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Research Article

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Pathogenic Antibodies Inhibit the Binding of Apolipoproteins to Megalin/gp330 in Passive Heymann Nephritis

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Abstract

Megalin/gp330 is an endocytic receptor that internalizes multiple ligands including apolipoproteins E (apo E) and B100 (apo B). Megalin is the main antigenic target in passive Heymann nephritis (pHN), where it binds circulating autoantibodies leading to the formation of subepithelial immune deposits (ID)—the hallmark of pHN. Apo E and apo B were found recently to accumulate within these IDs, and evidence was provided that their lipids may undergo peroxidation, causing glomerular basement membrane damage and proteinuria. Here we investigated if ID-forming antimegalin IgG can inhibit the binding and internalization of apo E–βVLDL (very low density lipoprotein) by megalin, and lead to their accumulation within IDs. By immunoelectron microscopy, apo E and apo B were detected in clathrin-coated pits and multivesicular bodies of podocytes in control rats, suggesting that the uptake of lipoproteins is a constitutive function of the glomerular epithelium. When pHN was induced by intravenous injection of antimegalin IgG, apo E and apo B were found within IDs by immunofluorescence and immunoelectron microscopy. Bound antibodies eluted from glomeruli of rats with pHN were found to inhibit the binding and internalization of apo E–enriched βVLDL by megalin. These results indicate that pHN-inducing antimegalin IgG is capable of interfering with the uptake of lipoproteins by megalin in vivo during the formation of IDs. (*J. Clin. Invest.* 1997. 100:2303–2309.) Key words: apolipoprotein • megalin gp330 • Heymann nephritis • lipid peroxidation

Introduction

Severe proteinuria in patients with membranous nephropathy causes the nephrotic syndrome with hyperlipidemia, accelerated atherosclerosis, and increased probability of thrombus formation (1). Moreover, high proteinuria is directly related to the development of chronic renal failure (2). Because direct analysis of the molecular pathogenesis of proteinuria is difficult to perform in human patients, we and others study an experimental rat model of human membranous nephropathy called Heymann nephritis (HN).[1] HN is characterized by the formation of subepithelial immune deposits (ID) in the glomerular basement membrane (GBM) and by the development of proteinuria (3).

Considerable progress has been made in the analysis of the pathogenic mechanisms of ID formation in HN (4) and, to a lesser extent, of GBM damage and proteinuria (5). The endogenous antigenic target of HN was identified as a large membrane glycoprotein called megalin (originally named gp330) (6). Megalin is a multiligand receptor of the LDL-receptor gene family (7, 8). Of the various ligands that have been identified for megalin, apolipoproteins B100 (apo B) and E (apo E) (9, 10) are of particular interest for the pathogenesis of passive HN (pHN) since they were localized recently within IDs of proteinuric rats with pHN (11). Moreover, evidence was provided that apo B was modified locally by lipid peroxidation (LPO) (11). LPO is a major cause of GBM damage and proteinuria (12), and apo E–enriched βVLDL may therefore provide polyunsaturated fatty acids to fuel this reaction.

These findings have raised questions about the molecular mechanisms leading to the accumulation of lipoproteins within IDs. In this study, we show that antimegalin antibodies eluted from glomeruli of pHN rats inhibit binding and uptake of apos by megalin. Our results suggest that during the induction of pHN, antimegalin antibodies bind to the receptor on the basal surface of podocytes and inhibit the endocytic clearance of apos from the glomerular filtrate. The inhibition of apo internalization leads to their accumulation within IDs of pHN rats, and may provide the necessary lipid environment for peroxidation and GBM damage.

Methods

Antibodies. Affinity-purified sheep antimegalin IgG was prepared from anti-Fx1A IgG (provided by Dr. W.G. Couser, University of Washington, Seattle, WA) by absorption onto a megalin/CNBr Sepharose 4B column. Rabbit antibodies to purified rat megalin (antimegalin, 070) (13) or to renal tubular microvilli (14) were prepared as described (15). Mouse mAb (20B) was raised against purified rat megalin (16). Mouse monoclonal IgGs specific for rat apo E and apo B were prepared and used as described (11). Briefly, mouse monoclonal IgG specific for rat apo B was raised against rat apo B purified by precipitation from isolated LDL fractions. Monoclonal anti–apo E IgG was produced by immunizing mice with isolated rat HDLs. The antibodies were screened and selected by immunoblotting purified apo B and apo E. FITC-conjugated rabbit anti–sheep $F(ab')_2$ fragments and FITC-conjugated goat anti–mouse IgG were from DAKO-

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1. *Abbreviations used in this paper:* HN, Heymann nephritis; ID, immune deposit; LPO, lipid peroxidation; pHN, passive HN.

PATTS (Copenhagen, Denmark). 10 nm gold-conjugated rabbit anti–sheep and sheep anti–mouse IgG were from Amersham International (Little Chalfont, UK).

