) activity at 37°C were determined. All measurements were carried out in triplicate.

Table 2. Ability of excess of unlabeled LDL to inhibit uptake of various ^{125}I-labeledlipoproteins in cultures of glomerular epithelial cells

^{125}I-lipoprotein *5 μg protein/ml*	Uptake in the absence of unlabeled LDL *ng/ml cell protein/5 hr*	% Inhibition of uptake in the presence of excess of unlabeled LDL
LDL	213 ± 24	89 ± 1
Chylomicron remnants (cell associated)	460 ± 21	21 ± 5
β-VLDL	270 ± 19	22 ± 5
VLDL-LCAT	150 ± 20	38 ± 14

After 48 hr in medium containing LDS, the monolayer cultures were incubated in fresh medium containing ^{125}I lipoproteins in the presence and absence of a 25-fold excess of unlabeled LDL. As measure of uptake, ^{125}I lipoprotein degradation was measured, except for chylomicron remnants where only cell associated activity was measured. The incubations were carried out in triplicate, and the results are given as mean ± SEM of three experiments.

Table 3. Down regulation of lipoprotein receptor activity by apo B and apo B, E-containing lipoproteins in cultures of glomerular epithelial cells

Unlabeled lipoprotein	Uptake of ^{125}I lipoproteins *ng/mg cell protein/5 hr*	
	^{125}I LDL	^{125}I chylomicron remnants
None	349.5 ± 20.5 (0%)	603.3 ± 36.7 (0%)
LDL	174.9 ± 19.5 (50 ± 5%)	404.3 ± 23.9 (30 ± 4%)
Chylomicron remnants	32.1 ± 8.6 (91 ± 2%)	239.7 ± 6.8 (60 ± 1%)
β-VLDL	92.0 ± 7.1 (74 ± 2%)	—

Monolayer cultures of glomerular epithelial cells were incubated for 48 hours in medium containing LDS to upregulate the LDL receptor activity. One milliliter of fresh medium containing LDS and unlabeled lipoproteins (10 μg protein/ml) was added to the cultures. After 24 hours incubation 10 μg of ^{125}I LDL or chylomicron remnants were added. Degradation of ^{125}I LDL and cell associated activity of ^{125}I chylomicron remnants served as measure of uptake. All incubations were carried out in triplicate and results are mean ± SEM of 3 experiments. Numbers in brackets indicate % inhibition.

because of their apo E content. Therefore we studied the uptake of ^{125}I β-VLDL to determine if the affinity of their uptake was similar to that observed with LDL or chylomicron remnants. The chemical composition of β-VLDL from cholesterol fed rabbits was 65% cholesterol (of which 16% was unesterified), 6.7% triglycerides, 20.3% phospholipids and 8% protein. SDS-PAGE showed the presence of apo B-48, apo B-100 and apo E as major apolipoproteins.

Like LDL and chylomicron remnants, these particles were taken up by specific, concentration dependent and saturable and receptor dependent pathway (Fig. 5). The analysis of data by Scatchard plot yielded K_m values for cell associated activity of about 7.3 μg/ml and 6.6 μg/ml for glomerular epithelial cells and cultured fibroblasts, whereas K_m values for degradation were 2.9 μg/ml and 5.6 μg/ml, respectively. However, the maximal capacities were lower (43% for cell associated activity and 19% for degradation) in glomerular epithelial cells when compared to cultured fibroblasts.

The VLDL from a patient with familial LCAT deficiency is an abnormal lipoprotein with regard to its chemical composition and apolipoprotein distribution, and therefore was also tested for its uptake. The chemical composition of this abnormal lipoprotein was 13% cholesterol (of which 2.4% was esterified), 60% triglycerides, 19.2% phospholipids and 7.7% protein. This VLDL had apo B-48, apo B-100, apo E as its major lipoproteins and traces of apo A-I. It was taken up by glomerular epithelial cells in a specific concentration dependent and saturable manner (Fig. 6). The Scatchard plot analysis of the data showed that, like other apo E-containing lipoproteins, LCAT-VLDL showed high affinity for glomerular cells.

