Successful Treatment of Recurrent Rejection in Renal Transplant Patients with Photopheresis

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Abstract. Photopheresis (ECP) is a new form of photochemotherapy that induces a selective inhibition of the host response to foreign histocompatibility antigens and reverses allograft rejection after organ transplantation. This report describes four adolescent patients with recurrent rejection episodes after renal transplantation, all uncontrolled using standard protocols of immunosuppression (intravenous steroids and OKT3), yet successfully treated with a 6-mo course of ECP. The ECP treatment was performed at weekly intervals during the first month, at 2-wk intervals during the second and third months, and then monthly for another 3 mo. Creatinine clearance improved throughout the treatment in three patients and remained unchanged in one. All patients had a pre-ECP biopsy with a grade 2 or 3 rejection (Banff) with a diffuse infiltrate CD8, CD14, LFA-1 (166 cells positive/0.048 mm²), and VLA-4 (51 cells positive/0.048 mm²) positive, as well as a tubular expression of HLA-DR (6.2 sections of tubule positive/0.048 mm²), ICAM-1, and VCAM-1 (3.1 and 2.9 sections of tubule positive/0.048 mm²). A strong reduction of cell infiltrate and expression of LFA-1 (6.6 cells positive/0.048 mm²), VLA-4 (0.7 cells positive/0.048 mm²), HLA-DR (0.2 section of tubules positive/0.048 mm²), ICAM-1 (0.3 section of tubules positive/0.048 mm²), and a disappearance of VCAM-1 staining were observed in the biopsies performed after 3 mo of ECP. All patients remained rejection-free during ECP, without infections or other complications commonly observed with increasing doses of standard immunosuppression. The clinical improvement allowed a progressive reduction of oral steroids in three of the four patients treated. (J Am Soc Nephrol 9: 121–127, 1998)

Allograft rejection is still one of the major issues in clinical transplantation. Almost 50% of graft failures are caused by rejection, and a large part of recipients of cadaver kidneys have at least one episode of rejection in the first 2 yr after transplantation (1). Several therapeutic and prophylactic measures, including high-dose steroids, antilymphocytic globulin, and a variety of monoclonal antibodies, have been used in the attempt to prevent and reverse allograft rejections (2,3). However, some major problems, such as a nonselective mechanism of action, high toxicity that limits the amount of drug often needed for rejection control, and an elevated risk of complications (i.e., infections and malignancies), are related to the administration of the usual immunosuppressive protocols. In addition, long-term allograft survival may be limited by the development of a chronic allograft nephropathy, in which several immunologic and nonimmunologic factors such as drug nephrotoxicity, hypertension, infections, and chronic rejection may play a role.

Photopheresis (ECP) is a new form of extracorporeal photopherotherapy currently used for the treatment of cutaneous T cell lymphoma (CTCL) and in some T cell-mediated diseases, including pemphigus vulgaris, scleroderma, rheumatoid arthritis, graft-versus-host disease, and systemic lupus erythematosus (SLE) (4–9). During ECP, 5 to 7 × 10⁹ peripheral lymphocytes are collected by apheresis and treated in an extracorporeal device with 8-methoxypsoralen (8-MOP) and UVA light (10,11). Because ECP has been demonstrated to downregulate both neoplastic and autoreactive T cell clones, respectively, in CTCL and autoimmune diseases, it has been hypothesized that it could even modulate alloreactive T cell populations responsible for allograft rejection after organ transplantation.

After preliminary experiments in animal models showing that its combination with standard immunosuppression prolongs the survival of transplanted organs, the efficacy of ECP has been demonstrated in cardiac transplant patients with acute rejection (12–14). ECP has also been used in subjects with a high risk of rejection, hypersensitized by previous transplantation or multiple pregnancies (15). Prolonged ECP treatment has also been shown to result in a significant reduction of coronary intimal hyperplasia after cardiac transplantation (16). More recently, a prospective multicenter study of 60 cardiac transplant patients showed that prophylactic treatment with ECP reduced the frequency of rejection episodes without increasing infection risk (17). Similarly, patients with recurrent rejection after cardiac transplantation experienced a reduction in the number and severity of rejection episodes without any complications after a 6-mo course of ECP (11).

ECP has been recently used in a few cases for the treatment of recurrent rejection in renal transplant patients, with promising results (18). In this report, we describe four patients with recurrent rejection episodes after renal transplantation, all uncontrolled using standard protocols of immunosuppression (intravenous steroids and OKT3), yet successfully treated with a 6-mo course of ECP. The ECP treatment was performed at weekly intervals during the first month, at 2-wk intervals during the second and third months, and then monthly for another 3 mo. Creatinine clearance improved throughout the treatment in three patients and remained unchanged in one. All patients had a pre-ECP biopsy with a grade 2 or 3 rejection (Banff) with a diffuse infiltrate CD8, CD14, LFA-1 (166 cells positive/0.048 mm²), and VLA-4 (51 cells positive/0.048 mm²) positive, as well as a tubular expression of HLA-DR (6.2 sections of tubule positive/0.048 mm²), ICAM-1, and VCAM-1 (3.1 and 2.9 sections of tubule positive/0.048 mm²). A strong reduction of cell infiltrate and expression of LFA-1 (6.6 cells positive/0.048 mm²), VLA-4 (0.7 cells positive/0.048 mm²), HLA-DR (0.2 section of tubules positive/0.048 mm²), ICAM-1 (0.3 section of tubules positive/0.048 mm²), and a disappearance of VCAM-1 staining were observed in the biopsies performed after 3 mo of ECP. All patients remained rejection-free during ECP, without infections or other complications commonly observed with increasing doses of standard immunosuppression. The clinical improvement allowed a progressive reduction of oral steroids in three of the four patients treated. (J Am Soc Nephrol 9: 121–127, 1998)
of rejection after renal transplantation (18–20). These reports prompted us to evaluate the beneficial effect of ECP in four renal transplant recipients with repeated rejection episodes uncontrolled by standard immunosuppression.

Materials and Methods

Patients

Four renal transplant recipients (two men) with a mean age of 16.5 yr (range, 15 to 17 yr) were entered in the study. Table 1 summarizes the patients’ clinical data. The patients were considered eligible for ECP because they all had experienced at least three acute rejection episodes that recurred after standard immunosuppressive therapies.

Routine immunosuppression consisted of oral methylprednisolone, in combination with cyclosporine and azathioprine. Acute rejection episodes were treated with high-dose methylprednisolone (1.5 g/m² per course), whereas steroid failure resulted in the use of OKT3 (5 mg/d) for 10 d.

Photopheresis Procedure

ECP was performed using the UVAR Photopheresis Instrument provided by Therakos (West Chester, PA), as described previously (11). Briefly, 240 ml of buffy coat and 300 ml of plasma were removed during each treatment by apheresis and diluted with 200 ml of saline solution. To this final, enriched lymphocyte solution containing ±6.8 x 10⁹ cells (range, 5.8 to 7.2 x 10⁹), 200 μg of 8-MOP (Oxsoralen, Gerot, Vienna, Austria) were added. The solution was exposed in an extracorporeal system for 90 min to a UVA light source (2 J/cm²) and then returned to the patient. A liquid psoralen preparation was used because effective blood concentrations of 8-MOP are often difficult to obtain after oral administration due to the high inter-and intraindividual variability of the absorption.

Treatment Protocol

ECP was performed with the following schedule: two consecutive treatments at weekly intervals during the first month, two treatments at biweekly intervals during the second and third months, and then monthly for another 3 mo.

Table 1. Clinical data of study patients*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient</th>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Age</td>
<td>17</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>F</td>
</tr>
<tr>
<td>Pretransplant renal disease</td>
<td>MPGN</td>
</tr>
<tr>
<td>Length of dialysis before Tx (mo)</td>
<td>18</td>
</tr>
<tr>
<td>Panel reactivity pre-Tx</td>
<td>0</td>
</tr>
<tr>
<td>Follow-up Tx pre-ECP (mo)</td>
<td>3</td>
</tr>
<tr>
<td>Rejection episodes pre-ECP</td>
<td>3</td>
</tr>
<tr>
<td>I.V. methylprednisolone courses pre-ECP</td>
<td>2</td>
</tr>
<tr>
<td>OKT3 treatment pre-ECP</td>
<td>+</td>
</tr>
</tbody>
</table>

* MPGN, membranoproliferative glomerulonephritis; NP, nephronophthisis; CU, complex urinary tract malformation; HY, renal hypoplasia; Tx, transplant; ECP, photopheresis; I.V., intravenous.

Laboratory Evaluation

Each patient received a baseline laboratory and immunologic evaluation, including complete blood cell count, liver and kidney function, electrolytes, and T cell subpopulations. Serum protein electrophoresis, quantitative immunoglobulin levels, and hepatitis A, B, and C markers were also monitored.

Renal Biopsies

A percutaneous biopsy was performed under ultrasound guidance at the beginning of the treatment and again 3 mo later. The histologic findings of the renal biopsies were graded according to Banff’s international criteria after classical staining with ematoxylin and eosin, periodic acid-Schiff, Silver periodic acid-Schiff, and Masson’s-Trichrome (21). The grade of interstitial inflammation was scored from 0 (<10% of parenchyma area inflamed) to 3 (>50% inflamed).

Immunohistochemical analysis in cryostatic sections was performed with the following mouse monoclonal antibodies: anti-CD4 for helper/inducer T cells, anti-CD8 for cytotoxic/suppressor T cells, and anti-CD14 for monocytes (Coulter, Miami, FL), anti-HLA-DR (Ortho-diagnostics, Raritan, NJ), CD11 anti-LFA-1, CD49d anti-VLA-4, CD54 anti-ICAM-1, and CD106 anti-VCAM-1 (Immunotech, Marseille, Cedex, France).

For each biopsy, a negative control was obtained by incubating the section with normal mouse serum (Dakopatts, Copenhagen, Denmark). The reaction was verified by the indirect immunoperoxidase method (22). Briefly, sections were fixed in cold acetone at 4°C for 5 min, incubated in a solution of bovine serum albumin in phosphate-buffered solution for 15 min, and subsequently with the primary antibody or control serum for 14 to 16 h at 4°C. After washing in phosphate-buffered solution and inhibiting endogenous peroxidase in a solution of hydrogen peroxide and methanol, samples were incubated with an anti-immunoglobulin monoclonal mouse antibody obtained from rabbit and with peroxidase antiperoxidase immunocomplex obtained from mouse (Dakopatts). Diaminobenzidine was used as a substrate for the peroxidase and, after staining with hematoxylin, samples were evaluated by two different observers. Infiltrating cells and tubular sections positively stained have been counted at a final magnification of x400, using a graticule in the eyepiece of the light microscope measuring an area of 0.048 mm². The mean of positive leukocytes or tubules for a surface area of 0.048 mm², observed in a minimum of 10 fields, was calculated for each biopsy.

Normal renal tissue from nephrectomies for renal tumors was used as control. Informed consent before enrollment in the trial was given by both the parents and the patients, and the treatment itself was approved by the local ethics committee.

Results

Three of the four patients enrolled in the study received ECP after a rise in the creatinine level and a biopsy-proven, acute rejection that occurred 2 to 6 wk after a previous course of intravenous steroid or OKT3. GFR improved, with a significant reduction in creatinine, after the first treatment (Figure 1). The fourth patient, with three rejection episodes in 4 mo after transplantation, was treated with ECP on the basis of a renal biopsy showing severe inflammatory infiltration. Throughout the study, creatinine remained stable between 150 and 166 μmol/L.

One year after ECP withdrawal, renal function remained unchanged in patients 1, 3, and 4. Patient 2, the only one with a persistent proteinuria of 2 to 2.5 g/L and a poor compliance
with immunosuppressive therapy, showed a progressive deterioration of renal function and returned to chronic dialysis.

**Histopathologic Evaluation**

Patient 1 had a pre-ECP biopsy showing a grade 3 acute rejection according to the Banff classification. The biopsies obtained from the other patients all showed grade 2 moderate acute rejection. The specimens evaluated after 3 mo of ECP documented a resolution of acute rejection features, with absent or mild inflammatory infiltrates only.

**Immunohistochemical Evaluation**

In normal renal tissue, the lymphocyte infiltrate was almost absent; HLA-DR was expressed by the endothelium of the glomerular and interstitial vessels and capillaries and never by tubular cells. Integrins ICAM-1 and VCAM-1 were expressed on endothelial cells of glomeruli and interstitial capillaries and on epithelial cells of Bowman’s capsule, respectively.

Table 2 shows the grade of interstitial inflammation according to the Banff criteria, the immunophenotype of infiltrating cells, and the mean of positive tubular cross-sections in the pre- and post-ECP biopsies. A rich inflammatory infiltrate (severe in three patients and moderate in one) was observed in the biopsies performed pre-ECP. The infiltrating lymphocytes were significantly reduced in the specimens obtained after 3 mo of treatment, with a decrease of CD4 (13 ± 3.3/0.048 mm² versus 1.7 ± 0.4/0.048 mm²), CD8 (57.5 ± 38/0.048 mm² versus 2.7 ± 1.8/0.048 mm²), and CD14-positive cells (62.5 ±

### Table 2. Immunophenotypic features of the inflammatory infiltrate and tubular cells in the renal biopsies before and after 3 mo of ECP

<table>
<thead>
<tr>
<th>Feature</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Grade of infiltrate (1 to 3)</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>CD4</td>
<td>14</td>
<td>1.2</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>CD8</td>
<td>91</td>
<td>2</td>
<td>89</td>
<td>5</td>
</tr>
<tr>
<td>CD14</td>
<td>87</td>
<td>1</td>
<td>76</td>
<td>12</td>
</tr>
<tr>
<td>LFA-1</td>
<td>187</td>
<td>10</td>
<td>168</td>
<td>9</td>
</tr>
<tr>
<td>VLA-4</td>
<td>80</td>
<td>0.5</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>DR</td>
<td>6.8</td>
<td>0.3</td>
<td>5.1</td>
<td>0.2</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>3.5</td>
<td>0.1</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>4</td>
<td>0</td>
<td>3.6</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* Mean number of leukocytes (CD4, CD8, CD14, LFA-1, VLA-4) and tubules (DR, ICAM-1, VCAM-1) staining positively per 0.048 mm² of surface area observing at least seven fields for each biopsy.
29/0.048 mm$^2$ versus $3.7 \pm 5.5/0.048$ mm$^2$). Similarly, the strong positivity for CD11a and CD49d in the inflammatory infiltrate was downregulated by the treatment: $166 \pm 82/0.048$ mm$^2$ versus $6.6 \pm 4.3/0.048$ mm$^2$ and $51.2 \pm 30/0.048$ mm$^2$ versus $0.7 \pm 0.9/0.048$ mm$^2$, respectively. In addition, the tubular immunoreactivity for ICAM-1 (3.1 ± 0.8 tubules/0.048 mm$^2$), VCAM-1 (2.9 ± 1), and HLA-DR (6.2 ± 1.2) observed in the pre-ECP specimens had mostly disappeared in the post-treatment specimens: 0.3 ± 0.3, 0, and 0.2 ± 0.1 tubules/0.048 mm$^2$, respectively (Figure 2).

**Laboratory Evaluation**

No significant changes in immunologic parameters (lymphocyte count, T cell subpopulations, autoantibodies, and immunoglobulins) were found in the patients throughout the study.

**Immunosuppressive Therapy**

ECP allowed a reduction of oral prednisolone in three patients, with a mean daily dose of steroids from $16 \pm 11$ mg pre-ECP to $8.5 \pm 1$ mg after 6 mo of treatment. Both cyclo-
sporin A (220 ± 58 versus 217 ± 40 mg/d) and azathioprine dosages (93 ± 12 versus 93 ± 12 mg/d) were maintained. Pulse intravenous methylprednisolone, increased oral dosages of steroids, or other drugs for the treatment of acute rejection episodes were not used during the treatment or in the 6-mo follow-up post-ECP period.

Complications
No complications or adverse effects were recorded during the treatments or in the course of the study.

Discussion
Multiple acute rejection is well known to be associated with poor, long-term prognosis after renal transplantation. This is due mainly to graft failure or complications such as infections and malignancies. In addition, repeated rejection episodes, in conjunction with the administration of high doses of nephrotoxic drugs such as cyclosporine, and viral infections as a result of a severe immunosuppression, allow the development of chronic allograft nephropathy.

ECP has been recently used in cardiac transplant patients with recurrent rejection. A 6-mo course of treatment allowed a reduction in the number and severity of rejection episodes without any complications (11). This experience prompted us to evaluate whether ECP could allow for a better control of recurrent rejection even in renal transplant patients.

Few patients with allograft rejection after renal transplantation have been treated with ECP. Horina et al. described three patients, two with chronic rejection and one with repeated rejection episodes, who received ECP at monthly intervals without any significant benefit. After a few months, all three patients returned to chronic dialysis (18). On the contrary, a more frequent schedule of treatment has been reported to improve GFR in four patients with allograft rejection (20). However, limited data are included about histopathologic changes induced by ECP in these reports.

In the present study, ECP was used in four adolescent patients with recurrent renal allograft rejection unsuccessfully treated by conventional protocols. Renal function improved in three patients, with a rise in serum creatinine before ECP that remained unchanged in the fourth patient. It is noteworthy that no rejection episodes have been observed during the 6 mo of treatment compared with 0.44 rejection episodes per month per patient before ECP.

A 3-mo course of ECP induced a remarkable reduction (more than 86%) of both lymphocytes and monocytes infiltrating the graft, and downregulated the expression of specific (HLA-DR) and nonspecific (ICAM-1 and VCAM-1) molecules on tubular cells that participate in the rejection process as both target and antigen-presenting cells. Further research is needed to determine whether this effect on tubular cells is exerted by a reduction of inflammation or by a specific release of cytokines induced by ECP. However, because cyclosporine and azathioprine dosages remained unchanged and oral steroid was reduced in three of the four patients treated, the clinical and histopathologic improvement is induced somehow by ECP.

The schedule of treatment that we used is very aggressive, since ECP is usually performed at monthly intervals in both CTCL and autoimmune diseases. However, in a previous study with cardiac transplant patients, we obtained a better resolution of rejection when ECP was performed at weekly intervals during the first month of treatment. For this reason, a similar protocol was chosen for the patients with renal allograft rejection.

The mechanism by which ECP exerts its positive effect in patients with allograft rejection is still unclear. Psoralens are hydrophobic compounds that intercalate with the DNA base pairs after UVA irradiation, forming photoadducts with pyrimidine bases, as well as with amino acids and fatty acids. The ability of the cells to repair DNA photoadducts is completely inhibited when the combined doses of 8-MOP (ng/ml) and UVA (J/cm²), as during ECP, are equal to or more than 50 (10).

Because only 5 to 10 × 10⁹ peripheral leukocytes are damaged by 8-MOP and UVA during ECP, the efficacy of the treatment cannot be attributed to a simple inactivation of these cells. It is likely that an immunomodulatory response against alloreactive T cell populations, exposed to 8-MOP plus UVA, could be triggered by the treatment (10).

In CTCL patients, a competent immune system seems to be crucial for the response to ECP. Several studies indicate that normal levels of CD8 (cytotoxic, suppressor) and CD19 (natural killer) T cells are required to produce an adequate immune response to irradiated and nonirradiated pathologic cells (23). However, no correlations were observed between clinical response and T cell subsets in our patients.

Studies in animal models have shown that the treatment of lymphocytes with 8-MOP and UVA light in an extracorporeal system inhibits the reactivity of the immunologic apparatus against transplanted tissues/organisms in an apparently specific way. Perez et al. reported that the infusion of syngeneic effector lymphocytes previously treated with 8-MOP and UVA (photoactivated effector T cells) selectively downregulated the host response to foreign histocompatibility antigens in a mice model. Skin of CBA/j mice grafted to BALB/c mice induces an acute rejection reaction. However, when the BALB/c mouse is injected before transplantation with photoinactivated effector T cells from a syngenic animal that previously rejected a skin graft, a longer survival of the transplanted tissue is induced. A parallel selective hypoproliferative response against graft antigens due to the generation of transferable induced CD8 T cells has also been shown (12).

In a primate cardiac xenograft model, Pepino et al. also reported that the extracorporeal treatment of recipient lymphocytes with 8-MOP and UVA immediately after transplantation increased graft survival by inducing a specific suppression of the response to the donor cells, as well as a suppression of antidonor lymphocytotoxic antibodies (13). This study has a clinical relevance because it demonstrates that a specific unresponsiveness may be induced after transplantation, treating recipient cells out of the body without any side effect.

Recently, after the demonstration in vitro that the combination of 8-MOP and UVA enhances synthesis and expression of MHC class I (24) and increases the number of empty class I
molecules on the surface of antigen-presenting cells, it has been hypothesized in CTCL patients that ECP may increase the antigenicity of the treated cells via an overexpression of MHC I molecules. Some of the cells lysed by 8-MOP and UVA may also release specific peptides ready to be combined to MHC-I that will fill empty class I molecules on the surface of antigen-presenting cells (23).

However, ECP exerts its beneficial effect in patients with an allograft rejection probably through different mechanisms, because in CTCL the response to the treatment occurs after 2 to 3 mo, and in allograft rejection often after only a few days (4,14). The production of cytokines, including tumor necrosis factor and interleukin-6, by leukocytes exposed to 8-MOP and UVA may be also involved in the ECP response (25).

In conclusion, our findings indicate that ECP is an effective and safe therapy for renal transplant patients with recurrent rejection episodes not responsive to standard immunosuppression. Better knowledge of the mechanism of action involved in the response induced by ECP and studies on a larger number of patients are needed to confirm the benefits of ECP in renal transplant recipients with allograft rejection and to permit a more systematic decision on the frequency of treatment, number of cells to be treated, and concomitant therapy.

Acknowledgments

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References