Purification of apo E–enriched βVLDL. Blood was collected from cholesterol-fed New Zealand White rabbits and supplemented with Na_2EDTA to a final concentration of 1 mg/ml. Plasma was cleared by centrifugation (500 *g*) for 20 min at 10°C. Plasma was then added to a centrifuge tube (Ultra Clear; Beckman Instruments, Inc., Fullerton, CA), overlaid with an equal volume of 0.9% NaCl, and centrifuged (25,000 *g*) for 20 min at 20°C. The top chylomicron layer was removed and replaced with an equal volume of 0.9% NaCl, and centrifuged (25,000 *g*) for 20 min at 20°C. Residual chylomicron remnants were then removed, and the remaining material was centrifuged (25,000 *g*) for 16 h at 20°C. The βVLDL layer was removed from the top, sterile-filtered, and protein concentration was determined by the BCA protein assay according to the manufacturer's instructions (Pierce Chemical Co., Rockford, IL). The apo E–enriched βVLDL fraction (Fig. 1, lane *B*) consisted of apo E (molecular mass ~ 34 kD) and apo B (molecular mass ~ 515 kD).

Megalin purification and radioiodination. Megalin was purified by mAb affinity chromatography as previously described (17) and radioiodinated using the Iodo-Bead method according to the manufacturer's instructions (Pierce Chemical Co.). Megalin migrated as a single intact band before (Fig. 1, lane *C*) and after (Fig. 1, lane *D*) labeling with ^{125}I.

Induction of pHN. 250-g male Sprague-Dawley rats were injected intravenously with 100 μg affinity-purified sheep antimegalin IgG and killed after 3 or 6 d. Other rats were injected with 10–15 mg rabbit IgG fractions raised against rat microvillar fractions (18), or purified megalin (19). Use of experimental animals was authorized by the Austrian Ministry of Science.

Glomerular eluates. IgG was eluted from isolated glomeruli or from homogenates of renal cortex of rats with pHN with 20 mM citrate buffer, pH 2.8, as described (15). IgG fractions were prepared by protein A or protein G affinity chromatography (Pharmacia Biotech AB, Uppsala, Sweden). The titers of IgGs eluted from glomeruli of pHN rats were determined by indirect immunofluorescence on cryostat sections of rat kidney. Activities were then normalized by dilution to give equal titers. Protein concentrations of eluted IgG were determined by ELISA (see below). The eluted glomerular IgG of 3- or 6-d pHN rats was analyzed by SDS-PAGE under reducing conditions followed by silver staining, and found to contain only IgG light (~ 25 kD) and heavy (~ 55 kD) chains (Fig. 1, lane *A*).

Determination of IgG concentrations by ELISA. Wells of microtiter plates (Corning Glassworks, Corning, NY) were coated by incubating with affinity-purified $F(ab')_2$ fragments of goat anti–rabbit or anti–sheep IgG (Accurate Chemical and Science Corp., Westbury, NY, 5 μg/ml) for 3 h at 20°C. Nonspecific sites were blocked with 2.5% BSA in PBS for 1 h at 20°C. Wells were rinsed with PBS containing 0.05% Tween 20, and incubated with varying concentrations of glomerular eluted IgG for 1 h at 37°C. After rinsing wells three times with PBS containing 0.05% Tween 20, wells were incubated with peroxidase-conjugated affinity-purified goat anti–rabbit or anti–sheep IgG $F(ab')_2$ (Accurate Chemical and Science Corp., 5 μg/ml) for 1 h at 37°C. After washing, bound peroxidase-conjugated antibodies were detected by incubating with DAB (0.04%) in 20 mM citrate buffer, pH 5.0. Color development was stopped by the addition of 8 N sulphuric acid and quantitated using an ELISA plate reader (Dynatech Laboratories, Inc., Chantilly, VA). Glomerular eluted IgG was quantitated by comparing directly to a standard curve generated in parallel using known concentrations of rabbit or sheep IgG.

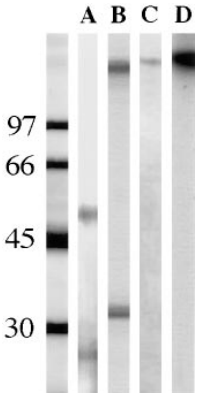

Figure 1. Analysis of reagents used for the solid-phase binding assay. Samples were mixed with Laemmli buffer containing 4% β-mercaptoethanol and separated by SDS-PAGE on 5–15% gradient gels, and proteins were detected by silver staining (lane *A*) or with Coomassie blue (lanes *B* and *C*), or processed for autoradiography (lane *D*). Lane *A*, Affinity-purified eluate prepared from isolated glomeruli of rats injected 3 d before with sheep antimegalin IgG. Only heavy (molecular mass ~ 55 kD) and light (molecular mass ~ 25 kD) chains of IgG are detected. Lane *B*, Rabbit apo E–enriched βVLDL contains only apo E (molecular mass ~ 34 kD) and apo B (molecular mass ~ 515 kD). Lane *C*, Affinity-purified megalin from rat renal microvilli (molecular mass ~ 600 kD). Lane *D*, Same preparation of megalin as in *C*, after radioiodination, demonstrating that there is no degradation of megalin as a result of the radiolabeling procedure.