The data with apo E-containing lipoproteins demonstrated that glomerular epithelial cells express a higher affinity towards these lipoproteins than LDL. Table 1 summarizes these data obtained from Scatchard plots. This is further substantiated by the data from displacement experiments of labeled LDL, β-VLDL, LCAT-VLDL and chylomicron remnants in the presence of 25-fold excess of unlabeled LDL. Excess of unlabeled LDL displaced ^{125}I-LDL by 89%, whereas labeled apo E-containing lipoproteins were displaced poorly (Table 2).

Effect of prolonged exposure to unlabeled apo B and apo B,E-containing lipoproteins on receptor activity

Next we examined the ability of unlabeled LDL chylomicron remnants and β-VLDL to down regulate the lipoprotein recep-

Table 4. Effects of various lipoproteins on suppression of sterol synthesis and formation of cholesteryl esters in cultures of glomerular epithelial cells

Lipoprotein	Sterol synthesis	Cholesteryl ester formation
LDS	1.642 ± 0.261 (100%)	0.243 ± 0.080
LDL	0.618 ± 0.188 (37.6%)	2.981 ± 0.250
β-VLDL	0.651 ± 0.262 (39.5%)	8.408 ± 0.51
HDL	1.706 ± 0.134 (103.9%)	0.298 ± 0.03

Monolayer cultures of glomerular epithelial cells were incubated in medium containing LDS for 48 hr. After this preincubation, 1 or 2 ml of fresh medium that contained lipoproteins (20 μg protein/ml) were added to the cultures. Values of sterol synthesis are given as nmol ^{14}C incorporation in sterols/mg cell protein/2 hr. Cholesterol esterification is given as nmol ^{3}H-oleate formed/mg cell protein/2 hr. All incubations were carried out in triplicate, and results are given as mean ± SD of three experiments.

tor activity in cultured human glomerular epithelial cells. After 48 hours in lipoprotein deficient serum (LDS) to upregulate their LDL receptor activity, cultures were incubated for 24 hours with 10 μg protein/ml of unlabeled lipoproteins. After washing the monolayer cultures, 15 μg of ^{125}I LDL or ^{125}I chylomicron remnants were added and incubations carried out for five hours. Data in Table 3 show unlabeled chylomicron remnants and β-VLDL inhibited the uptake of ^{125}I LDL by 91 and 74%, respectively. This inhibition was much stronger than by LDL. Using ^{125}I chylomicron remnants as ligand for the receptor showed that unlabeled LDL decreased the uptake of chylomicron remnants by only 30%. Thus apo B,E-containing lipoproteins were very effective in down regulating the apo B,E receptor activity in glomerular epithelial cells.

Effect of various lipoproteins on cellular sterol synthesis and cholesterol esterification in glomerular epithelial cells

Incorporation of [^{14}C] acetate into sterols is a measure of cholesterol synthesis in a variety of cultured cells. To determine whether lipoproteins that were taken up by glomerular cells could regulate cholesterol synthesis, we incubated cultures of glomerular epithelial cells in medium containing LDS to upregulate their lipoprotein receptor activity. After 48 hours cells were incubated with LDL, β-VLDL and normal HDL for five hours (Table 4). In the absence of lipoproteins, high sterol synthesis rates were noted. The presence of either LDL or β-VLDL at a lipoprotein concentration of 20 μg protein/ml inhibited sterol synthesis by about 60%. HDL from normal plasma was ineffective in suppressing cholesterol synthesis in cultured glomerular epithelial cells.

