Early changes in gene expression that influence the course of primary glomerular disease

LC Clement[1,4], G Liu[1,4], I Perez-Torres[3], YS Kanwar[2], C Avila-Casado[3] and SS Chugh[1]

[1]Department of Medicine, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA; [2]Department of Pathology, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA and [3]Department of Pathology, Instituto Nacional de Cardiologia, Mexico City, Mexico

Serial changes in glomerular capillary loop gene expression were used to uncover mechanisms contributing to primary glomerular disease in rat models of passive Heymann nephritis and puromycin nephrosis. Before the onset of proteinuria, podocyte protein-tyrosine phosphatase (GLEPP1) expression was transiently decreased in the nephrosis model, whereas the immune costimulatory molecule B7.1 was stimulated in both models. To relate these changes to the development of proteinuria, the time of onset and intensity of proteinuria were altered. When the models were induced simultaneously, proteinuria and anasarca occurred earlier with the collapse of glomerular capillary loops. Upregulation of B7.1 with the downregulation of GLEPP1, Wilms' tumor gene (WT1), megalin, and vascular endothelial growth factor started early and persisted through the course of disease. In the puromycin and the combined models, changes in GLEPP1 expression were corticosteroid-sensitive, whereas B7.1, WT1, vascular endothelial growth factor, and most slit diaphragm genes involved later in the combined model, except podocin, were corticosteroid-resistant. There was a very early increase in the nuclear expression of podocyte transcription factors ZHX2 and ZHX1 that may be linked to the changes in gene expression in the combined proteinuric model. Our studies suggest that an early and persistent change in mostly steroid-resistant glomerular gene expression is the hallmark of severe and progressive glomerular disease.

Kidney International (2007) **72,** 337–347; doi:10.1038/sj.ki.5002302; published online 25 April 2007

KEYWORDS: podocyte; minimal change disease; membranous nephropathy; ZHX proteins; WT1; glomerular collapse

Correspondence: *SS Chugh, Division of Nephrology, Tarry 4-753 Northwestern University, Feinberg School of Medicine, 320 East Superior, Chicago, Illinois 60611, USA. E-mail: s-chugh@northwestern.edu*

[4]These authors contributed equally to this work

Received 21 November 2006; revised 12 March 2007; accepted 20 March 2007; published online 25 April 2007

Proteinuria and glomerular disease results from a myriad of structural and as yet inadequately defined functional changes in components of the glomerular capillary loop. In different models, these changes can be variously initiated by inflammatory cells, complement, cytokines, circulating factors, or antibody toxicity. Regardless of the initiating event, changes in one or more of several putative pathways initiate a sequence of events within the intrinsic components of the glomerular capillary loop that eventually result in the development of proteinuria and glomerular disease.

There is a relative paucity of data on gene expression in experimental primary glomerular disease.[1] Most experimental studies, and all human kidney biopsy studies, have looked at time points coincident with, or after the onset of proteinuria. To understand the development of glomerular disease, it is important to study early changes, especially those just before the onset of proteinuria. Passive Heymann nephritis (PHN) and puromycin aminonucleoside nephrosis (PAN) are models of podocyte disease with a defined time point for the onset of proteinuria and a pre-proteinuric phase. Both models are suitable for the study of changes in gene expression before, and immediately after the onset of proteinuria. In this study, we induced both models in young rats, because they have very little baseline proteinuria, and onset of overt proteinuria is predictably on day 5 (occasionally on day 4).[1] Studies in these models indicate that transient changes in podocyte protein-tyrosine phosphatase (GLEPP1) and B7.1 occur early, and that changes in GLEPP1 expression are partially corticosteroid-sensitive. The potential importance of these gene changes in the development of proteinuria was assessed by changing the onset and intensity of proteinuria. Combining the two models results in the development of glomerular capillary loop collapse, which is associated with earlier onset of proteinuria, persistent changes in B7.1 and GLEPP1 expression, increased early podocyte nuclear expression of ZHX1 and ZHX2, additional downregulation of several mostly corticosteroid-resistant genes, and early death from severe anasarca.

RESULTS

Induction of animal models of proteinuria

Proteinuria data of a representative experiment for PAN, PHN, and PAN + PHN combination is shown in Figure 1. In

young rats, PAN and PHN cause proteinuria on day 5, whereas in the PAN + PHN combination, proteinuria develops on day 2. Lack of proteinuria on day 3 of the individual models and day 1.5 of the combination model was confirmed both by protein assay and confocal imaging of proximal tubules for rat albumin. Because there is a shift to the left in terms of the onset of proteinuria (i.e., day 2, compared to days 4–5 in the original models), day 1.5 is taken as a pre-proteinuric time point, and day 3 as the immediate post-proteinuric time point.

