

# Antibody-Mediated Rejection With a Striking Interstitial Monocyte/Macrophage Infiltration in a Renal Allograft Under FTY720 Treatment

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FTY720, a novel immunomodulator, causes rapid temporary depletion of peripheral-blood lymphocytes, inducing their sequestration in secondary lymphoid organs. FTY720 is effective in animal models of transplantation and is under evaluation for use in human transplantation. We report a 48-year-old renal transplant recipient who developed acute antibody-mediated rejection under a high-dose FTY720 (5 mg/d), low-dose cyclosporine A, and prednisone treatment protocol. A T-cell antihuman globulin and National Institutes of Health extended B-cell cross-match with donor cells were negative before transplantation. At 10 weeks posttransplantation, serum creatinine level increased and a renal biopsy showed a striking interstitial CD68<sup>+</sup> monocyte/macrophage infiltration with C4d staining of peritubular capillaries. Flow panel reactive antibody levels were positive in the recipient's serum for class I (9%) and class II (75%). The positive panel reactive antibody levels and presence of C4d in peritubular capillaries justified the diagnosis of antibody-mediated rejection. However, the presence of macrophage-rich interstitial infiltrate suggested a contribution of cellular rejection. The morphological characteristic of rejection with a striking interstitial CD68<sup>+</sup> monocyte/macrophage infiltration with paucity of T cells is very unusual and may reflect a unique effect of FTY720 therapy.

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**INDEX WORDS:** FTY720; lymphocyte depletion; acute allograft rejection; antibody-mediated rejection; C4d; pathology; kidney; transplantation.

**F**TY720 is the first in a new class of immunomodulators that are sphingosine-1 phosphate receptor (S1P-R) agonists. The efficacy of this agent was shown in experimental models of solid-organ and islet transplantation, autoimmune disease, and cancer.<sup>1-3</sup> FTY720 has a novel model of action in that stimulation of S1P-Rs produces peripheral lymphopenia by reducing the recirculation of lymphocytes from lymphatic tissue to blood and peripheral tissues.<sup>4-6</sup> It does not impair antigen-driven T-cell activation, proliferation, cytokine production, or signal transduction at clinically relevant concentrations and thus acts without inducing general immunosuppression.<sup>4</sup>

Administration of FTY720 prolonged graft survival in animal models of liver, kidney, and cardiac transplantation.<sup>7,8</sup> Two phase 1 clinical trials showed the efficacy and safety of FTY720 in stable renal transplant recipients.<sup>9-11</sup> A phase 2 study showed that FTY720, 2.5 mg, and cyclosporine A (CsA), or FTY720, 5 mg, combined with reduced-dose CsA was as effective in preventing acute rejection as mycophenolate mofetil and CsA.<sup>6,12-14</sup> However, the protective effect of FTY720 against antibody-mediated rejection (AMR) was unclear.

Supporting this concern, recent randomized controlled trials showed that 5 mg of FTY720 cannot compensate for a decrease in CsA with increased acute rejection rates.<sup>15,16</sup> Here, we describe a renal transplant recipient who developed AMR under high-dose FTY720 (5 mg/d), low-dose CsA, and prednisone treatment. Serial biopsies of the renal allograft showed an unusual rejection morphological state with striking interstitial monocyte/macrophage infiltration that may be a new rejection pattern under the influence of FTY720.

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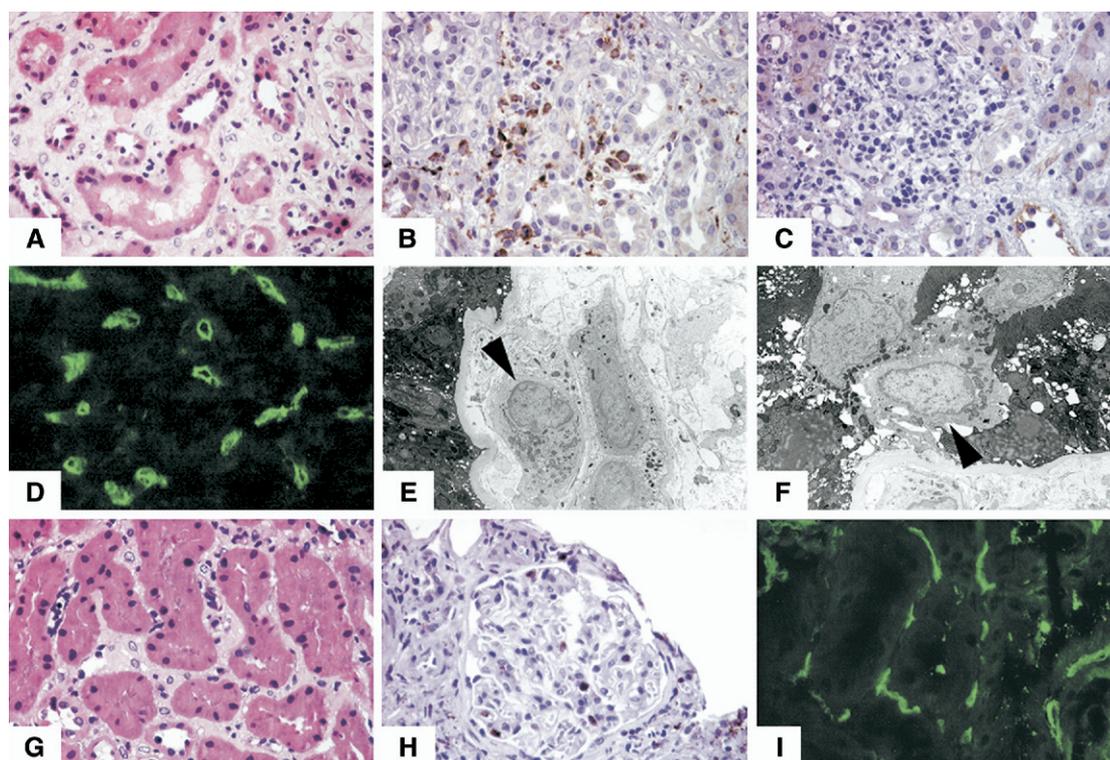
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**Figure 1.** (A to F) The first allograft biopsy specimen shows (A) an interstitial infiltrate of numerous large mononuclear cells with pale nuclei. (Hematoxylin and eosin; original magnification  $\times 400$ .) (B) These interstitial large mononuclear cells are positive for monocyte/macrophage lineage marker CD68 (immunoperoxidase; original magnification  $\times 400$ ) and (C) negative for CD31. Note the CD31 positivity in endothelial cells of an adjacent artery. (Immunoperoxidase; original magnification  $\times 400$ .) (D) Indirect immunofluorescence shows strong and diffuse linear peritubular capillary staining for C4d. (Cyanine; original magnification  $\times 250$ .) (E) Electron microscopy confirmed the presence of numerous large macrophage-like cells (arrowhead) in the interstitium. (Uranyl acetate–lead citrate; original magnification  $\times 3,000$ .) (F) Tubulitis lesion with macrophage-like cells (arrowhead) invading the tubular cells. (Uranyl acetate–lead citrate; original magnification  $\times 3,000$ .) (G) The second allograft biopsy specimen shows the persistent interstitial infiltrate of monocyte/macrophage lineage cells. (Hematoxylin and eosin; original magnification  $\times 400$ .) (H) The third allograft biopsy specimen (H–I) shows transplant glomerulitis with CD68<sup>+</sup> cells in glomerular capillary loops. (Immunoperoxidase; original magnification  $\times 400$ .) (I) Moderate diffuse C4d positivity along peritubular capillaries on the third biopsy specimen. (Cyanine; original magnification  $\times 250$ .)

### CASE REPORT

A 48-year-old man with end-stage renal disease caused by fibrillary glomerulonephritis received a living related kidney in August 2003. There were 2-antigen mismatches at B and DR loci. The T-cell antihuman globulin and National Institutes of Health extended B-cell cross-match with donor cells were negative before transplantation. Immunosuppressive treatment consisted of high-dose FTY720 (5 mg/d), low-dose CsA, and prednisone. Target CsA doses were C2 levels of 400 to 500  $\mu\text{g/L}$ . The graft functioned immediately. At 10 weeks posttransplantation, serum creatinine (SCr) level increased to 2.8 to 3.1 mg/dL (250 to 270  $\mu\text{mol/L}$ ). An allograft biopsy showed mild interstitial infiltrate of mononuclear inflammatory cells with mild tubulitis. The interstitial infiltrate had an unusual number of large mononuclear cells with pale nuclei presumed to be of monocyte/macrophage lineage (Fig 1A). By means of immunohistochemistry, these cells were positive for CD68 (Fig 1B) and negative for CD3 and CD31 (Fig 1C). No intimal arteritis or

glomerulitis or peritubular capillaritis was observed. By means of immunofluorescence, diffuse linear C4d staining of peritubular capillaries (PTCs) was observed (Fig 1D). Electron microscopy confirmed the presence of numerous interstitial macrophages (Fig 1E). Some of these macrophages had invaded the tubules (Fig 1F). Flow panel reactive antibody levels at the time of biopsy were 9% class I and 75% class II. The T-cell cross-match was weakly positive, and the B-cell cross-match was strongly positive. Donor-specific antibodies to class I B27 and class II DR4 and DR14 were identified. After finding this, a serum sample drawn 2 months pretransplantation was screened by using flow panel reactive antibodies. This was negative for both class I and II antibodies. The presence of donor-specific antibodies and C4d positivity meet criteria for a diagnosis of AMR.<sup>17</sup> However, the presence of prominent interstitial monocytic infiltrate and occasional tubulitis suggests a contribution of cellular rejection. Twelve weeks posttransplantation, SCr level was 2.8 mg/dL (250  $\mu\text{mol/L}$ ) and the patient was

treated with a course of methylprednisolone sodium succinate (Solu-Medrol; Pfizer Pharmacia & Upjohn Company, New York, NY) pulses and then a tapering dose of prednisone. SCr level measured 4 days after methylprednisolone sodium succinate administration was 1.9 mg/dL (164  $\mu$ mol/L) and ultimately decreased to 1.3 mg/dL (116  $\mu$ mol/L) at 14 weeks posttransplantation.