Measurement of [^{3}H] oleate incorporation into cholesteryl ester after incubation with various lipoproteins is a measure of activation of acyl:cholesterol-acyltransferase (ACAT). It also serves as a measure of effective cellular uptake of lipoproteins. Glomerular epithelial cells were therefore incubated with various lipoproteins and [^{3}H] cholesteryl oleate formation was measured. Both LDL and β-VLDL enhanced cholesteryl ester formation whereas HDL was ineffective. The higher values of cholesteryl ester formation with β-VLDL are due to the higher cholesterol/protein ratio in β-VLDL as compared to LDL.

Discussion

Pathogenetic factors involved in glomerular sclerosis which lead to progression of renal disease are still insufficiently elucidated. Among various factors such as high protein diet, impaired coagulative processes, increased glomerular intracapillary pressure, the plasma lipoprotein pattern and ultimately the deposition of lipids in glomeruli, have been suggested to contribute to sclerosis of glomerular capillary tufts [1, 2, 25–27]. Several observations in experimental animals and microscopic examination of human renal biopsies lend support to the concept of involvement of lipids in glomerular sclerosis [27]. Foam cells and lipid deposits have been observed in renal tissue in rats with puromycin aminonucleoside nephrosis. Histochemical analyses show that cholesterol and phospholipids constitute the bulk of these lipid deposits [28]. Also in human glomerular disease renal lipoidosis can be observed [29]. Patients with nephrotic syndrome can accumulate lipids in glomeruli and the renal interstitium [3]. In the plasma of these patients VLDL and chylomicron remnants accumulate. Further, these lipoproteins show an abnormal lipid-protein composition [30]. Whether normal as well as abnormal lipoproteins are taken up by glomerular cells by a receptor mediated or receptor independent pathway remained still unclear.

We therefore investigated the uptake of apo B-100 and apo E-rich lipoproteins in visceral epithelial cells cultured from human glomeruli. These cells synthesize basement membrane components and are involved in the genesis of sclerotic foci; they have characteristic features that may be relevant in disease states but not under normal conditions [31, 32]. This cell type has been shown to accumulate lipids in glomerular disease. In healthy kidneys visceral epithelial cells rest on the semipermeable glomerular basement membrane and are surrounded by plasma ultrafiltrate devoid of lipoproteins. In glomerular proteinuric states, high molecular weight proteins as well as lipoproteins are filtered and can thus directly interact with visceral epithelial cells.

In the present study, visceral epithelial cells showed receptor mediated binding, internalization, and degradation of ^{125}I-LDL. This uptake was specific and could be displaced by increasing concentrations of unlabeled LDL. Analyses of the data by Scatchard plot revealed that maximum capacity for uptake (binding, internalization and degradation) was only about 10% of that noted in cultured fibroblasts. However, the K_m values for uptake were comparable in cultures of epithelial cells and fibroblasts. Thus, glomerular epithelial cells take up LDL via a receptor mediated pathway.

In various renal diseases apo E containing triglyceride-rich lipoproteins, such as VLDL, β-VLDL and chylomicron remnant particles, accumulate in the plasma of these patients [30]. Apo E has been reported to be a ligand for their cellular uptake [33]. We next examined the uptake of chylomicron remnants by glomerular epithelial cells. These remnant particles show high affinity binding in hepatic as well as peripheral cells [33, 34]. Cultured glomerular epithelial cells expressed high affinity but low maximal capacity for uptake of chylomicron remnants. As in the case of LDL, the K_m values for uptake were similar to those obtained in cultured fibroblasts. Cholesteryl ester-rich VLDL (β-VLDL) which circulate in the plasma of cholesterol-fed rabbits are also rich in apo E and utilize apo E as a ligand for

their binding. Like chylomicron remnants, these lipoprotein particles were also taken up with high affinity by cultured glomerular epithelial cells.

