Reduced Immunoglobulin A Transcytosis Associated with Immunoglobulin A Nephropathy and Nasopharyngeal Carcinoma*

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Background: The A580V human plgR polymorphism is associated with IgA nephropathy and nasopharyngeal carcinoma.

Results: A580V mutation reduces plgR/plgA transcytosis and seemingly plgR cleavage and release from the apical surface.

Conclusion: The A580V polymorphism regulates plgR and IgA-plgR complex transcytosis across cells.

Significance: Defects in plgR trafficking and processing may underlie the pathogenesis of IgA nephropathy and nasopharyngeal carcinoma.

The polymeric immunoglobulin receptor (pIgR) is a single-spanning transmembrane glycoprotein expressed on many epithelial cells lining mucosal surfaces (1–3). At the basolateral (BL) cell surface, the pIgR binds its ligand, polymeric IgA (plgA). The plgA can in turn bind to its antigen. The plgR–plgA complex is endocytosed and transcytosed through a series of endocytic vesicles to the apical (AP) surface. There, the extracellular, ligand binding domain of pIgR is proteolytically cleaved and released together with the plgA into external secretions. This cleaved fragment of pIgR is termed secretory component (SC).

A missense mutation (pIgR-A580V) in the extracellular region of human pIgR is associated with increased risk of IgA nephropathy (IgAN) in Japan (4). IgAN is the main cause of primary glomerulonephritis worldwide, especially in east Asia; 20–40% of patients progress to end stage renal failure (5). The pIgR-A580V mutation has also been associated in two studies with increased risk of nasopharyngeal carcinoma (NPC) in Thai and southern Chinese populations, where NPC is a leading form of cancer (6–8). This result is intriguing, because Epstein-Barr virus (EBV), the causative agent of NPC, can enter epithelial cells via binding to the plgR of plgA antibodies directed against EBV (9, 10).

EXPERIMENTAL PROCEDURES

Plasmids, Viral Production and Transduction, and Cell Culture—Human plgR in pcDNA3.1 was kindly provided by C. Kaetzel (University of Kentucky). The A580V point mutation was made by QuikChange mutagenesis (Stratagene). plgR (WT and A580V) coding sequence was transferred to plLZRS-MS-IRES-GFP retroviral vector (A. Reynolds, Vanderbilt University), giving expression of hplgR and GFP under IRES control. Viral production and transduction were performed as described (11), with some modifications. plLZRS vectors were transduced into 293-GPG packaging cells (O. Weiner, University of California San Francisco). The following day, fresh medium was added, and viral supernatants collected 3 days after transfection. For retrovirus transduction, subconfluent Madin-Darby canine kidney (MDCK) cells 16–24 h after plating were incubated with virus-containing supernatants for 48 h at 37 °C. Stable lines were made using two complementary procedures: (i) direct transfection of pcDNA3.1-plgR followed by antibiotic-mediated selection (0.5 mg/ml G418 for 2–3 weeks) and screening for hplgR-positive clones, or (ii) viral transduction (see below) using plLZRS-MS-IRES-GFP-plgR (WT and A580V) followed by FACs to enrich for pools of GFP-expressing cells. All assays were verified using cell lines made by both methods, ensuring differences noted were due to the A580V plgR mutation and not clonal variation. plgR-expressing MDCK cells were grown as described (11, 12), on 12 or 24 mm polycarbonate Transwell filters (Corning) for 3–4 days for all experiments.

