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Immune Mediators in Idiopathic Nephrotic Syndrome: Evidence for a Relation Between Interleukin 8 and Proteinuria

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ABSTRACT: The pathogenesis of idiopathic nephrotic syndrome (INS) remains unknown. Several findings suggest a role for the immune system. This study aimed to evaluate immune mediators in INS by measuring plasma and urinary levels of transforming growth factor β_1 (TGF-β_1), monocyte chemoattractant protein-1 (MCP-1/CCL2), regulated on activation normal T-cell expressed and secreted (RANTES/CCL5) and IL-8 (IL-8/CXCL8) in pediatric patients with INS and in age-matched healthy controls. Patients were divided according to their response to corticosteroids: steroid-sensitive (SS, $n = 8$), or steroid-resistant (SR, $n = 24$). Immune mediators were also compared in regard with disease activity (relapse and remission). Immune mediators were measured by ELISA. Plasma TGF-β_1 levels in SR patients were approximately 2.8-fold higher than control values ($p < 0.05$). Urinary IL-8/CXCL8 was 2.9-fold higher in INS patients in relapse (proteinuria >100 mg/m^2/24 h) when compared with patients in remission ($p < 0.05$), and levels had a positive correlation with individual proteinuria values ($p < 0.05$). Urinary IL-8/CXCL8 was significantly higher in relapsed SR than in SS patients in remission. No changes in MCP-1/CCL2 and RANTES/CCL5 levels were detected. Our findings suggest that IL-8/CXCL8 and TGF-β_1 are involved in the pathogenesis of INS: IL-8/CXCL8 associated with local changes in glomerular permeability and TGF-β_1 could be related to worse response to corticosteroids. **(*Pediatr Res* 64: 637–642, 2008)**

Idiopathic nephrotic syndrome (INS), characterized by heavy proteinuria, edema and hypoalbuminemia, is the most common glomerular disease in childhood (1). Common histologic variants are minimal change nephrotic syndrome (MCNS) and focal segmental glomerulosclerosis (FSGS) (2). The main predictive factor for disease evolution is not histologic diagnosis, but the response to treatment with corticosteroids (1,3). Indeed, children with steroid-resistant (SR) nephrotic syndrome have a greater chance to develop end-stage renal disease than those with steroid-sensitive (SS) nephrotic syndrome (1–3).

The pathogenesis of INS is not yet fully understood. Many studies have proposed a role for the immune system (2,4–6), and this hypothesis is supported by a favorable response to anti-inflammatory drugs (2), a relation between relapses and viral infections or allergic reactions (5), the recurrence of the disease in transplanted patients (1) and an association with immunologic disorders (2).

More recently, it has been proposed that alterations in the cytokine and chemokine profile of INS patients might contribute to proteinuria and glomerular damage (7,8). Cytokines are a group of proteins produced by several kinds of cells that function as soluble mediators with intercellular signaling functions (4). Chemokines constitute a large family of low-molecular-weight cytokines whose main action is the recruitment and activation of leukocyte subsets in various models of inflammation—the word "chemokine" is a contraction of the terms "chemoattractant" and "cytokine" (9,10).

A cytokine that is often associated with the progression of kidney disease is transforming growth factor-β (TGF-β) (11,12), which exhibits fibrogenic and proinflammatory properties in the kidney (12–14). One of the actions of TGF-β is to induce the accumulation of monocytes and stimulation of fibroblasts by increasing the expression of two C–C-chemokines: monocyte chemoattractant protein-1 (MCP-1/CCL2) and regulated on activation normal T-cell expressed and secreted (RANTES/CCL5) (15–17). Studies have found increased levels of MCP-1/CCL2 and RANTES/CCL5 in progressive glomerular diseases, allograft rejection and interstitial nephritis (18,19). Another mediator of interest when studying the INS pathogenesis is IL-8 (IL-8/CXCL8), a chemokine produced by endothelial cells and macrophages that attracts neutrophils and lymphocytes to the inflammation site (17,18), and may be involved in the pathogenesis of proteinuria in INS (7,20).