One of the lipoprotein metabolic disorders which leads to deposition of lipids in renal tissue is the familial LCAT deficiency [4, 5]. The plasma lipoprotein pattern and as well as lipid-protein composition of plasma lipoproteins is abnormal in these patients. Most of these abnormal lipoproteins are thought to originate from surface components of chylomicrons and are apo E rich [34]. We next examined the uptake of VLDL from LCAT deficiency patients. VLDL obtained from plasma of normal fasting donors is a poor ligand for apo B,E receptors in cultured fibroblasts. However, VLDL with abnormal lipid composition from a LCAT deficiency patient was taken up by receptor mediated pathway.

The uptake of lipoproteins by receptor mediated pathway leads to suppression of intracellular sterol synthesis and enhanced cholesteryl ester formation [19]. Glomerular epithelial cells in cultures showed both the suppression of [^{14}C] incorporation into sterols and increased formation of cholesteryl esters. Thus, the data obtained on the uptake of radioiodinated lipoproteins and the metabolic effects of cholesterol delivered by lipoproteins to these cells suggest that glomerular epithelial cells are capable of receptor-mediated lipoprotein uptake.

The high affinity for the uptake of apo E containing lipoproteins by glomerular epithelial cells may have clinical significance, since in kidney diseases accumulation of these lipoproteins may be pronounced. In nephrosis in man, apo E-rich VLDL and their remnants show increased content of cholesterol and triglycerides. These lipoproteins could be taken up by a receptor mediated pathway into glomerular visceral epithelia. In glomerular proteinuric states the avid uptake of apo E-containing lipoproteins could then lead to accumulation of lipids in visceral epithelia. Intracellular lipid deposition may further be pronounced by a significant reduction in plasma LCAT activity in nephrosis [35]. In fact, a recent case report shows that apo B- and apo E-containing lipoproteins with β-electrophoretic mobility accumulate in glomeruli and may thus contribute to sclerosis in nephrotic syndrome [36]. Although the present study does not provide conclusive data on the role of plasma lipoproteins in intracellular lipid accumulation in glomeruli, it attempts to conceptualize the mechanisms of lipid deposition in visceral epithelium, one of the three major glomerular cell types. Similar mechanisms of receptor mediated lipoprotein uptake may apply to endothelium and to mesangial cells. Recent preliminary reports on rat and human mesangial cells support this concept [9, 10].

In conclusion, our data show that glomerular visceral epithelial cells in cultures can take up normal lipoproteins as well as apo E-rich abnormal lipoproteins via a receptor mediated and not by a scavanger, that is, receptor independent, non-saturable pathway. The affinity for the uptake of apo E-containing lipoproteins is high and comparable to data of these lipoproteins in cultured fibroblasts. Apo E-rich lipoproteins accumulate in plasma in nephrotic syndrome, which results in the exposure of visceral epithelial cells to relatively high concentrations of these lipoproteins. Avid uptake of apo E-containing lipoproteins could contribute to glomerular lipid accumulation and sclerosis.

Acknowledgments

This work is dedicated to Professor Dr. Fritz Scheler, Department of Internal Medicine, University Hospital, Göttingen, on his 65th birthday. This work was supported by a grant from the Deutsche Forschungsgemeinschaft SFB 330, C7, to Dr. H.-J. Gröne. Mouse monoclonal vimentin antibody (V9) and mouse monoclonal antibody against keratin intermediate filaments (LU 5) used in this study were a gift from Dr. M. Osborn, Max Planck Institute of Biophysical Chemistry, Göttingen, FRG. We appreciate the help of H. Bartels, M.D., Head of the Department of Urology, Weender Hospital, Göttingen, FRG, in obtaining unfixed kidneys excised because of renal carcinoma; M. Osborn, Ph.D., Max Planck Institute of Biophysical Chemistry, 3400 Göttingen, FRG, who provided the monoclonal antibodies to intermediate filaments. We thank Ms. U. Kneissler for help in preparing the transmission electron micrographs, and Ms. A. Henniger and Ms. C. Wise for technical assistance.