Immunofluorescence. Kidneys of normal rats and of rats with 3 and 6 d pHN were perfused with MEM for 5 min. The cortex was minced, and small pieces of cortex were snap-frozen in liquid N_2. Cryostat sections (4 μm) were prepared, dipped in −20°C acetone, and air dried. Some sections were incubated directly with FITC-conjugated anti–sheep IgG, and others were incubated with murine mAbs directed against rat apo E or apo B (10 μg/ml) followed by FITC-conjugated goat anti–mouse IgG (11). As controls, primary IgGs were omitted or replaced by irrelevant mAbs. Micrographs were taken on a microscope equipped for epifluorescence (Axiophot; Carl Zeiss, Oberkochen, Germany).

Immunoelectron microscopy. Kidneys of rats injected with antimegalin IgG were flushed with MEM followed by perfusions with freshly prepared 4% formaldehyde in PBS. Fixation was continued for 6 h at 20°C, followed by embedding in Lowicryl K4M (Chemische Werke LOWI, Waldkraiburg, Germany) (20). Ultrathin sections were blocked with 1% ovalbumin in PBS for 1 h, followed by incubation with rabbit antimegalin IgG or with the murine mAbs against rat apo E or apo B (~ 50 μg/ml) and an anti–mouse 10-nm gold conjugate (1:50). Sections were stained with lead citrate (21).

Solid-phase binding assays. Wells of microtiter plates (Falcon Labware, Becton Dickinson, Oxnard, CA) were coated for 16 h at 4°C with 100 μg/ml of apo E–enriched βVLDL in 20 mM Hepes, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$ (buffer A). Wells were rinsed three times with buffer A, and nonspecific sites were blocked by incubation with buffer A containing 3% BSA for 1 h at 23°C. The immobilized apo E–βVLDL was then incubated with ^{125}I-megalin (4×10^5 cpm, 1.5 μg/ml) in buffer A containing 3% BSA for 3 h at 23°C in the presence or absence of the following reagents: apo E–βVLDL (10 μg/ml), sheep antimegalin (10 μg/ml), rabbit antimegalin (30 μg/ml), or glomerular eluates from rats with pHN induced with either sheep (10 μg/ml), or rabbit antimegalin (3 μg/ml), rabbit antimicrovillar IgG from 3-d pHN rats (20 μg/ml), rabbit antimicrovillar IgG glomerular eluate from 6-d pHN rats (20 μg/ml), and monoclonal antimegalin 20B (24 μg/ml). Wells were rinsed three times with buffer A, and bound ^{125}I-megalin was released by the addition of 2 N NaOH and quantitated in a gamma counter (5500B; Beckman Instruments, Inc.). SD and statistical significances of the mean values of replicate points were calculated by the Student's *t* test.

L2 cell internalization and degradation assay. L2 rat yolk sac carcinoma cells, which are known to express megalin (13, 22), were grown to confluency on 1% gelatin-coated cell culture plates in DME supplemented with 10% FCS, 100 μg/ml streptomycin sulfate, and 100 U/ml penicillin G. Cells were rinsed three times with DME containing 0.1% BSA and incubated with ^{125}I-apo E–βVLDL (3×10^6 cpm, 200 ng/ml, specific activity of 24,000 cpm/ng) in the presence of the indicated antibodies for 3 h at 37°C in an incubator adjusted to 95% air/5% CO_2. The medium was then removed, and adjusted to 15% TCA at 4°C. After 1 h, precipitated proteins were sedimented (10,000 *g*, 15 min), and the TCA-soluble counts released into the supernatant were quantitated by gamma counting. TCA-soluble cpm was divided by specific activity (24,000 cpm/ng) to determine amount of ^{125}I-apo E–βVLDL degraded.