In this context, the present study aimed to evaluate circulating and urinary immune mediators in INS patients and to compare their levels according to steroid sensitiveness and disease activity at the time of blood and urine collection. We specifically measured plasma and urinary levels of MCP-1/

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Abbreviations: ACEi, angiotensin converting enzyme inhibitors; **FSGS,** focal segmental glomerulosclerosis; **IL-8/CXCL8,** Interleukin-8; **INS,** idiopathic nephrotic syndrome; **MCNS,** minimal change nephrotic syndrome; **MCP-1/CCL2,** monocyte chemoattractant protein-1; **RANTES/CCL5,** regulated on activation normal T-cell expressed and secreted; **SR,** steroid-resistant; **SS,** steroid-sensitive; **TGF-β_1,** transforming growth factor-β_1

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CCL2 and IL-8/CXCL8, plasma TGF-β_1 and urinary levels of RANTES/CCL5 in pediatric patients with INS, to disclose possible changes that might contribute to the pathogenesis of this syndrome.

SUBJECTS AND METHODS

Study design. The present cross-sectional study used a convenience sample of children and adolescents with INS, followed-up at the Pediatric Nephrology Unit of our institution from 2005 to 2007. Diagnostic criteria for INS were based on the International Study of Kidney Disease in Children (21). Our Pediatric Nephrology Unit was established in 1980 and has followed-up approximately 300 children with nephrotic syndrome, according to a protocol that includes definition of disease etiology, assessment of clinical course and laboratory alterations, institution of treatment protocols and indication of renal biopsy based on clinical (corticosteroid unresponsiveness) and laboratory findings.

Patients with nephrotic syndrome. Inclusion criteria included children and adolescents with well-established INS with still preserved renal function, followed-up from 2005 to 2007, whose parents gave their consent to participate in the study protocol. Children and adolescents with congenital or secondary forms of nephrotic syndrome and INS patients at stages 2–5 of chronic kidney disease were automatically excluded from the study.

Controls. The control group consisted of sex and age-matched healthy subjects from our Pediatric Primary Care Center. Healthy status was determined through the subjects' medical history and either a parental report or self-report to rule out the presence of chronic or acute diseases.

Ethical aspects. The Ethics Committee of the Federal University of Minas Gerais approved the study. Informed consent was obtained from parents of all included subjects. The research protocol did not interfere with any medical recommendations or prescriptions. Our Ethics Committee did not allow 24-h urine collection in healthy controls. Blood samples in control group were only drawn simultaneously to other routine blood exams. The follow-up of the INS patients and healthy controls was guaranteed even in cases of refusal to participate in the study.

Study protocol. We allocated INS patients into two groups according to their response to treatment with corticosteroids. Steroid-sensitive (SS group), if complete remission of INS was obtained after an 8-wk course of corticosteroids, or SR group, if no remission or only partial remission occurred with steroids and if the patient needed to use other medications as an attempt to achieve disease control (3). We also subdivided SR and SS patients in two subgroups, according to disease activity (SR in relapse, SR in remission, SS in relapse and SS in remission) at the time of blood and urine collection for immune mediators measurements. In accordance to standard recommendations (2,3), our INS patients were considered in remission if their proteinuria levels were below or equal to 100 mg/m^2/24 h, and in relapse if their proteinuria levels were above 100 mg/m^2/24 h (2,3).

Clinical characteristics and casual measurements. Clinical characteristics and casual measurements were obtained at the same time of blood and urine collection. The clinical variables analyzed were age, gender, height, weight, body mass index, and systolic and diastolic blood pressure. In INS patients, serum levels of urea, creatinine, albumin, cholesterol, triglycerides, and uric acid were assessed using the same blood sample obtained for the measurements of immune mediators. Urinary determinations of creatinine levels and 24-h protein excretion were also performed simultaneously to the measurements of urine chemokines levels. GFR was estimated using the Schwartz *et al.* (22) formula in INS patients and in healthy controls. Renal biopsy results and medications used at the time of blood sampling are also provided (Table 1).

Blood sampling. After informed consent, all subjects (INS patients and controls) were submitted to blood collection for the measurement of immune mediators. Blood sampling occurred at only one occasion, simultaneously to other routine exams, as mentioned before. The samples were collected into sterile citrate tubes, which were immediately immersed in ice, and processed within 30 min after collection. Cells were sedimented by centrifugation at 700 g for 10 min at 4°C; then supernatant plasma was collected and respun for another 20 min at 1300 g to sediment platelets (23). Cell-free plasma was aliquoted into 0.5 mL samples and stored at −80°C until measurements.

Urine sampling. According to the recommendations of our local Ethics Committee, 24-h urine samples were not allowed to be collected from healthy controls. For this reason, 24-h urine samples were obtained only from patients with INS, at the same day of blood collection. After homogenization, 10 mL of the collected urine were centrifuged at 4°C for 20 min at 1300 g. Cell-free urine was aliquoted into 0.5 mL tubes and stored at −80°C until measurements.

Cytokines and chemokines measurement. Plasma and urinary levels of MCP-1/CCL2 and IL-8/CXCL8, plasma TGF-β_1 and urinary RANTES/CCL5 were measured by specific enzyme-linked immunoassay (ELISA) kits (R&D Systems, Minneapolis, MN), following the manufacturer's instructions, as described elsewhere (24). Urine chemokine levels were standardized to urine creatinine measured in the same spot urine and expressed as pg/mg cr. All samples were assayed in duplicate in a single assay to avoid interassay variation. Our intra-assay variation for the ELISA measurements was below 3%. For measurement of TGF-β_1, we used a Quantikine kit (R&D Systems, Minneapolis, MN), and the samples were activated before the assay. The detection limits were 6 pg/mL for TGF-β_1, 8 pg/mL for MCP-1/CCL2, 2 pg/mL for RANTES/CCL5, and 6 pg/mL for IL-8/CXCL8.

Statistical analysis. The values are expressed as medians or means and SD, when appropriate. Analysis of variance, followed by the Newman-Keuls test, was used for multiple comparisons of means. The Mann-Whitney test was used to compare medians between two groups and Kruskal-Wallis test for multiple medians comparisons. Spearman test was used to test correlations. The level of significance was set at $p < 0.05$.

RESULTS

Subject characteristics and casual measurements. The control group ($n = 12$) included 5 boys and 7 girls ranging in age from 6.1 to 13.5 y. The mean values of weight, height, body mass index, systolic and diastolic pressures, and renal function parameters were within normal range (Table 1).

INS patients were divided according to steroid-responsiveness (SS or SR). The clinical and laboratorial features of each group at the time of blood and urine sampling are shown in Table 1. No differences were detected in age, sex distribution, weight, height, body mass index, nitrogen waste levels (urea and creatinine), uric acid, albumin, triglycerides, total cholesterol and GFR among INS groups and controls ($p > 0.05$, Table 1). Diastolic blood pressure was higher in the SR group than in SS or controls ($p < 0.05$). In addition, triglycerides, proteinuria and percentage of relapsing patients were significantly higher in the SR group when compared with SS ($p < 0.05$, Table 1).

As expected, almost all SR patients (22 among 24) were submitted to renal biopsies, which showed focal segmental sclerosis in 16 patients (73%) and diffuse mesangial proliferation in six (27%). In sharp contrast, only one SS patient was biopsied and histologic pattern evidenced MCNS (Table 1).

The majority of SR patients had previously received a course of cyclophosphamide (19 patients—79%) and/or cyclosporine (four patients—17%) according to our protocol recommendations as an attempt to induce disease remission. However, only one of them was still using cyclophosphamide associated with a low dose of corticosteroid at the time of blood and urine collection. As shown in Table 1, the others were receiving the combination of corticosteroids with angiotensin converting enzyme inhibitors (ACEi) (46%), or the isolated use of corticosteroids (25%) or ACEi (25%). None of the SS patients was treated with other medications rather than corticosteroids and only 3 among 8 patients were taking corticosteroids at the time of blood and urine collection due to present or recent disease relapse (Table 1).

As also evidenced in Table 1, the majority of our SR group (19 among 24 patients) still had an active disease at the time of collection, contrasting with the SS group in which only 2 out of 8 patients were in relapse.

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Table 1. *Subject characteristics, casual measurements and clinical features of age-matched healthy controls and of idiopathic nephrotic syndrome patients divided according to corticosteroid responsiveness*

Characteristics and measurements	SR group ($n = 24$)	SS group ($n = 8$)	Controls ($n = 12$)	p
Age (y)	12.8 ± 3.3	10.4 ± 4.9	11.0 ± 1.8	NS
Sex (%; male:female)	54:46	75:25	42:58	NS
Systolic BP (mm Hg)	108 ± 14	96 ± 13	101 ± 6	0.051
Diastolic BP (mm Hg)	73 ± 13*	63 ± 15	59 ± 6	<0.05
Weight (kg)	44.1 ± 12.7	32.3 ± 16.1	41.0 ± 8.0	NS
Height (cm)	145.6 ± 17.2	133.1 ± 28.5	144.6 ± 10.3	NS
BMI (kg/m^2)	20.4 ± 3.3	17.2 ± 2.0	19.6 ± 3.2	NS
Urea levels (mg/dL)	25.5 ± 13.8	23.4 ± 4.0	—	NS
Creatinine levels (mg/dL)	0.64 ± 0.23	0.50 ± 0.17	0.48 ± 0.10	NS
Triglycerides (mg/dL)	145 [98–187]†	65 [52–76]	—	<0.05
Total cholesterol (mg/dL)	193 [151–277]	153 [129–173]	—	NS
Albumin (g/dL)	3.9 [2.8–4.4]	4.2 [3.9–4.5]	—	NS
Uric acid (mg/dL)	5.2 ± 1.2	4.6 ± 0.7	—	NS
Glomerular filtration rate	146 ± 43	162 ± 26	174 ± 26	NS
Biopsy	22	1	—	—
MCD	0	1	—	—
FSGS	16	0	—	—
DMP	6	0	—	—
Medication in use			—	—
Steroid only	6	3	0	—
ACEi only	6	0	0	—
Steroid plus ACEi	11	0	0	—
Cyclophosphamide	1	0	0	—
None	0	5	12	—
Proteinuria (mg/m^2/24 h)	282 [107–875]†	50 [39–82]	—	<0.05
Disease activity (relapse:remission)	19 [79%]:5 [21%]‡	2 [25%]:6 [75%]	—	<0.05

Values are expressed as mean ± SD for all variables, except for triglycerides, cholesterol and albumin where medians, 25 and 75% percentiles are shown, and for biopsy and medication data, where the number of occurrences is shown.

Glomerular filtration rate was estimated using the Schwartz formula (22).

* $p < 0.05$ (SR *vs* SS and SR *vs* Controls, ANOVA test); † $p < 0.05$ (Mann-Whitney test); ‡ $p < 0.05$ (Fisher's test).

NS, non-significant; BMI, body mass index; MCD, minimal change disease; DMP, diffuse mesangial proliferation.