Reprint requests to Hermann-Josef Gröne, M.D., Department of Pathology University Hospital, Robert-Koch-Str. 40, 3400 Göttingen, Federal Republic of Germany.

References

1. Olson JL, Heptinstall RH: Nonimmunologic mechanisms of glomerular injury. *Lab Invest* 59:564–578, 1988
2. Diamond JR, Karnovsky UJ: Focal and segmental glomerulosclerosis: Analogies to atherosclerosis. *Kidney Int* 33:917–924, 1988
3. Alexander RW: Focal glomerular sclerosis, in *Nephrotic Syndrome*, edited by Brenner BM, Stein JH, New York, Churchill Livingstone, 1982, pp 175–197
4. Norum KR: Familial lecithin cholesterol acyltransferase deficiency, in *Clinical and Metabolic Aspects of High-Density Lipoproteins*, edited by Miller NE, Miller GJ, Amsterdam Elsevier Publishers, 1984, 297–324
5. Hovig T, Gjone E: Familial plasma lecithin:cholesterol acyltransferase (LCAT) deficiency. *Acta Path Microbiol Scand* (Section A) 81:681–697, 1973
6. Norum KR, Gjone E: Lecithin:cholesterol acyltransferase. Recent research on biochemistry and physiology of the enzyme. *Scand J Clin Lab Invest* 33:191–197, 1974
7. Brown MS, Goldstein JL: Lipoprotein metabolism in the macrophage: Implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem* 52:223–261, 1983
8. Kashgarian M, Sterzel RB: Mesangial function in normal and diseased animals, in *Nephrology Proceedings of the Xth International Congress of Nephrology*, edited by Robinson RR, New York, Springer-Verlag, 1988, 552–557
9. Wassermann J, Santiago A, Holthofer H, Epstein M, Schlondorff D: Interactions of low density lipoprotein with rat mesangial cells. (abstract) *Kidney Int* 35:439, 1989
10. Wheeler DC, Persand J, Kingstone D, Sweny P, Warghese Z, Moorhead JF: Receptor mediated binding of human low density lipoprotein (LDL) to rat mesangial cells in vitro. (abstract) *Kidney Int* 35:439, 1989
11. Olivetti G, Anversa P, Melissari M, Loud AV: Morphometry of the renal corpuscle during postnatal growth and compensatory hypertrophy. *Kidney Int* 17:438–454, 1980
12. Walli AK, Seidel D: Role of lipoprotein-X in the pathogenesis of cholestatic hypercholesterolemia. *J Clin Invest* 74:867–879, 1984
13. Havel RJ, Eder HA, Bragdon HJ: The distribution and clinical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 34:1345–1353, 1955
14. Weber P, Owen JS, Desai K, Clemens MR: Hereditary lecithin-cholesterol acyltransferase deficiency. *Am J Clin Pathol* 88:510–516, 1987
15. Thiery J, Seidel D: Fish oil feeding results in an enhancement of cholesterol-induced atherosclerosis in rabbits. *Atherosclerosis* 63: 53–56, 1987
16. Bilheimer DW, Eisenberg S, Levy RJ: The metabolism of very low density lipoproteins. I. Preliminary in vitro and in vivo observations. *Biochim Biophys Acta* 260:212–221, 1972