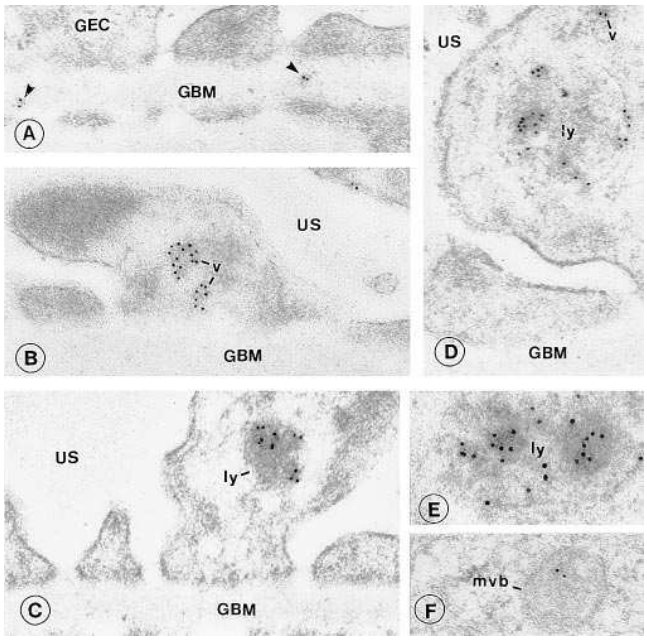

Figure 2. Localization of apo E in the GBM and endocytic system of podocytes from normal rat kidneys by indirect immunogold labeling. Ultrathin sections were prepared from Lowicryl-embedded kidneys and incubated sequentially with an anti–apo E mAb and anti–mouse 10-nm gold conjugate. (*A*) apo E is seen in small electron-dense granules in the GBM that may correspond to lipoprotein particles (*arrowheads*). (*B–D*) Gold particles are found within endocytic vesicles (*v*), lysosomes (*ly*), and multivesicular bodies (*mvb*) of podocytes. *US*, Urinary space. *GEC*, Glomerular epithelial cell. ×18,000.

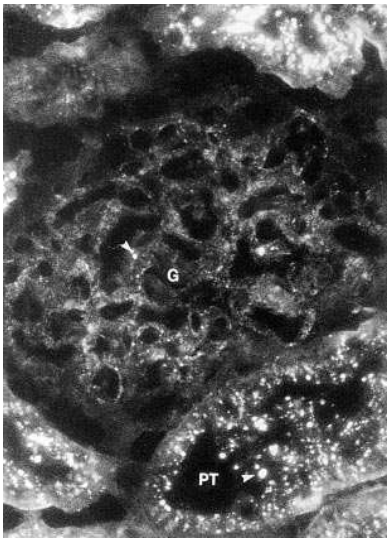

Figure 4. Localization of apo E by indirect immunofluorescence on a cryostat section of a kidney from a rat with pHN induced by injecting affinity-purified sheep antimegalin IgG 3 d before. apo E is detected in a granular pattern in peripheral capillary loops resembling the distribution of IDs. apo E is also found in lysosomes (*arrowheads*) of epithelial cells of the glomerulus (*G*) and proximal tubule (*PT*). ×600.

Results

apo E and apo B are endocytosed by podocytes of normal kidneys. To determine if podocytes normally internalize apo E and apo B, glomeruli from control animals were analyzed for the presence of these proteins by immunoelectron microscopy. Minimal granular staining of glomeruli of normal rats has been observed by indirect immunofluorescence in a previous study (11). By immunoelectron microscopy, apo E was found occasionally within the GBM in association with electron-dense granules (Fig. 2 *A*) and also within endocytic vesicles (Fig. 2 *B*) and lysosomes (Figs. 2, *C–F*) of podocytes. apo B was found in clathrin-coated pits at the base of foot processes (Fig. 3, *A–C*), within intracellular vesicles (Fig. 3, *C* and *D*), and occasionally associated with the endothelial cell surface (Fig. 3 *C*) and the GBM (Fig. 3 *D*). These findings demonstrate that apo E– and apo B–containing lipoproteins or their fragments can cross the GBM and be endocytosed by podocytes, suggesting that uptake of lipoproteins occurs as a regular housekeeping function of the podocyte.

apo E and apo B accumulate within IDs of rats with pHN. To assess the fate of apo E and apo B in pHN, affinity-purified antimegalin IgG was injected into normal rats (23) to induce pHN, and the animals were killed 3 or 6 d after injection. By immunofluorescence, apo E (Fig. 4) and apo B (Fig. 6 *A*) were found in glomeruli of pHN rats in a granular distribution resembling that of IDs. By immunogold labeling, apo E (Fig. 5) and apo B (Fig. 6 *B*) were localized within IDs, lysosomes, and multivesicular bodies of podocytes. As IDs do not show up well due to low contrast in the Lowicryl sections used for immunoelectron microscopy, their presence was confirmed by detecting the antigenic target megalin (Fig. 5, *D* and *E*), which is found consistently in ID of HN (24, 25). No labeling was seen in controls using an irrelevant primary antibody (data not shown). These results provide direct evidence that apo E and apo B accumulate within IDs of rats with pHN induced with monospecific antimegalin IgG.