plgR Transcytosis Assay by BL Biotinylation—Biotinylation was performed at 17 °C (11, 12). Cells (on 12-mm filters) were washed three times with Hanks’ balanced saline solution (HBSS) containing 20 mM HEPES and biotinylated basolaterally
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with 0.5 mg/ml LC-NHS-biotin (Pierce) in HBSS for 30 min; 500 μl of MEM with 0.6% BSA (w/v), 20 mM HEPES, 100 units/ml penicillin, and 100 μg/ml streptomycin (MEM/BSA/HEPES) was added to the AP side. Cells were washed with MEM/BSA/HEPES buffer three times for 20 min at 17 °C to remove excess biotin. Transwells were transferred to 150-μl drops of MEM/BSA/HEPES ± pIgA (0.3 mg/ml) in a humid box for 10 min at 17 °C (with 250 μl of MEM/BSA/HEPES on the AP side). Cells were then transferred to a 37 °C water bath. Another 250 μl of 37 °C MEM/BSA/HEPES buffer was added to the AP chamber of filters to change the temperature to 37 °C. After the chase, 350 μl of cold MEM was added to the BL side, and filters were placed onto an ice-cold metal plate. AP medium was collected, and cells were lysed in 500 μl of 0.5% SDS (w/v) lysis buffer containing 100 mM NaCl, 50 mM triethanolamine HCl, pH 8.6, 5 mM EDTA HCl, pH 8.0, 0.2% Trasylol (v/v), 0.02% NaN₃ (w/v). Samples were boiled for 10 min and vortexed for 15 min at room temperature. Samples were then precleared with Sepharose CL2B beads, and 500 μl 2.5% (v/v) Triton dilution buffer containing 100 mM NaCl, 100 mM triethanolamine HCl, pH 8.6, 5 mM EDTA HCl, pH 8.0, 1.0% Trasylol (v/v), 0.02% NaN₃ (w/v) was added before immunoprecipitation with 30 μl of protein G beads (and 1 μg/sample of rabbit anti-human SC antibody (Dako and Santa Cruz Biotechnology) overnight at 4 °C. Immunoprecipitated samples were analyzed by gel electrophoresis and Western blotting using IRD800-streptavidin. Protein bands were visualized by LI-COR Biosciences Odyssey NIR Imager scanner.

pIgA Transcytosis by ELISA Analysis—Cells on a 12-mm filter were treated with 100 μg/ml biotinylated pIgA (biotinylation procedure was based on the manufacturer’s instructions (Pierce) from the BL surface at 37 °C for 20, 40, and 80 min (11). AP media were collected, and cells were lysed in 0.5% Nonidet P-40 (v/v) lysis buffer containing 125 mM NaCl, 20 mM HEPES, 4 mM MgCl₂, and biotinylated pIgA. Protein bands (Pierce) were visualized by LI-COR Biosciences Odyssey NIR Imager scanner.

Statistics—Data are expressed as mean ± S.E. Student’s t test was utilized to compare differences between wild-type and mutant cells. p and n values are presented in the figure legends.

RESULTS
To investigate how the pIgR-A580V mutation can underlie both IgAN and NPC, we expressed wild-type human pIgR (pIgR-WT) and pIgR-A580V in polarized MDCK cells, which have been used extensively for studies of pIgR trafficking (13). Both pIgR-WT and pIgR-A580V were uniformly expressed at similar levels (Fig. 1).

We measured transcytosis of pIgR by biotinylating it at the BL surface and quantitating the subsequent release of biotin-SC into the AP medium in the absence or presence of pIgA. AP SC release was reduced from 49.81 ± 1.47% (pIgR-WT) to 37.84 ± 3.79% (pIgR-A580V), p = 0.008 in the absence of pIgA (Fig. 2A, top).

Binding of pIgA to the pIgR is known to increase the rate of transcytosis of the pIgR. We therefore determined the effect of the mutation on AP SC release in the presence of pIgA added to the BL medium. Under this condition, AP SC release was also decreased by the mutation, i.e. 54.6 ± 1.29% (pIgR-WT) and

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The N-terminal, extracellular region of plgR consists of five immunoglobulin-like domains, which become SC after proteolytic cleavage. This is connected by a stalk region to the single membrane-spanning segment of plgR. The exact site(s) of the cleavage that converts plgR to SC has not been definitively determined (14, 15). The Ala-580 mutation is in the stalk region of plgR and close to the likely cleavage site(s). It is possible that the plgR-580V mutation decreases the rate of cleavage of plgR at the AP surface. Our measurement of plgR transcytosis scores release of SC into the AP medium, and so a decrease in cleavage would decrease the apparent rate of transcytosis. The same holds true for measurement of transcytosis of plgA bound to the plgR. Furthermore, decreased cleavage could increase the amount of plgR that enters the AP endocytic pathway rather than undergoing cleavage and thereby increase the apparent rate of endocytosis.