17. ARMSTRONG VW, WALLI AK, SEIDEL D: Isolation, characterisation and uptake in human fibroblasts of an apo(a)-free lipoprotein obtained on reduction of lipoprotein(a). *J Lip Res* 26:1314–1323, 1985
18. GOLDSTEIN JL, BASU JK, BROWN US: Receptor-mediated endocytosis of low density lipoprotein in cultured cells, in *Methods in Enzymology* (Vol 98), edited by FISCHER S, FLEISCHER B, New York, Academic Press Inc., 1983, pp 241–260
19. GOLDSTEIN JL, DANA SE, BRUNSCHEDE GY, BROWN MS: Genetic heterogeneity in familial hypercholesterolemia. Evidence of two different mutations affecting functions of low density lipoprotein receptor. *Proc Natl Acad Sci USA* 72:1092–1096, 1975
20. SPRECHER DL, HVEG JM, SCHAEFER EJ, ZECH LA, GREGG RE, LAHATOS E, BREWER B: The association of LDL-receptor activity, LDL-cholesterol levels and clinical course in homozygous familial hypercholesterolemia. *Metabolism* 34:294–299, 1985
21. LOWRY OHN, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the folin phenol reagent. *J Biol Chem* 193: 265–275, 1951
22. WALLI AK, SEIDEL D: Effects of clofibric acid and bezafibrate administration on activities of alkaline phosphatase and other enzymes in livers of rats. *Res Exp Med* 179:153–161, 1981
23. WIELAND H, SEIDEL D: Quantitative lipoprotein electrophoresis, in *Handbook of Electrophoreses* (Vol III), edited by LEWIS LA, Philadelphia, CRC Press Inc, 1983, pp 83–102
24. WEBER K, OSBORN M: The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J Biol Chem* 244:4406–4412, 1969
25. BRENNER BM, MEYER TW, HOSTETTER TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation and intrinsic renal disease. *N Engl J Med* 307:652, 1982
26. PURKERSON ML, JOIST JH, GREENBERG JU, KAY D, HOFFSTEN PE, KLAHR S: Inhibition of anticoagulant drugs of the progressive hypertension and uremia associated with renal infarction in rats. *Thromb Res* 26:227–240, 1982
27. GRÖNE H-J, WALLI AK, GRÖNE E, NIEDMANN P, THIERY J, SEIDEL D, HELMCHEN U: Induction of glomerular sclerosis. A functional and morphological study in the rat. *Lab Invest* 60: 433–446, 1989
28. GROND J, VAN GOOR H, ERKELENS DW, ELENA JD: Glomerular sclerotic lesions in the rat. Histochemical analysis of their macromolecular and cellular composition. *Virchows Arch* (B) 51:521–534, 1986
29. FARRAGIANA T, CHURG J: Renal lipoidoses: A review. *Human Pathol* 18:661–679, 1987
30. GERARDI E, ROTA E, CALANDRA S, GENOVA R, TAMBORINO A: Relationship among the concentrations of serum lipoproteins and changes in their chemical composition in patients with untreated nephrotic syndrome. *Eur J Clin Invest* 7:563–570, 1977
31. CHURG J, SOBIN LH: *Renal disease: Classification and atlas of glomerular diseases*. Tokyo, Jgahu Shoin, 1982, pp 298–307
32. SCHWARTZ MM, BIDANI AK, LEWIS EJ: Glomerular epithelial cell function and pathology following extreme ablation of renal mass. *Am J Pathol* 126:315–324, 1987
33. MAHLEY RW, INNERARITY TL: Lipoprotein receptors and cholesterol homeostasis. *Biochim Biophys Acta* 737:197–222, 1983
34. SHEVRILL BC, INNERARITY TL, MAHLEY RW: Rapid hepatic clearance of the canine lipoproteins containing only the E apoprotein by a high affinity receptor. Identity with the chylomicron remnant transport process. *J Biol Chem* 255:1804–1807, 1980
35. COHEN JL, CRAMP DG, LEWIS AD, TICKNER TR: The mechanism of hyperlipidaemia in nephrotic syndrome—role of low albumin and the LCAT reaction. *Clin Chim Acta* 104:393–400, 1980
36. WATANABE Y, OZAKI J, YOSHIDA F, FUKATSU A, ITOH Y, MATSUO S, SAKAMOTO N: A case of nephrotic syndrome with glomerular lipoprotein deposition with capillary ballooning and mesangiolysis. *Nephron* 51:265–270, 1989

Published by Springer-Verlag, New York Inc.