Antimegalin and eluted glomerular IgGs inhibit the binding of megalin to apo E–enriched βVLDL. The presence of apo E and apo B within IDs of pHN rats induced with antimegalin

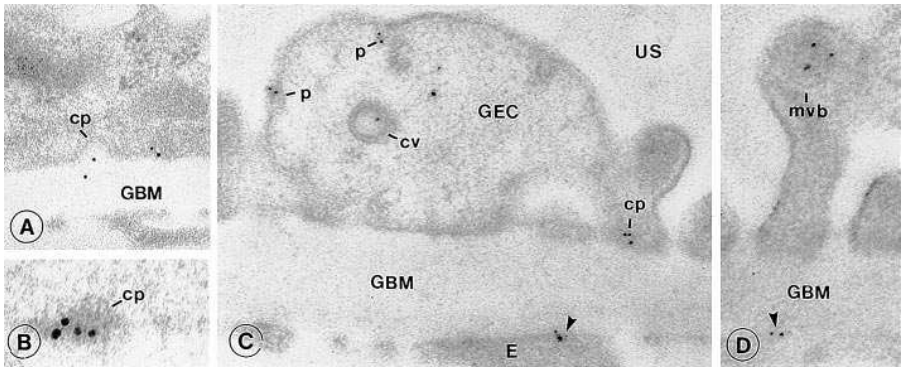

Figure 3. Localization of apo B in endocytic elements of normal rat kidney by indirect immunogold labeling. apo B is seen within the GBM (*A* and *D*), in coated pits on the basal (*cp*) or lateral (*p*) cell membrane, and in multivesicular bodies (*mvb*) of podocytes (*GEC*). *US*, Urinary space. *GEC*, Glomerular epithelial cell. *cv*, Coated vesicle. ×18,000.

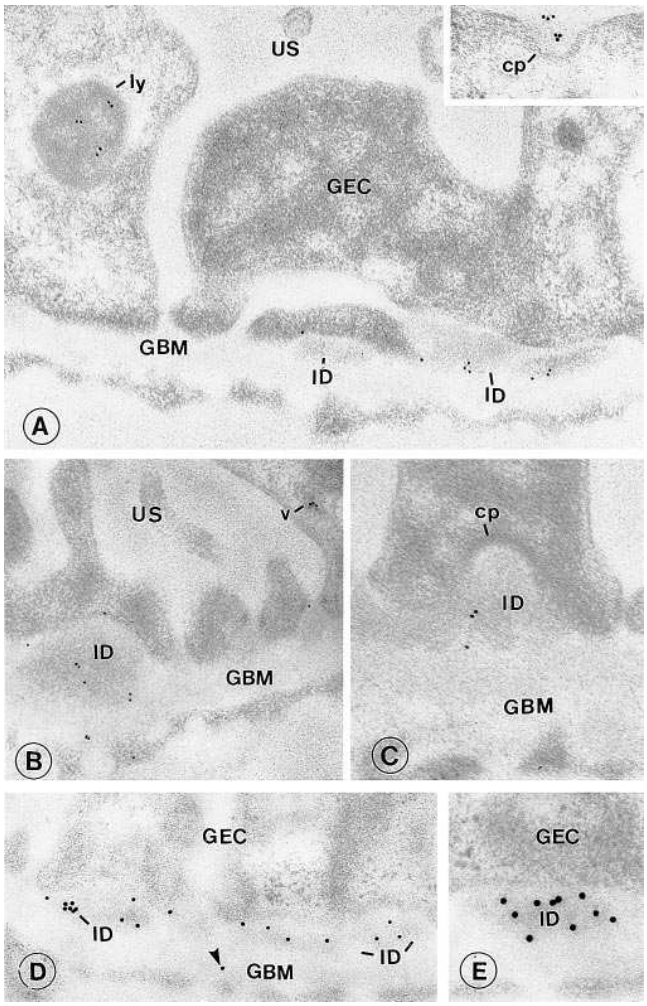

Figure 5. Localization of apo E in IDs in glomeruli of 3-d pHN rats by indirect immunogold labeling. Sections of Lowicryl-embedded kidneys of rats injected with affinity-purified sheep antimegalin IgG were incubated with anti–apo E mAb followed by an anti–mouse 10-nm gold conjugate. (*A–C*) apo E is localized within subepithelial IDs and on the base of the foot processes in association with IDs. It is also found in lysosomes (*ly*) and small vesicles (*v*) within glomerular epithelial cells (*GEC*) and in clathrin-coated pits (*cp*) on the urinary surface (*US*) of glomerular epithelial cells (*A*, *inset*). (*D* and *E*) The presence of subepithelial IDs is verified by immunogold labeling with affinity-purified rabbit antimegalin IgG. *A*, *B*, and *D*, ×18,000; *C*, *E*, and *inset* in *A*, ×30,000.

