Glomerular Epithelial Protein 1 and Podocalyxin-like Protein 1 in Inflammatory Glomerular Disease(Crescentic Nephritis) in Rabbit and Man

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The podocyte is the cell responsible in large part for maintaining the glomerular filtration barrier. Glomerular epithelial protein 1(GLEPP1) is a glomerular epithelial cell(podocyte) foot process transmembrane protein tyrosine phosphatase that we have recently identified, colned, and sequenced. The function of this molecule is not yet known, but we speculate that it acts as a receptor which plays a role in the regulation of podocyte foot process structure and function. The human counterpart of GLEPP1 has also been cloned and sequenced, localized to chromosome 12p12-13, and monoclonal antibodies have been raised against a fusion protein made from part of its extracellular domain that can be used in formalin-fixed paraffin embedded human kidney tissue. Podocalyxin-like protein 1(PCLP1) identifed in rabbit has many of the characteristics of podocalyxin identified in rats as the major anionic protein of the glomerular podocyte. Podocalyxin is thought to function to maintain foot process structure and function on the basis of its negative charge. The relationship between podocalyxin and PCLP1 is uncertain at the present time, pending molecular cloning of the rat podocalyxin and comparison of nucleotide sequence with that of rabbit PCLP1. however, the distribution of PCLP1 in the glomerulus and renal cortex is as described for podocalyxin, being predominantly on the apical surface of foot processes but also present on the endothelial cell surface in glomeruli, peritubular capillaries, and in other organs. PCLP1 is a highly posttranslationally modified transmembrane glycoprotein, as shown by sequencing and transfection experiments wherein the core protein has a predicted size of 55 kd and the observed size by SDS-PAGE is 140 kd. GLEPP1 and PCLP1 are thought to play a role in regulating the structure and function of podocyte foot processes. Glomerular injury affecting the podocyte is likely to be reflected by changes in these proteins.

We report results of experiments in which we have examined the changed distribution of GLEPP1 in human glomerular inflammation and crescent formation. To better understand the metabolism of GLEPP1 in glomerular inflammation, we have performed studies in a rabbit model of crescentic nephritis induced by guinea pig anti-rabbit basement membrane(anti-GBM) antibody. We used PCLP1 as a second glomerular cell marker with which to compare the observed changes in GLEPP1 in the rabbit model. GLEPP1 distribution in human renal biopsy with inflammatory glomerular disease and crescent formation was examined by immunocytochemistry. A model of inflammatory glomerular injury induced by guinea

pig anti-rabbit basement membrane(anti-GBM) antibody was used to examine the distribution and amount of GLEPP1 and PCLP1 mRNA and protein.

A biopsy study was done to determine whether the extent of GLEPP1 depletion from glomeruli at early time points(Day 7) would predict the severity of crescent formation at Day 30. Glomeruli from human renal biopsies with crescentic nephritis showed focal to diffuse disappearance of GLEPP1 protein. No GLEPP1 was present within the cellular crescent. By Day 4 of the rabbit anti-GBM model, before cellular crescents had formed, GLEPP1 protein was reduced from $127 \pm 28 \times 10^7$ to $30 \pm 5 \times 10^7$ molecules per glomerulus($p<0.001$), and GLEPP1 mRNA was reduced by 62%($p<0.05$). In contrast, at this time there was no significant reduction of PCLP1 protein from the normal number of 309×10^9 molecules per glomerulus and the PCLP1 mRNA level had not decreased. At Day 4, podocyte foot processes were effaced and proteinuria was present. Glomerular culture supernatants from Day 4 rabbits caused a reduction in GLEPP1 but not PCLP1 protein expression by cultured normal glomeruli, showing that a soluble factor was produced at Day 4 which reduced the number of GLEPP1 molecules in glomeruli. There was no detecable proteolysis of GLEPP1 or PCLP1 in glomeruli and no increase in GLEPP1 or PCLP1 excretion in urine. Therefore, the reduction in glomerular GLEPP1 was associated with reduced synthetic capacity. The proportion of glomenuli with reduced GLEPP1 at Day 7 of the model was significantly associated with the percent of glomeruli which had formed crescents at Dat 30($r=0.86$, $p< 0.0001$).

The data provided suggests that the extent of the decrease in GLEPP1 likely reflects the extent of glomerular immune-driven inflammation and injury to the glomerulus. Injury to podocytes may be very important in determining outcome for a glomerulus. A normally functioning podocyte population is likely to be essential for glomerular survival. Thus, the presence of GLEPP1 as a marker of podocyte integrity in various forms of glomerular injury may prove to be clinically useful. The finding that GLEPP1 is absent or reduced may be an indicator of poor prognosis. (Lab Invest 1996, 74:571-584)