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Differential chemokine expression in tubular cells in response to urinary proteins from patients with nephrotic syndrome

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Abstract

Background and aim: Comparison of urine proteins in idiopathic minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) patients has been previously conducted, but the relationship between the severity of tubular injury and the composition of urine proteins in various kidney diseases is unknown. This study aimed to investigate the chemokine expression in human tubular cells in response to urine proteins from patients with nephrotic syndrome. *Methods:* Urine proteins collected from patients with MCD or FSGS were extracted by ultrafiltration and coincubated with HK-2 cells. The expression of the *RANTES* and *MIF* genes and the activation of p38 and extracellular regulated kinase were determined. *Results:* The urine proteins from both MCD and FSGS patients contained a primary band of proteins with Mr of ~62 kDa. The major cytokines present in urine proteins from MCD and FSGS patients were IL-6 and IL-8, while IL-1β, IL-10, IL-12p70 and TNF-α were only detectable. We observed time- and dose-dependent increases in RANTES and MIF expression with urine protein treatment in HK-2 cells. The urine proteins from FSGS patients induced a higher expression of these two chemokines in HK-2 cells compared to the urine proteins from MCD patients. ERK and p38 were activated by urine proteins from either MCD or FSGS patients. Pretreatment with SB203580 or PD98059 abolished the increase in RANTES and MIF expression induced by urine proteins from FSGS patients, while only SB203580 suppressed the high expression induced by urine proteins from MCD patients. *Conclusion:* These findings indicate that the urine proteins from MCD and FSGS patients induce a differential expression of RANTES and MIF in tubular cells through distinct activation of MAPK-related signaling pathways.

Keywords: Nephrotic syndrome; Human serum albumin; Proteinuria; Chemokine; MAPK

1. Introduction

Progressive nephropathies are characterized by a highly enhanced glomerular permeability to proteins, in turn leading to proteinuria and concomitant tubulointerstitial damage [1–3]. How proteinuria results in such precipitating interstitial changes is not fully understood, but it is certainly multifactorial and involves numerous pathways of cellular damage [2–4]. Earlier studies in vitro have already confirmed that certain serum protein components (e.g. albumin and transferrin) can stimulate proximal tubular cells to secrete a large number of chemokines, such as regulated upon activation, normal T cell expressed and secreted (RANTES/CCL5, chemokine (C–C motif) ligand 5) [2] and macrophage immigration inhibitory factor (MIF) [5] into the basolateral medium, and the polarized secretion of these chemoattractants in vivo is considered to promote monocyte and lymphocyte recruitment into the renal interstitium [6,7]. Additionally, it is well established that activation of the extracellular signal regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) signal transduction pathway plays an important role in the inflammatory response [8]. p38 and ERK as important signaling molecular have been proved to mediate release of MIF and RANTES in tubular cells exposed to a single component such as albumin or IgG, suggesting

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a possible link between proteinuria and inflammatory damage of tubular cells involving MAPK pathway [9,10]. However, these studies have a significant limitation. They have generally used a single serum component (e.g. albumin) at very high and physiologically irrelevant concentrations to study the effect of protein overload on proximal tubular epithelial cells (PTECs) [11,12]. However, the glomerular ultrafiltrate often contains a mixture of proteins and a single component might not be responsible for the damage of tubular cells in renal disease development [13].

Focal segmental glomerulosclerosis (FSGS) is one of the most prevalent histopathological lesions in idiopathic nephrotic syndrome. It is distinguished clinically from minimal change disease (MCD) by non-selective proteinuria, steroid resistance and progressive renal insufficiency. Furthermore, it has been shown that FSGS patients presented more often with progressive tubular degeneration and interstitial fibrosis compared to MCD patients even though both diseases had a similar degree of proteinuria [14–17]. Thus in an effort to find therapeutic targets in proteinuria, comparison of urine proteins in MCD and FSGS patients has been previously conducted [18–20], but the relationship between the severity of tubular injury and the composition of urine proteins in various kidney diseases is unknown.