To measure plgR cleavage directly, we biotinylated plgR at the AP surface at 4 °C and then warmed the cells to 37 °C for 5 min and quantitated the release of biotinylated SC. Indeed, the rate of cleavage of plgR to SC was decreased by the plgR-A580V mutation. Cleavage of WT versus A580V, respectively, was 24.03 ± 0.94% versus 17.26 ± 0.61%, p = 0.03 (Fig. 4). This suggests that the reduction in plgR-A580V transcytosis is likely due to reduced cleavage (13, 16, 17).

DISCUSSION

The plgR has been studied extensively as a model for membrane trafficking and transcytosis in polarized epithelial cells. Its 103-residue cytoplasmic domain contains sorting signals for BL delivery, endocytosis, avoidance of degradation, and transcytosis (13, 16). The plgR is also a signaling receptor in that binding of the plgA ligand to the plgR leads to activation of a signaling network involving the Src family kinase Yes, EGFR, ERK, FIP5, rab3b, retromer, phospholipase C¢y1, and increased intracellular free calcium (2, 11). All of these processes involve the C-terminal cytoplasmic domain of the plgR. In contrast, the plgR-A580V mutation is located in the extracellular region of the plgR. In particular, this mutation is in the membrane proximal “stalk” region of the plgR where proteolytic cleavage of SC occurs. Indeed, the mutation likely reduces this cleavage, which probably accounts for the observed decreased rates of transcytosis of plgR and plgA, as well as the increased AP endocytosis of plgA.

Our data do not exclude the possibility that the mutation might instead alter the conformation of the plgR in a way that increases AP endocytosis. As cleavage probably occurs at the AP surface, this increased endocytosis could cause a decrease in the apparent rate of cleavage of the plgR, thereby accounting for all of our data. However, given the proximity of the mutation to the cleavage site on the plgR, we tend to favor the explanation that the mutation directly reduces the rate of cleavage.

IgAN and NPC are both complex, poorly understood diseases. Many genetic and environmental risk factors are likely to be involved and may account for the different outcomes (IgAN versus NPC) in different populations. IgAN is characterized by mesangial deposits of IgA (mainly polymeric), often complexed with antigen. Several loci have been identified with IgAN in a genome-wide association study (18). Another factor is altered
galactosylation of IgA1 (5). IgAN is likely a heterogeneous group of diseases, and pIgR-A580V may play a role only in select populations, such as in Japan. We have shown that the pIgR A580V mutation results in a decrease in AP SC release and suggest that this is an additional factor contributing to both diseases. IgAN often follows mucosal infections, which cause increased production of pIgA and pIgA-antigen complexes. Ordinarily, such pIgA-antigen complexes are efficiently excreted into mucosal secretions by pIgR (1). The reduced efficiency of transcytosis by pIgR-A580V could lead to buildup of pIgA-antigen complexes, which would then be filtered out in the kidney, resulting in IgAN (20). Although the effect of pIgR-A580V on transcytosis is modest (but statistically significant), this could be sufficient to account for a slow accumulation of pIgA-antigen complexes, consistent with the multiyear time course of IgAN.

EBV is associated with infection and cancer in lymphocytes, e.g. Burkitt lymphoma, as well as NPC, an epithelial carcinoma (19). Complexes of pIgA bound to a surface protein of EBV are efficiently transcytosed across pIgR-expressing hepatocytes and polarized MDCK cells without causing EBV infection (9). However, nonpolarized pIgR-expressing HT29 human colon carcinoma cells are infected by EBV, which enters the cells via pIgA-EBV complexes bound to pIgR. We suggest that the reduced efficiency of transcytosis of EBV by pIgR-A580V could lead to infection, even in otherwise well polarized epithelial cells. Oncogenic transformation of one cell by EBV may be sufficient to result in NPC. It is thus possible that even a slight increase in the rate of infection of epithelial cells expressing the mutant pIgR could lead to clinical NPC. Therefore, the modest decrease in transcytosis caused by pIgR-A580V could be sufficient to account for the association of this mutation with NPC.

Our results provide a unified model of how a slowing in cleavage of pIgR by a missense mutation and the resultant decreased transcytosis of pIgA may contribute to two distinct diseases, IgAN and NPC, in vulnerable populations. This provides potentially important clues in both understanding the pathogenesis of these diseases and perhaps how they might be better diagnosed, prevented, or treated.

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