Plasma and urinary levels of cytokines and chemokines according to steroid responsiveness. No significant differences were detected in plasma concentrations of IL-8/CXCL8 and MCP-1/CCL2 in SR, SS or controls (Table 2). No differences were observed in the comparison of TGF-β_1 levels in SS patients and healthy controls. In contrast, plasma TGF-β_1 levels were 2.8-fold higher in SR when compared with controls ($p < 0.05$; Table 2 and Fig. 1*A*). Additionally, plasma TGF-β_1 levels were slightly but not significantly increased in SR patients when compared with SS group (Table 2 and Fig. 1*A*). We further divided the groups into four subgroups, according to disease activity (SR in relapse, SR in remission, SS in relapse and SS in remission). Although the highest TGF-beta levels occurred in relapsed SR patients, they only reached statistical significance when compared with control values ($p < 0.05$, Fig. 1*B*). Unfortunately, the Quantikine kit was not able to detect urinary TGF-beta 1 levels in the majority of urine samples from our INS patients. On the other hand, urinary concentrations of IL-8/CXCL8, MCP-1/CCL2 and RANTES/CCL5 were adequately measured and did not differ between SR and SS patients (Table 2).

Plasma and urinary levels of cytokines and chemokines according to disease activity. As shown in Table 3, plasma concentrations of TGF-β_1, IL-8/CXCL8 and MCP-1/CCL2, and urinary levels of MCP-1/CCL2 and RANTES/CCL5 were not significantly different between patients during remission and in relapse. However, the urinary measurements showed a significant elevation of IL-8/CXCL8 levels in INS patients in relapse, reaching values 2.9-fold higher than patients during disease remission ($p < 0.05$; Table 3 and Fig. 2*A*). We also divided the disease activity groups into subgroups according to steroid responsiveness (SR in relapse, SR in remission, SS in relapse and SS in remission). Figure 2*B* shows a clear trend of higher urine urinary IL-8/CXCL8 levels in the subgroups in relapse when compared with remission ones, but the small number of relapsed SS patients and SR in remission did not allow conclusive statistical comparisons. On the other hand, urinary IL-8/CXCL8 levels of relapsed SR patients were significantly higher than those of SS patients in remission ($p < 0.05$; Fig. 2*B*). Additionally, urinary levels of IL-8/CXCL8 positively correlated to proteinuria ($p < 0.05$; Fig. 3).

DISCUSSION

The pathogenesis of INS still remains obscure. The immune system is thought to play a pivotal role, and there is a lot of evidence that supports this theory (2,4–6). In 1974, Shalhoub (25) developed a hypothesis that INS was an immune disorder, with increased levels of a lymphocyte-derived permeability factor. Since then, several groups have studied possible factors that could be responsible, at least in part, for the physiologic abnormalities of INS (4,26). In this study, we specifically focused on the evaluation of blood and urinary concentrations of TGF-β_1, MCP-1/CCL2, RANTES/CCL5 and IL-8/CXCL8

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Table 2. *Cytokines and chemokines measurements in nephrotic patients according to corticosteroid responsiveness and in age-matched healthy controls*

ELISA measurements	SR group (n = 24)	SS group (n = 8)	Controls (n = 12)
Blood			
TGF-β_1 (ng/ml)	8.0 [3.8–20.1]*	5.0 [3.6–7.4]	2.8 [1.3–4.8]
IL-8/CXCL8 (pg/ml)	236 [144–293]	270 [187–329]	246 [154–314]
MCP-1/CCL2 (pg/ml)	117 [92–246]	59 [32–84]	38 [9–355]
Urine			
IL-8/CXCL8 (pg/mg cr)	18.7 [12.6–66.9]	19.7 [7.7–46.5]	—
MCP-1/CCL2 (pg/mg cr)	95.4 [54.1–262.7]	133.7 [78.2–180.3]	—
RANTES/CCL5 (pg/mg cr)	30.3 [16.6–53.3]	36.2 [28.2–43.1]	—

Values are expressed as medians, 25 and 75% percentiles.
* $p < 0.05$ (SR *vs* controls, Kruskal-Wallis test).
mg cr, milligrams of creatinine.