To seek further evidence, we have hypothesized that the use of a wide range of proteins extracted from proteinuria might closely resemble the glomerular ultrafiltrate in tubular lumen and serve as a model for further study in the mechanism of tubular damage induced by proteinuria in kidney diseases. This study was undertaken to assess and compare the expression of two important chemokines, RANTES and MIF, in cultured proximal tubular cells treated with urine proteins extracted from MCD or FSGS patients and to explore the possible role of ERK and p38-MAPK signaling transduction pathways in differential chemokine production.

2. Materials and methods

2.1. Reagents and drugs

HK-2 cells, a primary human proximal tubular cell line, was purchased from American Type Culture Collection (Manassas, VA); Dulbecco's Modified Eagle's medium (DMEM)/F-12, fetal bovine serum (FBS) and trypsin/ethylenediamine tetraacetic acid (EDTA) solution were purchased from Gibco (Langley, OK); TRIzol and reverse transcriptase were from Invitrogen (Carlsbad, CA); PCR buffer, Taq polymerase and dNTPs were from Takara Bio Inc. (Shiga, Japan), and ELISA Quantikine kit for RANTES was from R&D Systems (Minneapolis, MN). The specific inhibitors, SB203580 and PD98059, were from CalBiochem (San Diego, CA). Primary antibodies of p38 and ERK were from Cell Signaling Technology (Danvers, MA). Purified human serum albumin (HSA, A5843), Triton X-100, and dimethyl sulfoxide (DMSO) were from Sigma–Aldrich (St. Louis, MO). Polymyxin B-immobilized columns (Detoxi-GelTM) were from Pierce Chemical (Rockford, IL). The ultrafilter (Labscale TTF system, PXC 100C 50) was purchased from Millipore Co. (Billerica, MA). The human inflammation Cytometric Bead Array kit for determining interleukins (IL-8, IL-1b, IL-6, IL-10 and IL-12p70), and tumor necrosis factor-α (TNF-α) and BD LSR II flow cytometer were from BD Pharmingen (San Jose, CA).

2.2. Patients and urine collection

Informed consent was obtained from all patients participating in this study, after local Ethics Committee approval. Diagnosis was established based on the criteria of ICD-11 for 10 patients with MCD and 12 patients with FSGS through biopsy using light and electron microscopy. Only patients who have never been treated with steroid or any other immunosuppressive agents were enrolled in this study. The spontaneous urine was collected in a clean container on ice (~4 °C) and stored at −80 °C until analysis. Some key clinical data of the patients studied are summarized in Table 1. The mean urine protein concentration in MCD and FSGS patients was 3.5 ± 1.4 mg/ml and 3.7 ± 2.0 mg/ml, respectively. Thus, a concentration range of 0.1–10 mg/ml for the urine proteins was used for our in vitro experiments.

2.3. Urine protein ultrafiltration and preparation

Following initial centrifugation (4000*g* for 15 min at 4 °C) the supernatant of urine sample was collected and filtered by a 0.45-μm filtration membrane to remove non-soluble deposits and cellular particles. The samples were then applied to an ultrafilter with polyethersulfone membrane (5 kDa molecule weight cut-off and low protein binding membrane). After lyophilization using a freeze dryer to remove extra water, the urine protein extract was stored at −80 °C. In all in vitro experiment, dried and powdered urine proteins were dissolved in sterile phosphate-buffered saline (PBS) and then added to cells. Endotoxin in urine proteins was removed by polymyxin B-immobilized columns and cleared reagents contained <0.1 EU/ml of endotoxin as determined by the limulus amebocyte lysate assay. Urine proteins were eluted in 0.5 mM NaCl and 50 mM Tris at pH 7.5. Coomassie-stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was used to verify that there was no gross degradation of the proteins prepared using this protocol.

Table 1
Clinical data of patients

Patient characteristic	MCD	FSGS
Number	10	12
Sex (male/female)	9/1	9/3
Mean age (years)	21 ± 10	35 ± 17
Proteinuria (g/day)	3.5 ± 1.4	3.7 ± 2.0
Serum creatinine (μmol/L)	73.0 ± 8.8	92.0 ± 86.7

Abbreviations: FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease.