T lymphocyte subsets and cytokine production by graft-infiltrating cells in FSGS recurrence post-transplantation

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Abstract

Background. Focal segmental glomerulosclerosis (FSGS) aetiology remains undefined although a derangement of lymphocytes and monocytes–macrophages, at least, has been strongly suspected. We report the graft-infiltrating phenotypes and their cytokine production in a case of FSGS recurrence post-transplantation.

Methods. The kidney transplant recipient suffered immediate FSGS recurrence. Aspiration biopsies were done at the first and second week post-surgery and were analysed by flow cytometry. The cytokine analysis was done on aspiration sample culture supernatants and serum by enzyme-linked immunosorbent assay.

Results. High expression of CD3CD69, CD3CD71 and CD4CD29 was found on infiltrating lymphocytes. Biopsy cultures pointed to a Th₀/Th₁ pattern of cytokine production as well as significant synthesis of transforming growth factor-β₁. Interestingly, monocyte chemokines were absent.

Conclusion. We report evidence of intragraft lymphocyte activation in the early days of FSGS recurrence. Aspiration biopsy cultures showed failure of cyclosporin A to inhibit interleukin-2 (IL-2) production by infiltrating lymphocytes. If our findings are confirmed in similar patients, a trial with anti-IL-2-receptor antibody could be warranted.

Key words: cytokines; flow cytometry; focal segmental glomerulosclerosis; recurrence; transplantation

Introduction

Focal segmental glomerulosclerosis (FSGS) recurs in 20–40% of patients receiving a first kidney transplant, with ensuing graft failure in half of them [1]. In a recent paper, Savin et al. [1] described a circulating factor in serum from recurrent FSGS patients which induces increased glomerular permeability to albumin. While both the biochemical characteristics and eventual targets of this factor remain unknown, it should reflect a disorder of lymphocyte function, most probably of T lymphocytes [2]. Saito and Atkins reported an increased number of monocytes–macrophages and lymphocytes in the interstitium throughout the evolution of experimental FSGS [3].

Previously, our group studied graft-infiltrating lymphocytes (GIL) in kidney transplant recipients, both stable and acutely rejecting patients [4–7]. We hypothesized that the study of GIL in a case of FSGS recurrence post-transplantation would give additional information concerning early phase pathogenesis.

Subject and methods

The patient presented with nephrotic syndrome when he was 33 years old, and renal biopsy showed FSGS. He was treated with oral steroids without any success, and reached end-stage renal failure 5 years later, still suffering from unremitting nephrotic proteinuria. He was started on regular haemodialysis and, 11 months later, he received a cadaver renal transplant. Donor and recipient HLA were A22, B8, DR1, DQ1, and A28, B40, DR5, DQ3, respectively. The patient was treated from the beginning with a new cyclosporin formulation, azathioprine and prednisolone, and his graft started to function immediately. However, as he remained nephrotic with proteinuria from 5 to 10 g/day, a core renal biopsy was done on day 14 and repeated at 5 weeks post-transplantation. He received a course of plasmapheresis from the third week on without any success.

GIL were obtained by fine-needle aspiration biopsy (FNAB) following the methods described by Häyry [8] on days 7 and 14 post-surgery. The samples were aspirated into 6 ml of RPMI medium with 125 U/ml of lithium heparin. A 500 μl aliquot was submitted to a brief cytocentrifugation and, after staining by a modified Romanowsky method, GIL were classified as described [8].

One ml of each sample was analysed by flow cytometry using a FACScan from Becton-Dickinson® as was the corresponding peripheral blood sample; monoclonal antibodies were purchased from Coulter® and Becton-Dickinson®. After red blood cell lysis, the cell concentration in the
remaining 4.5 ml of each aspiration sample was adjusted to 5 \times 10^{6} \text{ cell/200 ml}, and cultured on RPMI medium supplemented with 10% autologous serum and recombinant interleukin-2 (rIL-2) at 10 Un/ml. The incubation was done at 37°C and 5% CO₂ and, after 48 h of culture, six wells were pulsed with [³H] thymidine at 1 μCi/well. The assessment of tracer incorporation was done by a Beckman beta-counter 16 h later. From the remaining wells, we collected supernatants at 48 h of culture, that were kept at −70°C until tested for the following cytokines: IL-2, IL-4, IL-10 and interferon-γ (IFN-γ), measured by enzyme-linked immunosorbent assays (ELISAs) provided by Endogen®. Cambridge, MA, and IL-1ra, IL-4-sRα, IL-6, IL-8, IL-12, IL-13, granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage CSF (M-CSF), macrophage inflammatory peptide-1α (MIP-1α), transforming growth factor-β₁ (TGF-β₁) and TGF-β₂ evaluated by ELISAs from R&D Systems®. MN. Serum from day 7 and day 14 was also tested for IL-2 and IL-10. After 96 h of incubation, cells were aspirated from the wells and, following a brief cytocentrifugation, they were stained by a modified Romanowsky method.

Trough blood cyclosporin levels were measured by a monoclonal TDx® assay from Abbott. The patient gave his informed consent and this study is part of a research project approved by local Ethics Committee.

Results

A second core renal transplant biopsy carried out in the fifth week post-transplantation showed FSGS. Electron microscopy of the first biopsy evidenced mesangial enlargement and effacement of visceral cell foot processes.

Serum creatinine on days 7 and 14 was 2.0 and 2.3 mg/dl, respectively, and blood cyclosporin levels were 223 and 250 μg/ml.

Both fine-needle aspiration biopsies were classified as grade III, i.e. a borderline probability of rejection; no blasts were seen. Proliferation results on days 7 and 14 were (median \pm SEM) 1116 \pm 100.4 and 299 \pm 64.2 c.p.m., respectively.

At the end of 96 h of incubation, on light microscopy, the day 7 culture displayed well preserved renal cells and blast transformation in 10% of lymphocytes; no macrophages were seen. Day 14 culture did not show signs of lymphocyte activation, and half of the kidney cells exhibited mild to moderate vacuolization.

In Table 1, we present the cytofluorometric analysis of T subsets present in FNAB samples and in peripheral blood lymphocytes (PBL). While the markers identifying early T cell activation were highly expressed on FNAB T cells, DR expression (late activation marker) on CD3 and CD8 subsets, which has shown to correlate with acute rejection [6], was present at the same order of magnitude as in a large group of stable kidney transplant patients previously analysed by us [6]. Interestingly, CD4CD29 expression on GIL was prominent by day 14.

On day 7, circulating IL-2/IL-10 were 0/8 pg/ml; on day 14 they were 0/24 pg/ml, respectively.

The first week aspiration biopsy culture supernatant was evaluated for IL-2, IL-4, IL-10 and IFN-γ; the values measured were 381, 0, 8 and 0 pg/ml, respectively. From the day 14 aspiration biopsy culture supernatant, we confirmed a very high value for IL-2, 1188 pg/ml, curiously followed by a small increase in IL-10, 24 pg/ml. We also measured small amounts (pg/ml) of IL-1ra, 30; IL-4-sRα, 6; IL-8, 263; and IL-12, 4. TGF-β₁ increased to 780 pg/ml, but TGF-β₂ was not present. Interestingly, we did not find any trace of IL-6, GM-CSF, M-CSF or MIP-1α, and IL-4 was also absent.

Discussion

Our patient suffered an early recurrence of FSGS post-transplantation. (The recipient of the other kidney of this pair is enjoying good graft function without any abnormalities in urinary sediment.) The first core renal biopsy done on day 14 failed to provide enough tissue for analysis by light microscopy, but electron microscopy showed expansion of the mesangial area.

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Discussion

Our patient suffered an early recurrence of FSGS post-transplantation. (The recipient of the other kidney of this pair is enjoying good graft function without any abnormalities in urinary sediment.) The first core renal biopsy done on day 14 failed to provide enough tissue for analysis by light microscopy, but electron microscopy showed expansion of the mesangial area. The second core renal biopsy at 5 weeks post-transplantation displayed a full-blown FSGS picture. As our patient has been free of any rejection episodes, we believe that our findings on graft-infiltrating lymphocytes, unlike what we have reported in stable kidney transplant recipients [6], may reflect mainly changes brought about by FSGS recurrence.