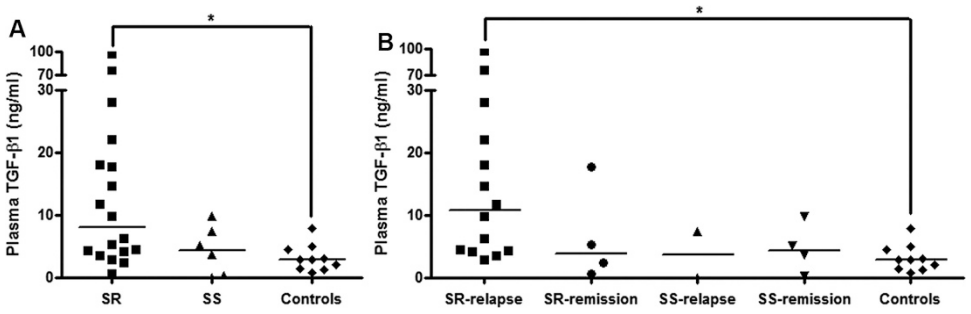

Figure 1. *A*, Comparison between plasma TGF-β_1 levels in steroid-resistant (SR, ■), steroid-sensitive (SS, ▲) nephrotic patients and age-matched healthy controls (Controls, ♦). *B*, Comparison between plasma TGF-β_1 levels in steroid-resistant patients in relapse (SR-relapse, ■), steroid-resistant in remission (SR-remission, ●), steroid-sensitive patients in relapse (SS-relapse, ▲), steroid-sensitive in remission (SS-remission, ▼) and in age-matched healthy controls (Controls, ♦). *$p < 0.05$ (Kruskal-Wallis test).

Table 3. *Cytokines and chemokines measurements in nephrotic patients according to disease activity*

ELISA measurements	Relapse (n = 21)	Remission (n = 11)
Blood		
TGF-β_1 (ng/ml)	7.4 [4.2–20.1]	5.0 [1.5–13.7]
IL-8/CXCL8 (pg/ml)	217 [121–267]	274 [187–366]
MCP-1/CCL2 (pg/ml)	100 [92–275]	100 [59–125]
Urine		
IL-8/CXCL8 (pg/mg cr)	45.9 [14.6–72.5]*	15.7 [10.9–37.5]
MCP-1/CCL2 (pg/mg cr)	98.8 [60.8–262.2]	126.9 [59.2–190.6]
RANTES/CCL5 (pg/mg cr)	30.3 [16.6–36.7]	40.0 [26.7–57.9]

Values are expressed as medians, 25 and 75% percentiles.
* $p < 0.05$ (Mann-Whitney test).
mg cr, milligrams of creatinine.

in children with INS, since previous reports described alterations of these immune mediators in diverse renal diseases, including glomerulopathies (8,12,15,19,27).

Steroid resistance in children with INS is considered a stronger factor for a poor prognosis than histologic findings (28). In this context, it has been suggested that TGF-β might contribute to the progression of renal disease, leading to accumulation of extracellular matrix (ECM) and tissue fibrosis (12,15). High TGF-β expression and an elevation in its urinary concentration were previously detected in patients with FSGS, but not in those with MCNS (15,27,29). In our study, plasma TGF-β_1 was found to be elevated in children with SR nephrotic syndrome when compared with healthy controls. It was also higher than the levels of SS patients, although the difference was not statistically significant. The absence of statistical difference between SR and SS could be due to the intrinsic limitations of the convenience sample. The small number of SS patients as well as the use of other medications in SR group such as ACEi and prednisone could interfere with the results. As previously reported, the treatment with ACEi reduced plasma TGF-β levels in experimental models of proteinuria and diabetic nephropathy (30,31). In addition, steroid administration also decreased renal expression of TGF-β in patients with severe proteinuria (15). Indeed, the use of these medications might have reduced plasma TGF-β_1 concentration in our SR patients, thus attenuating the differences between INS groups in our study. Another important aspect was the collection of samples from patients at different time-points during disease evolution. This fact could also interfere with the measurements. Our findings were not conclusive enough to establish a role for TGF-β in INS. However, we suggest that increased plasma TGF-β_1 in SR patients could be another pathogenic factor for the progression of renal disease. Further studies are obviously necessary to address this issue.