Histological characterization of the animal models

Histological assessment of each group confirmed the induction of disease (Figures 2 and 3). PAN and PHN were histologically similar to previous descriptions extensively published in the literature. Evidence of significant foot process effacement was present in the proteinuric PHN, PAN, and combination rats. This PAN + PHN combination model has features of both PAN and PHN, for example extensive foot process effacement as typical of PAN, and subepithelial deposits as seen in PHN. In the day 6 combination rats, about a third of the glomeruli had collapsed capillary loops (see detailed analysis later). Day 1.5 and 3 combination rats had no evidence of capillary loop collapse. Most PAN + PHN combination rats develop progressive anasarca early, and die by days 7–8. The expression of several podocyte markers was studied in various study groups by immunostaining. Only results for selected proteins are presented (Figure 4, Figure S6). As is also noted in human collapsing glomerulopathy,[2] Wilms' tumor gene (WT1), a podocyte-specific transcriptional factor in the adult kidney, was significantly decreased in glomeruli with collapsed loops, and only mildly decreased in glomeruli with normal appearing capillary loops in the combination day 6 rats. WT1 staining was unchanged in PAN and marginally decreased in PHN at later time points. Expression of GLEPP1 was moderately decreased even in glomeruli with noncollapsed loops in the combination day 6 group and severely depleted in those with collapsed loops. In

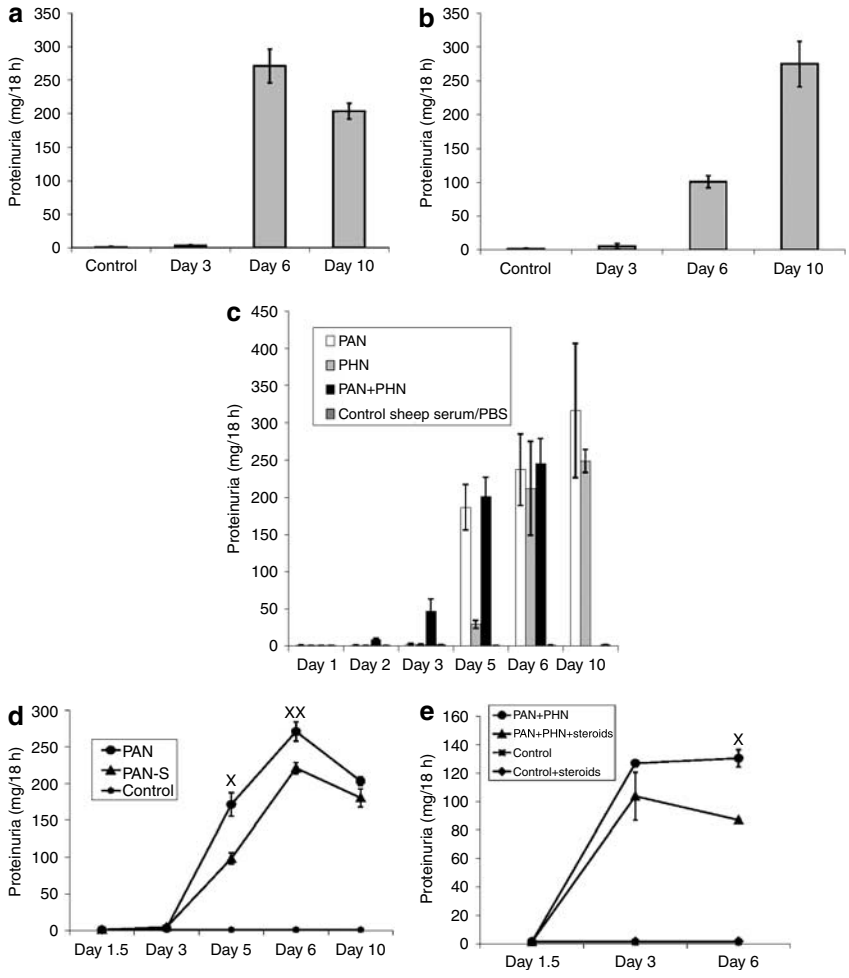

Figure 1 | Induction of proteinuria in the PAN, PHN, and PAN + PHN combination models. (**a**) PAN and (**b**) PHN rats develop proteinuria on day 5 (occasionally on day 4), whereas (**c**) PAN + PHN combination rats become proteinuric on day 2. $P < 0.001$ for all proteinuric time points vs controls in **a–c**. (**d**, **e**) Effect of corticosteroids therapy on proteinuria in (**d**) PAN and (**e**) PAN + PHN combination rats is shown. Rats were treated on alternate days with corticosteroids (see text), resulting in mild decrease in proteinuria. ($n = 4$ rats/group in all experiments). x indicates $P < 0.001$; xx indicates $P < 0.01$.