IgG suggested that the antibodies might inhibit binding and uptake of these ligands. Next, we tested if antimegalin antibodies are able to inhibit directly ^{125}I-megalin binding to immobilized apo E–βVLDL using a solid-phase assay (26). Both the affinity-purified sheep and rabbit antimegalin IgGs inhibited the binding of ^{125}I-megalin to apo E–βVLDL by > 80% (Fig. 7). Moreover, the sheep and rabbit antimegalin IgGs and anti-microvillar IgG eluted from glomeruli of pHN rats also inhibited the binding of ^{125}I-megalin to apo E–βVLDL by 50–75%. By contrast, antimegalin monoclonal 20B (16), which failed to induce IDs, also failed to inhibit this interaction. These data show that all antimegalin IgGs capable of inducing IDs and all preparations of IgG eluted from glomeruli of rats injected with different antimegalin IgGs are capable of inhibiting the binding of apo E–βVLDL to megalin. This suggests that the accumulation of apo E and apo B within IDs could be due to the inhibition of uptake of these ligands by megalin.

Internalization of apo E–βVLDL by megalin is inhibited by eluted glomerular antibodies in vivo. We next tested if the antibodies eluted from glomeruli of rats with pHN are able to inhibit the endocytosis of apo E–βVLDL. To this end, we incubated rat L2 cells, a cell line known to express megalin (13, 22, 27), with radioiodinated apo E–βVLDL in the presence of varying amounts of antimegalin IgG, IgG eluted from glomeruli of pHN rats, or nonimmune IgG at 37°C. We then determined the amount of ^{125}I-apo E–βVLDL degraded, by quantitating the TCA-soluble radioactivity released into the medium after 3 h of incubation. As shown in Fig. 8, cells incubated with nonimmune IgG took up and degraded considerable amounts of apo E–βVLDL, as indicated by release of non–TCA-precipitable radioactivity. By contrast, when cells were incubated with either antimegalin IgG or glomerular eluted antibodies, the degradation of ^{125}I-apo E–βVLDL was inhibited in a concentration-dependent manner. These results demonstrate that antibodies eluted from glomeruli of pHN rats induced with antimegalin IgG are able to inhibit the binding and internalization of apo E–enriched βVLDL by megalin in vivo.

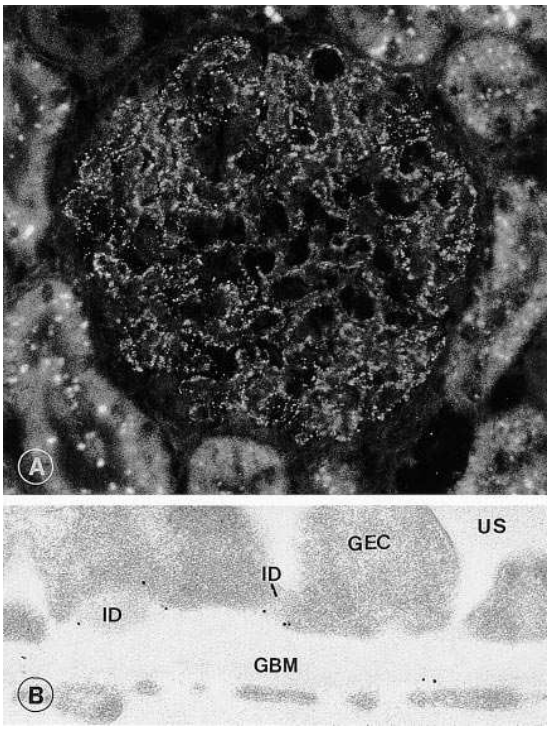

Figure 6. Localization of apo B to ID in kidneys of pHN rats injected with affinity-purified sheep antimegalin IgG. Specimens processed as in Fig. 4 for indirect immunofluorescence (*A*) or as in Fig. 5 for immunogold labeling (*B*). The overall distribution of apo B is similar to that of apo E (see Figs. 4 and 5). (*A*) apo B is concentrated in peripheral capillary loops in a fine granular pattern, similar to the distribution of ID. It is also seen in lysosomes of proximal tubule cells. (*B*) By immunoelectron microscopy, apo B is found in subepithelial IDs. *US*, Urinary space. *GEC*, Glomerular epithelial cell. *A*, ×600; *B*, ×18,000.