Because SCr level subsequently increased to 1.8 mg/dL (158  $\mu$ mol/L) at week 16 posttransplantation, a second biopsy was performed. The biopsy specimen showed peritubular capillaritis and glomerulitis and substantial numbers of interstitial macrophages (Fig 1G) with diffuse PTC C4d positivity. The biopsy specimen was consistent with recurrent AMR; therefore, 3 doses of intravenous immunoglobulin, 2 g/kg/mo, were administered; FTY720 therapy was withdrawn; and mycophenolate mofetil therapy was introduced.

At 26 weeks posttransplantation, SCr level was stable at 1.7 mg/dL (150  $\mu$ mol/L) and the third biopsy was performed. The biopsy specimen showed CD68<sup>+</sup> macrophages in glomerular capillary loops (Fig 1H) and interstitium with persistent diffuse PTC C4d positivity (Fig 1I). Graft function subsequently stabilized. However, at 24 months posttransplantation, SCr level increased to 1.7 mg/dL (153  $\mu$ mol/L). The fourth biopsy showed chronic active AMR with transplant glomerulopathy, C4d positivity in PTCs, and PTC basement membrane multilayering. Graft function is still stable at 35 months posttransplantation with an SCr level of 1.6 mg/dL (145  $\mu$ mol/L).

## DISCUSSION

FTY720 transiently decreases mature lymphocytes in peripheral blood, affecting T cells more than B cells.<sup>4,5</sup> The phosphorylated FTY720 metabolite acts as an agonist at G protein-coupled S1P-Rs on lymphocytes and induces internalization/degradation of the receptor. This hinders S1P-R1-dependent egress from lymphoid organs. Thus, lymphocytes are trapped in lymph nodes and are not available in the peripheral circulation to localize to graft sites.<sup>1</sup>

In contrast to phase 2 studies, phase 3 studies showed increased rates of acute rejection in patients treated with FTY720, 5 mg, and decreased CsA dose.<sup>15,16</sup> Here, we report that high-dose FTY720 combined with decreased-dose CsA/prednisone failed to protect against AMR in a renal transplant recipient. This recipient was not presensitized because pretransplantation serum was negative for HLA antibodies. However, at 10 weeks posttransplantation, donor-specific antibodies were present. We conclude that FTY720 in the context of low calcineurin inhibitor concentrations is not efficacious in preventing AMR. Furthermore, caution is warranted in

the use of this agent in highly sensitized transplant recipients.

C4d<sup>+</sup> rejections with a monocyte/macrophage-rich interstitial infiltrate in patients administered therapeutic agents causing peripheral lymphopenia should be regarded as mixed rejections, and both cellular and humoral rejection components may require treatment. The current case is unlikely to be pure AMR<sup>17,18</sup> because there was a constant interstitial infiltrate of macrophages and the patient responded to pulse steroid therapy. This histological characteristic is unusual for pure AMR, which includes aggregation of inflammatory cells in capillaries, rather than in interstitium.<sup>17</sup> Macrophages recently were shown as effectors of tissue damage in acute rejection.<sup>19</sup> Furthermore, a clinical trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab, which induces lymphocyte sequestration to the lymph nodes, showed that rejection episodes in transplant recipients treated with alemtuzumab were characterized by predominant infiltration by macrophages, and in most patients, dysfunction was seen without lymphocytic tubulitis and Banff criteria for rejection were not met.<sup>20</sup> KRP-203, another novel S1P-R agonist, was reported to be effective to prevent rejection in rat skin and heart allografts,<sup>21</sup> but pathological characteristics with this agent are unclear.

Given that pretransplantation panel reactive antibody was negative in this patient, there appears to have been a de novo humoral response that would require T-cell help. Antigen-specific T and B cells are essential for humoral immune responses to many antigens.<sup>22,23</sup> Because FTY720 causes lymphocyte sequestration into secondary lymphoid organs and does not impair T-cell functions, it is possible to have an ongoing cognate T-cell-B-cell interaction helping B cells produce antibodies.

Here, we report an unusual AMR pattern with striking interstitial CD68<sup>+</sup> macrophage infiltration in a renal allograft in a patient administered FTY720. This observation may reflect a unique effect of FTY lymphocyte sequestration to the lymph nodes. Thus, we suggest that morphological characteristics of renal allograft rejection with the new drug FTY may be different from those encountered before and new rules for the diagnosis of rejection may apply.

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