Transplantation

Potential Role of Soluble ST2 Protein in Idiopathic Nephrotic Syndrome Recurrence Following Kidney Transplantation

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Background: Corticosteroid-resistant idiopathic nephrotic syndrome (INS) recurs rapidly after transplantation in 30% to 50% of transplant recipients, suggesting the presence of 1 or more circulating factors that alter the glomerular filtration barrier. We investigated the possible role in INS recurrence of soluble ST2 (sST2) protein, a marker of T helper type 2 (T_H2) cells and a factor predicted to be regulated by the transcription factor c-Maf; involvement of sST2 protein would be consistent with the observation that both T_H2 cells and c-Maf appear to be activated during INS relapse.

Study Design: Retrospective observational study.

Setting & Participants: Patients with biopsy-proven corticosteroid-resistant INS who had undergone kidney transplantation between September 1983 and April 2007 (n = 71). A control group consisting of proteinuric transplant recipients with kidney failure unrelated to INS (n = 34).

Predictor: Patients who developed INS recurrence after transplantation (n = 31) were compared with those in whom INS did not recur (n = 40) and the control group. Recurrence of INS was defined as urine protein excretion greater than 2 g/d immediately after transplantation that persisted at greater than 1 g/d despite treatment or a kidney graft biopsy showing minimal change glomerulonephritis or focal segmental glomerulosclerosis.

Outcomes & Measurements: Urine protein excretion in the 3 groups was 5.0 g/d (range, 1.3 to 10.5), 0.14 g/d (range, 0 to 0.46), and 4.3 g/d (range, 3 to 6.2). The sST2 protein was analyzed both quantitatively and qualitatively in patient sera, and its activity was tested in vitro on a mouse podocyte cell line and in vivo in rats.

Results: sST2 protein levels were significantly increased after transplantation in patients with INS recurrence compared with the 2 other groups (617.5 versus 23 pg/mL; $P < 0.001$ and 158.5 pg/mL; $P < 0.01$ respectively). However, patients with recurrence expressed a normal sST2 isoform, and the sST2 protein was unable to induce podocyte injury in vitro or trigger proteinuria in rats.

Limitations: Pretransplantation and posttransplantation sera do not always represent paired samples.

Conclusions: These data suggest that sST2 protein is a marker of INS recurrence that does not seem to be involved in the development of INS.

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INDEX WORDS: Idiopathic nephrotic syndrome; kidney transplantation; recurrence; sST2.

Editorial, p. 406

Idiopathic nephrotic syndrome (INS) is a glomerulopathy of unknown cause characterized by massive albuminuria without histological evidence of inflammatory injury or immune complex deposits. Recently, genetic abnormalities have been shown to be involved in INS. However, patients with treatment-resistant INS who progress to end-stage renal failure with focal and segmental glomerular sclerosis usually do not show genetic alterations.[1] After transplantation, recurrence of the initial disease is observed in 30% to 50% of patients, leading to graft loss in approximately 50% of cases.[2,3] In 90% of these patients, nephrotic syndrome recurs within the first few hours after transplantation, suggesting involvement

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of a circulating albuminuric factor. The beneficial effect of plasmapheresis also supports this hypothesis.[2,4,5] Several attempts have been made to determine the nature of this putative albuminuric factor, but its molecular characterization has remained elusive.

The recent observation of an association between INS relapse and an atypical T helper type 2 (T_H2) cell polarization, characterized by c-Maf activation and interleukin 4 (IL-4) downregulation,[6] raises the question of the implication of T cells in the pathophysiological process of this disease. In this study, we investigated the role of a soluble factor, soluble ST2 (sST2) protein, for which the promoter contains the c-Maf recognition element (D. Sahali, unpublished data), as a putative albuminuric factor in patients with INS who experience recurrence after transplantation. In humans, the ST2 gene codes for 2 main products by means of alternative splicing. One is a heavily glycosylated transmembrane protein of 61.5 to 80 kDa called ST2L, which is composed of an extracellular region with 3 immunoglobulin domains and an intracellular Toll–IL-1 receptor domain. The other is a soluble secreted protein of 50 to 60 kDa, sST2, which is made up of only the extracellular part of ST2L. The soluble form is secreted by activated T_H2 cells that express ST2L on their surface.[7] In mice, the ST2 protein has been described as a stable marker of a subset of activated T_H2 cells independent of the production of IL-4, IL-5, and IL-10,[8] although it is not a universal marker of this T-cell phenotype. Interestingly, several investigations have suggested an important role for ST2 in allergic airway inflammation,[9-11] and a relationship between respiratory allergies and proteinuria in some patients with nephrotic syndrome is well known.[12] That sST2 is associated with a T_H2-biased immune response and that its expression may be inducible by the c-Maf transcription factor indicate its potential role in INS pathological states, particularly in recurrence. In this report, we investigate the possible role of sST2 in human kidney transplant recipients with INS recurrence.

METHODS

Patients

Seventy-one patients with biopsy-proven corticosteroid-resistant INS who had undergone kidney transplantation between September 1983 and April 2007 were included. All patients were treated with an immunosuppressive regimen that included calcineurin inhibitors and antimetabolic drugs (mycophenolate mofetil or azathioprine) and/or corticosteroids, plasmapheresis, or immunoadsorption. Pretransplantation serum samples were collected within the 12 hours before surgery and kept frozen at −20°C. Patients who presented with proteinuria with protein greater than 2 g/d immediately after transplantation that persisted at greater than 1 g/d despite treatment during follow-up and who had a kidney graft biopsy specimen showing minimal change glomerulonephritis or isolated focal and segmental glomerular sclerosis lesions without other transplant-specific lesions were defined as patients with recurrent disease (n = 31). On the contrary, patients with INS with nonrecurrent disease (n = 40) had proteinuria with protein less than 1 g/d 1 week after transplantation, and this remained at less than 0.5 g/d thereafter. The control group consisted of 34 proteinuric transplant recipients with non–INS-related end-stage renal failure (diabetes, obstructive uropathy, reflux nephropathy, immunoglobulin A glomerulonephritis, nephroangiosclerosis, chronic interstitial nephropathy, and polycystic kidney diseases; Table 1). In this group, proteinuria was related to different kidney graft lesions: allograft glomerulonephritis, recurrence of immunoglobulin A nephritis, or diabetes. In these 3 cohorts, serum samples from 20 patients were collected both before and after transplantation (9 for the INS-recurrence group, 10 for the INS-nonrecurrence group, and 1 for the non-INS group).

All patients gave informed consent to participate in this study according to French legislative guidelines.

Quantification of sST2 in Patient Sera

The concentration of sST2 protein in serum samples of patients with INS and controls was determined by using a commercial enzyme-linked immunosorbent assay (Human ST2/IL-1 R4 DuoSet kit; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

In Vitro Studies

Analysis of sST2 Binding to Protein A

Full detail is provided in the supplementary methods (Item S1, available as online supplementary material with this article at www.ajkd.org). In brief, human sST2-containing supernatants from transiently transfected COS-7 cells were collected and passed through a protein A column. Bound proteins were eluted with 0.1 mol/L of glycine, and sST2 was detected by means of a Western blot using an anti–human ST2 antibody.

Two-Dimensional Gel Electrophoresis and Mass Spectrometry

Full detail is provided in Item S1 of the supplementary material. In brief, sST2 was purified from patient-derived plasma by using an immunoaffinity column and subjected to 2-dimensional gel electrophoresis. Spots of interest were excised, trypsin digested, and analyzed by using liquid chromatography–tandem mass spectrometry at the Biopolymers–Interactions–Structural Biology Platform at the INRA research center (Nantes, France).