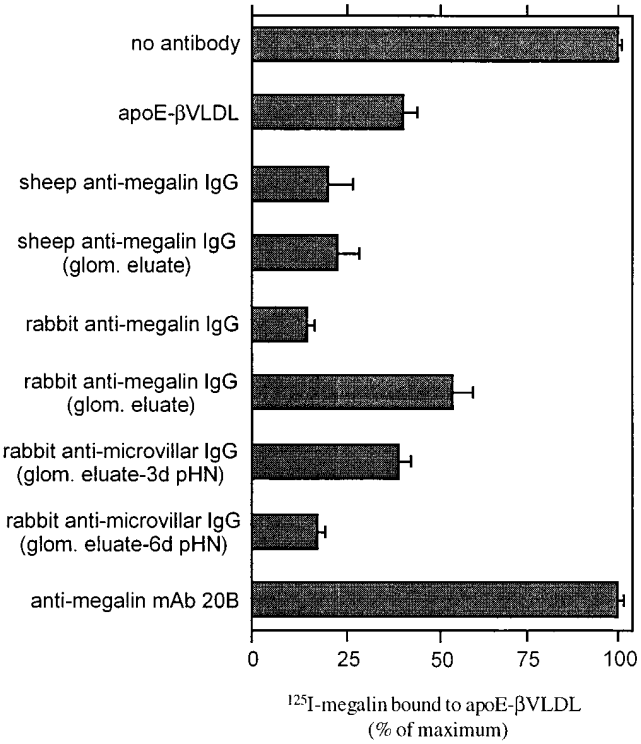

Figure 7. IgG eluted from glomeruli of pHN rats inhibits the binding of ^{125}I-megalin to immobilized apo E–βVLDL. Results are expressed as percentage of the total ^{125}I-megalin bound to apo E–βVLDL in the absence of inhibitors. Sheep and rabbit antimegalin IgG used to induce pHN and the corresponding IgG eluted from isolated glomeruli of pHN rats all effectively inhibited the binding of ^{125}I-megalin to apo E–βVLDL. Both the 3- and 6-d glomerular eluates from the pHN rats induced with rabbit antimicrovillar IgG also inhibited binding. Binding was also inhibited by apo E–βVLDL. The non–ID-inducing mAb 20B failed to block the receptor–ligand interaction. The concentration of each IgG preparation in this assay was adjusted to equal titers as determined by indirect immunofluorescence on cryosections of normal rat kidney. Total IgG concentrations of glomerular eluates (see Methods) were determined by an ELISA. Experiments were performed twice in duplicate. Bars indicate SDs, consistently < 10%.

Discussion

Knowledge of the molecular mechanisms of glomerular capillary wall damage and resulting proteinuria in membranous nephropathy is less advanced than our understanding of the pathogenesis of ID formation (for review, see references 5 and 25). It is established that C5b-9 complement membrane attack complexes form within IDs (28), and that their insertion into cell membranes of podocytes (29) triggers the development of proteinuria. The generation of reactive oxygen species (ROS) within the glomerulus has been identified as a critical C5b-9–dependent step (30). ROS were found to induce local LPO, forming reactive compounds that damage GBM matrix proteins, particularly type IV collagen (12). This sequence of events appears to be a major pathogenic mechanism leading to damage to the GBM in pHN, because proteinuria can be abrogated by treatment with the potent LPO-inhibitor, probucol (12). These findings raised the question as to the source of polyunsaturated fatty acids required for LPO. We hypothesized that lipoproteins originating from the plasma could serve as a source for these lipids (11), similar to their role in atherosclerotic lesions (31), and, in keeping with this idea, we showed that apo B and apo E were localized within IDs of rats with pHN (11). In addition, apo B extracted from isolated glomeruli in pHN showed signs of oxidative damage, such as fragmentation and malondialdehyde adduct formation (11).

Collectively, these recent studies suggest that the accumulation of apo E– and apo B–containing lipoproteins within IDs could provide the necessary environment for LPO and play a critical role in the development of proteinuria in HN. Since megalin is a receptor for apo E (10) and B100 (9), we have investigated if antimegalin IgG, when injected into rats to induce pHN, inhibits ligand binding to the receptor during the formation of IDs, which in turn results in an accumulation of apo E– and apo B–containing lipoproteins and/or their fragments (11). In this study, we provide evidence that podocytes internalize apo E and apo B as a normal housekeeping function, and that the injection of antimegalin IgG results in the deposition of these lipoprotein carriers within IDs. Using a solid-phase binding assay, we showed further that IgG fractions prepared from several independent pHN-inducing sera are capable of inhibiting the binding of apo E–βVLDL to purified megalin. Moreover, the antimegalin IgG eluted from glomeruli of pHN rats also inhibited strongly both ligand binding to megalin and megalin-mediated ligand internalization in a cultured cell line. From these findings, we conclude that antibody binding to megalin during the induction of pHN interferes with the bind-