TGF-β_1 is known to induce production of MCP-1/CCL2 and RANTES/CCL5, chemokines that have been associated to ECM deposition and proteinuria (16,17,19). We evaluated these chemokines in INS patients, but we did not find differences in their plasma and urinary levels. However, this result did not exclude a role for TGF-β1 in INS, since the increase in expression of MCP-1/CCL2 and RANTES/CCL5 is only one of the proposed mechanisms of TGF-β action in progressive renal diseases. This cytokine is also implicated in the accumulation of extracellular matrix via increased synthesis and decreased degradation of its components and via up-regulation of integrins on cell surface, thus facilitating the deposition of matrix in the interstitial space (12).

There has been a long debate as to whether or not MCNS and FSGS are part of the same disease spectrum, since MCNS can evolve into FSGS (32). There have been reports

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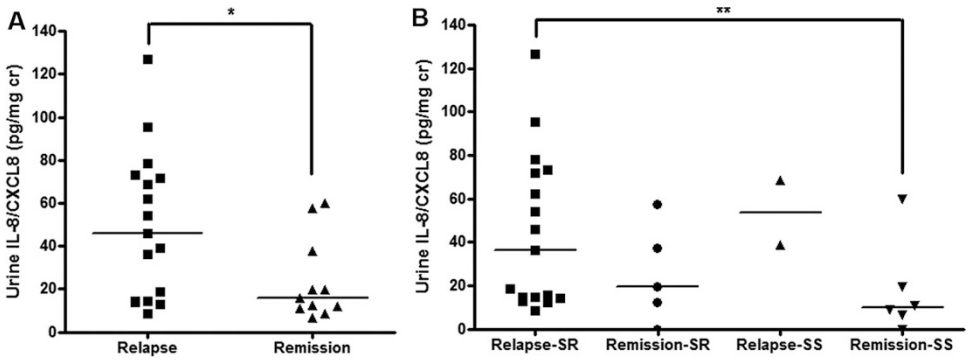

Figure 2. *A*, Comparison between urinary IL-8 (IL-8/CXCL8) levels in nephrotic patients in relapse (■) and remission (▲). *B*, Comparison between urinary IL-8 (IL-8/CXCL8) levels in relapsed steroid-resistant patients (Relapse-SR, ■), steroid-resistant in remission (Remission-SR, ●), relapsed steroid-sensitive patients (Relapse-SS, ▲), and steroid-sensitive in remission (Remission-SS, ▼). $*p < 0.05$ (Relapse *vs* Remission, Mann-Whitney test); $**p < 0.05$ (Relapse-SR *vs* Remission-SS, Kruskal-Wallis test).

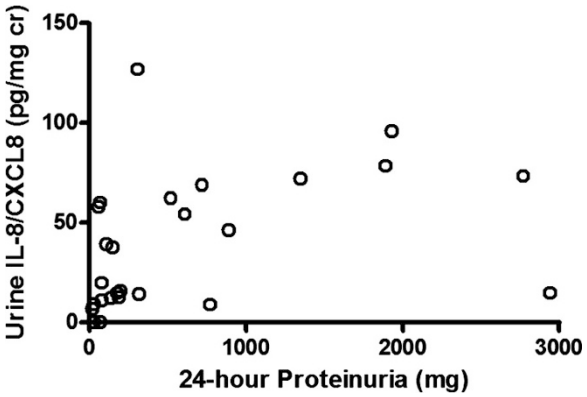

Figure 3. Correlation between urinary IL-8 (IL-8/CXCL8) levels and 24-h proteinuria in patients with idiopathic nephrotic syndrome. $*p < 0.05$, $r = 0.558$ (Spearman test).

