論文の内容の要旨

論文題目 Podocyte albumin handling in the nephrotic syndrome

和訳 ネフローゼ症候群における足細胞アルブミン処理機構の解明

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Background: There has been considerable controversy over whether the glomerular basement membrane (GBM) or the slit diaphragm is the most important restriction barrier of glomerular filtration of proteins. Electron microscopy studies visualizing the “zipper structure” of the slit diaphragm and the discovery of many slit diaphragm proteins such as nephrin, whose deficiency leads to nephrotic syndrome has led many to believe that the slit diaphragm is the main filtration pathway for serum proteins in both physiological and pathologic states. However, the number of slit membranes is reduced and slit diaphragms are altered to a tight junction-like structure in minimal change nephrotic syndrome in spite of massive proteinuria, suggesting other pathways of protein filtration. We have obtained results suggestive of non-slit diaphragm routes for albumin filtration in previous studies.

In this study, we aimed to elucidate the glomerular filtration pathways of albumin with various methods. We utilized 2nm thiocyanate gold which can be clearly detected by electron microscopy with silver enhancement, and Evans blue dye, which strongly and specifically binds to albumin, as tracers for albumin, and were able to observe the passage of albumin through the glomerulus and speculate the filtration pathways of proteins in detail.

Methods: Nephrotic syndrome was induced by intraperitoneal injection of puromycin aminonucleoside (PAN). Kidney specimens from PAN rats and controls were obtained after intravenous administration of 2nm gold-labeled albumin, fixed by glutaraldehyde and examined on electron microscopy following silver enhancement. To visualize the glomerular filtration pathway of albumin, We continuously infused rats with Evans blue-labeled human serum albumin (EB-HSA), and examined the glomerular filtration of albumin by confocal microscopy of kidney lowicryl sections, immunogold scanning electron microscopy (SEM) of kidney vibratome sections, and clearance measurement.

To elucidate whether the production of reactive oxygen species (ROS) by NADPH oxidase in podocytes is involved in the pathophysiology of this disease model, we performed pre-embedding

immunocytochemistry for NADPH oxidase and cerium chloride histochemistry. We also tested the effects of apocynin, a NADPH oxidase inhibitor.

Results: Few 2nm gold particles were observed in the the GBM, and between foot processes and tubular epithelial cells in control rats, while rarely seen under the slit membrane. In contrast, in PAN rats, increased gold labeled albumin uptake was observed in the paramesangial areas and podocyte cell bodies. EB-HSA was observed in the podocytes of PAN rats by confocal microscopy, and urinary EB-HSA excretion showed a 132-fold increase compared to controls. Immunogold-SEM showed a clear increase in the number of albumin molecules in podocyte vesicles (33.3±6.0 vs. 0.3±0.2 particles per podocyte cross section, $p < 0.01$) and on the podocyte apical membrane (113.8±21.3 vs. 5.2±0.8 particles per micrograph, $p < 0.01$), suggesting that a endocytic pathway for albumin is enhanced in the podocyte of nephrotic rats. With apocynin treatment, the number of gold particles localizing EB-HSA was significantly decreased with reduced podocyte superoxide production and urinary EB-HSA excretion. Immunoprecipitation of isolated glomeruli with anti-FcRn antibody showed a considerable increase in albumin bound to FcRn in PAN rats, which decreased in apocynin-treated PAN rats,

Conclusion: In conclusion, we have demonstrated that an albumin filtration pathway through the podocyte cell body via endocytosis and exocytosis mediated by FcRn may exist, resulting in selective albuminuria. Overproduction of ROS by NADPH oxidase precedes the onset of proteinuria, and NADPH oxidase inhibition with apocynin suppressed podocyte albumin endocytosis and exocytosis and ameliorated proteinuria, shedding light on apocynin as a new therapeutic approach for the nephrotic syndrome.