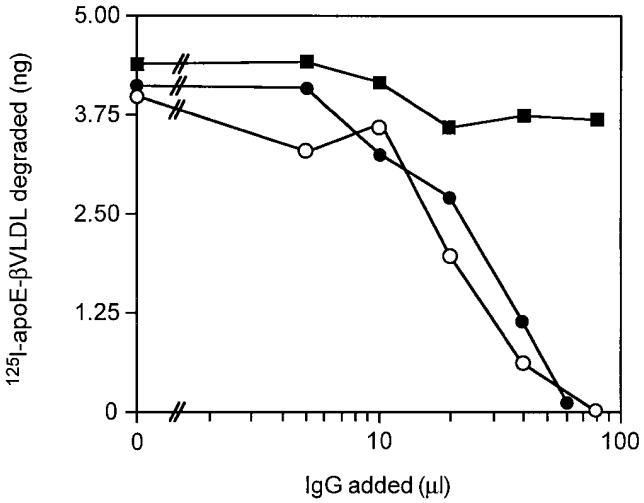

Figure 8. IgG eluted from glomeruli of pHN rats inhibits megalin-mediated internalization and degradation of ^{125}I-apo E–βVLDL. Cultured L2 rat yolk sac cells that express megalin were incubated with ^{125}I-apo E–βVLDL in the presence of varying amounts of either rabbit antimegalin IgG (●, 3 μg/ml), IgG eluted from glomeruli of pHN rats (○, 10 μg/ml) injected with rabbit antimegalin IgG, or nonimmune IgG (■, 3 μg/ml). After 3 h, the culture medium was adjusted to 15% TCA to precipitate undegraded proteins, and the TCA-soluble radioactivity released into the supernatant was measured to quantitate intracellular degradation. Both antimegalin and glomerular eluted IgG inhibited the internalization and degradation of radiolabeled apo E–βVLDL in a concentration-dependent manner. Nonimmune IgG showed no effect on intracellular degradation of ^{125}I-apo E–βVLDL. Experiments were performed twice in duplicate. SD of replicate points was consistently < 5%.

ing and internalization of apo E– and apo B–containing lipoproteins by the receptor on the podocyte cell surface. Therefore, these results provide a logical explanation for the series of events in HN that leads to the accumulation of apos within IDs and the GBM.

These findings are consistent with recent studies by us (14) and others (32) in which a pathogenic epitope has been identified in megalin that binds IgGs eluted from glomeruli of pHN rats, and therefore is directly involved in the development of pHN. Interestingly, this epitope was found to reside in the fifth cysteine-rich domain of the second cluster of ligand-binding motifs (14) and to overlap with a recently identified apo E–βVLDL binding site on megalin (33). Thus, the binding sites for both the eluted glomerular IgGs and apo E–βVLDL are located within this short 46–amino acid domain of megalin.

Before the availability of monospecific antibodies, pHN was induced by injection of polyspecific IgG raised against crude kidney cortical fractions, such as Fx1A (34) or isolated rat microvilli (35). These sera contained IgGs directed not only against megalin, but also against complement C5b-9–activating lipids (23) and several other microvillar constituents. Injection of these mixed IgG into rats resulted in ID formation, capillary damage, and heavy proteinuria, which could account for the penetration of large lipoprotein particles through the GBM and their accumulation within the GBM and IDs. By contrast, in this study, we have induced IDs by injecting affinity-purified antimegalin IgG without inducing capillary damage (23), and have found a similar deposition of apo B and apo E within IDs. Our results demonstrate that binding of ID-forming antimegalin IgG is sufficient for accumulation of lipoproteins within IDs in vivo and does not require capillary damage or C5b-9 activation. However, it remains to be determined how such large lipoprotein particles or their fragments are able to penetrate the GBM without the gross GBM damage that occurs upon activation of the complement.

Are nephritogenic HN antibodies in general capable of interfering with megalin–ligand interactions, and is this property a prerequisite for the stability and growth of IDs and thus for progression of the disease? Accumulation of megalin-immune complexes in the lamina rara externa of the GBM can be explained by inhibition of endocytosis, which occurs normally when ligands bind to megalin. It seems likely that rapid shedding of the immune complexes from the podocyte cell surface and their immobilization within the GBM (24) would make it impossible for these cells to internalize efficiently and to clear the complexes by endocytosis. At present, no experimental answers to these questions are available.

An important finding from this study indicates that one physiological function of megalin in podocytes is receptor-mediated uptake of apo E– and apo B–containing lipoproteins. It is intriguing that all ID-forming antimegalin IgG tested to date inhibit specifically the binding of these lipoproteins to megalin. This may suggest that biologically active sites on proteins, such as those for ligand binding, could serve preferentially as autoantigenic targets, as proposed by the recent finding for autoantigens in systemic lupus erythematodes (36).

In pHN, ID-forming antibodies are apparently a crossroad for pathogenic mechanisms of ID formation and proteinuria, in that the antibody-mediated inhibition of lipoprotein binding to megalin results in their accumulation within IDs, where they provide fatty acids needed for LPO, a major cause for GBM damage and proteinuria in pHN (12). Similar pathogenic mechanisms may also operate within ID in human membranous nephropathy, as apo E and B100 have been found in human glomeruli by immunofluorescence (37) and by immunoelectron microscopy (our unpublished results).

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