Flow cytometry analysis showed signs of intra-graft activation, manifested by high expression of CD3CD25, CD3CD71, CD3CD69 and CD2CD54, at the peripheral blood level this was less clear, as expression of CD3CD69 and CD3CD71 was considerably lower. While our patient presented low expression of CD3CD71 at the periphery, it was expressed in 39% of intra-graft lymphocytes. This is one of the highest values we have observed; in a group of 52 stable renal transplant patients, CD3CD71 expression
was found in 7.5 ± 6.2% of cells [6]. Also, CD4CD29, which identifies memory T cells, showed much higher expression on FNAB T cells from our patient on day 14 compared with what we found in stable patients (26 ± 10.8) [6]. This could reflect expansion of memory T cell clones specifically engaged in FSGS recurrence. On the other hand, we previously have defined an activation score for GIL studied by flow cytometry that enabled us to differentiate between stable vs acutely rejecting renal transplant recipients [6]. This score is based on FNAB T cell subsets that change most during an acute rejection crisis and it is obtained as the sum of the percentage of phenotypes multiplied by empiric coefficients: DR × 8 plus CD8DR × 32 plus CD3DR × 16 plus CD8CD57 × 4 plus the ratio FNAB/PBL CDS25 × 40 plus FNAB/PBL CDS3 × 100 minus CD4CD45RA × 4 (this subset is significantly down-regulated during acute rejection). This activation score reaches a sensitivity of 83.9% and a specificity of 90.5% for acute rejection using a cut-off of 630 points [6]. With regard to our patient, this score reached 478 points on day 7 and came down to zero on day 14 post-transplantation, both well below the cut-off value for acute rejection, thus suggesting that different T subsets were involved in this FSGS recurrence compared with acute rejection, namely CD3DR and CD8DR, both significantly up-regulated during rejection [6].

Cytokine analysis of serum showed the absence of circulating IL-2 and a small amount of IL-10, which is in agreement with previous observations in a group of 43 transplant patients [7]. On the contrary, there was a significant production of IL-2 by aspiration biopsy cultures. Actually, both IL-2 values, measured from day 7 and day 14 samples were >250 pg/ml, which according to our experience is a reliable cut-off value for acute rejection crisis in kidney transplants reaching 100% sensitivity and 87.2% specificity [7]. Others have reported elevated circulating IL-2 receptor levels in children with FSGS and nephrotic syndrome [9]. Our patient presented an up-regulation of CD3CD25 expression on PBL in the second week post-transplantation despite the absence of IL-2 in serum, probably related to cyclosporin A. The absence of IL-6 is interesting as this cytokine, besides being produced by Th1 type lymphocytes, is also a product of mesangial cells [10] and this could be the counterpart to the absence of mesangial proliferation seen in our patient, as well as in other cases of FSGS. Actually, IL-6 synthesis by day 7 FNAB cultures in stable patients reached 1164 ± 440 pg/ml [5]. This different IL-6 behaviour in our patient could reflect both a strong Th1 deviation and a lack of IL-6 production by monocytes/macrophages that were absent from both aspiration samples. Interestingly, IL-1ra synthesis by our patient aspiration biopsy culture was down-regulated compared with what we found previously in stable recipients, 313 ± 80 pg/ml [5]. The lower IL-1ra observed may allow a stronger T cell stimulation as well as higher expression of intercellular adhesion molecules [11], as we documented 32% for FNAB CD2CD54 on day 7, compared with 19 ± 16.4% found in 51 stable patients previously studied [6]. The high value measured for TGF-β1 could reflect the initiation of fibrogenesis [12] although cyclosporin A is thought to be a stimulant of TGF-β1 synthesis.

The proliferation results, much higher on day 7 than on day 14, probably reflect two phases, a dividing mode followed by a synthetic phase. Actually, the proliferation measured on day 7 is within the values found in 28 patients with acute rejection, 1374 ± 108 c.p.m. [4], which we regard as another indirect sign of GIL activation. Alternatively, this could reflect the start of monocyte–macrophage proliferation involved in the early phases of FSGS [3].

In summary, we describe an FSGS recurrence in the first 2 weeks post-surgery in a kidney transplant patient. As far as we are aware, this is the first report of clear signs of intragraft lymphocyte activation during the early days of FSGS recurrence post-transplantation with a strong IL-2 production, although cultures also showed IL-10. An enhancement of GIL CD4CD29 expression was seen on day 14, probably reflecting specific memory T cell clones involved in the process. Altogether, our findings could mark the involvement of lymphocytes on the procedure escaping cyclosporin A immunosuppressive (IL-2 inhibition) effects. Furthermore, we measured important TGF-β1 synthesis that could well be associated with early fibrigenogenesis. Provided a future study involving more FSGS recurrence cases confirms that a IL-2 rise is both involved in early stages of recurrence and, at the same time, constitutes a marker of the inability of cyclosporin A to suppress lymphocyte response, a trial with anti-IL-2-receptor antibody could be warranted.

References

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