on some cases of INS in children who were initially corticosteroid responsive with MCNS histology, but progressed to FSGS over a 10-y period of repeated renal biopsies (32). It has also been suggested that long-standing proteinuria may contribute to the transformation of MCNS to FSGS (33). In this context, the involvement of a circulating permeability factor in the pathogenesis of proteinuria has been suggested by several findings. In patients with FSGS, proteinuria often recur after receiving a normal kidney transplant (34), although kidneys with FSGS were successfully transplanted in other subjects with total remission of the disease (35). In addition, there have been reports of successfully treated FSGS by plasmapheresis (36), and induction of proteinuria in a newborn of a mother with FSGS (37). A potential candidate for a permeability factor is IL-8/CXCL8. Most studies that assessed this chemokine in INS found increased serum levels in patients during relapse, and normal concentrations in remission (7,8,38). IL-8/CXCL8 was also found to have effects on the metabolism of the glomerular basement membrane, possibly increasing glomerular permeability (7). Infusion of this chemokine in rats induced proteinuria, an effect that could be reversed by the infusion of anti-IL-8/CXCL8 neutralizing antibodies (7). In our study, plasma levels of IL-8/CXCL8 were not significantly different between groups, although the urinary levels were increased in INS patients in relapse. Increased urinary IL-8/CXCL8 was also described in patients with lupus nephritis and IgA nephropathy (39). On the other hand, in the same study, the levels of this chemokine remained unchanged in nephritic patients compared with controls (39). Although, it should be mentioned that all nephritic patients were in remission (39). More recently, Cho *et al.* (40) reported for the first time high concentrations of IL-8/CXCL8 in the urine and plasma of pediatric patients with MCNS during relapse. Accordingly, in our study, the increase of this chemokine was independent of histology or response to corticosteroids, and was also related to urinary protein excretion. Moreover, urinary levels of IL-8/CXCL8 were positively correlated to proteinuria. This finding cannot be attributed merely to the increased permeability itself, because the same was not found for MCP-1/CCL2 or RANTES/CCL5, proteins with similar functions and molecular weights (9,10).

We are aware of the limitations associated with the cross-sectional design of our study. The main possible weakness was the use of a convenience sample, which makes homogeneity among the selected groups very difficult to obtain, since the levels of proteinuria, the presence and previous use of immunosuppressive drugs and the stage of disease activity at the time of collection may interfere with cytokine and chemokine measurements. Nevertheless, some aspects of the study may increase the strength of our findings, such as the utilization of strictly defined inclusion and exclusion criteria and a well-established protocol for the measurements of cytokines and chemokines with very low intra-assay variability (24).

In summary, we found increased plasma TGF-β_1 in SR patients with preserved renal function when compared with healthy controls, suggesting that, at least in relapsed SR patients, some degree of fibrogenesis may be underway even at early stages of the disease. On the other hand, no changes in plasma TGF-beta levels were detected in the comparison of SS groups and controls. In addition, the cytokine levels in SR patients had a trend to be higher than in SS patients, but did not reach statistical difference probably due to the interference of disease treatment (corticosteroids and ACEi). Further studies are obviously necessary to confirm this possibility. More importantly, we also detected increased urinary IL8/CXCL8 in relapsed SR children when compared with SS patients in remission, with a positive correlation with urinary protein levels. This finding could be related to the effect of this chemokine on glomerular basal membrane and protein excretion, as described by other groups (7). Since plasma levels of IL8/CXCL8 were not different between groups, this result

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probably indicates an inflammatory process localized to renal tissue. Finally, our findings suggest that IL-8/CXCL8 and TGF-β_1 could be involved in the pathogenesis of INS: IL-8/CXCL8 associated with glomerular inflammation and local changes in permeability and TGF-β_1 probably correlated with worse response to corticosteroids.

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