Early Experience with Anti-Tac in Clinical Renal Transplantation

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THE SEARCH for more effective and less broadly toxic immunosuppressive agents remains a major goal of transplantation research. One approach to achieving more specific immunosuppression is to target only those lymphocytes responding to an allograft by directing therapy at activation antigens. Of these antigens, the interleukin-2 receptor (IL-2R) has proven of particular interest, both because of its important biological role in the activation T-cells[1] and because of extensive animal model experience with anti-IL-2R monoclonal antibody (Mab) therapy.[2-6]

Anti-Tac is a murine Mab with specificity for the 55KD beta subunit of the human IL-2R.[7] Anti-Tac blocks binding of IL-2 to its receptor and prevents association of the alpha and beta chains of the receptor to form the high affinity IL-2R.[8] Recent work from our laboratory has shown that anti-Tac as a single agent will significantly delay rejection of renal allografts in cynomolgus monkeys.[6] Encouraged by these results, we have initiated a randomized trial of prophylactic therapy with anti-Tac in clinical renal transplantation. This study presents our early experience with three protocols for the use of this agent.

MATERIALS AND METHODS

Preparation and Administration of Anti-Tac

Anti-Tac, a murine IgG2a Mab has been extensively characterized elsewhere.[1,7] The antibody was purified from ascites of Balb/c mice inoculated with the hybridoma, suspended in saline at a concentration of 2 mg/ml, sterilized by filtration, and stored at −20°C. Prior to the first administration of the Mab, all patients were injected intradermally with 0.1 ml of a 1:1000 dilution of anti-Tac in saline to exclude hypersensitivity. Each dose of 20 mg was infused intravenously over two hours in 50 ml normal saline containing 1% human serum albumin.

Patient Protocols

Three protocols for the use of anti-Tac were examined. In each protocol, only patients receiving a first cadaver allograft were eligible, and patients were randomized to experimental or control groups by a sealed envelope technique. There were no significant differences between groups with respect to age, sex, or degree of HLA AB or DR matching. All protocols were approved by the clinical studies committtees of both hospitals.

In the first protocol, patients were randomized to receive anti-Tac plus conventional immunosuppression (n = 12) or conventional immunosuppression alone (n = 9). Anti-Tac was given at a dose of 20 mg qd for 10 days beginning on posttransplant day 1. Conventional immunosuppression consisted of either cyclosporine 12 mg/kg/day and prednisone 30 mg/day or cyclosporine 8 mg/kg/day, azathioprine 2 mg/kg/day, and prednisone 30 mg/day. In both groups cyclosporine doses were adjusted by blood level and clinical evidence of nephrotoxicity. First rejection episodes were treated with a methylprednisolone pulse 1 gm IV qd for 3 days.

In the second protocol, no cyclosporine was used in the experimental group in the first week. Patients were randomized to receive either anti-Tac 20 mg/day for 10 days, azathioprine 2 mg/kg/day, and prednisone 30 mg/day, with cyclosporine 8 mg/kg/day added on day 8, or conventional triple therapy with cyclosporine 8 mg/kg/day, azathioprine 2 mg/kg/day, and prednisone 30 mg/day. This protocol was terminated prematurely, as noted below.

In the third protocol, low dose cyclosporine is being used in the experimental group. Patients are randomized to receive either anti-Tac 20 mg/day for ten days, cyclosporine 4 mg/kg/day, azathioprine 2 mg/kg/day, and prednisone 30 mg/day, or conventional triple therapy as in the second protocol. In the experimental group, the cyclosporine dose is increased to 8 mg/kg/day at the conclusion of anti-Tac treatment. This protocol is ongoing, with nine patients entered in the experimental group and ten in the control group to date.

During treatment, serum and peripheral blood mononuclear cells were obtained on days 0, 2, 4, 6, 8, 10, and 14 to monitor anti-Tac serum levels, the development of anti-mouse immunoglobulin antibodies, T-cell subsets and expression of IL-2R on circulating lymphocytes, and the effect of therapy on the ability of circulating lymphocytes to participate in mixed lymphocyte reactions and cell-mediated lympholysis.

RESULTS

Protocol 1

The first protocol was designed to ascertain the safety of anti-TAC administration and to obtain preliminary evidence of efficacy. Patients were randomized to receive anti-TAC plus conventional immunosuppression (n = 12) or conventional immunosuppression alone (n = 9). In the group receiving anti-TAC, all skin tests were negative and no complications of antibody administration were identified.

In this protocol, administration of anti-TAC reduced the frequency of early rejection episodes and delayed the onset of the first rejection episode. In the first ten days posttransplant, during which anti-TAC was given to the treatment group, only one of 12 patients receiving anti-TAC experienced a rejection episode, compared with five of nine patients in the control group ($p < 0.05$). The single treated patient with rejection had primary nonfunction of the graft, and

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rejection was diagnosed by biopsy on postoperative day 7. This patient was subsequently demonstrated to have cyclosporine nephrotoxicity, and it is uncertain if a clinical rejection episode would have been noted had there been immediate renal function.

Rejection episodes eventually occurred in seven of twelve patients receiving anti-TAC and in eight of nine control patients (p = ns). However, the mean time to the first rejection episode was 24.7 days in the anti-TAC group, compared with 9.4 days in the control group ($p < 0.01$, Mann-Whitney rank sum testing). Four patients with rejection in the anti-TAC group responded to a steroid pulse, while three required OKT3; one of the latter patients eventually lost his graft to uncontrolled rejection. A single control patient also lost his graft, with accelerated acute rejection leading to removal of a ruptured allograft on postoperative day 4.

All patients in this initial protocol have now been followed 12-21 months, with no subsequent rejection episodes or graft losses. Graft survival is 92% in the treated group and 89% in the control group. Mean creatinine at last follow-up was 2.1 mg/dl, for the anti-TAC group and 1.7 mg/dl for the control group (p = ns). No deaths have occurred in either group.

Protocol 2

As the initial protocol suggested that anti-TAC would prevent rejection during its administration, the second protocol was designed to determine if the use of cyclosporine could be avoided in the early posttransplant period. Patients were randomized between quadruple therapy, consisting of anti-TAC for ten days, azathioprine and prednisone, with cyclosporine added on day 8, or triple therapy. By happenstance, five of the first six patients entered in this protocol were randomized to the anti-TAC group. None of these patients completed the anti-TAC protocol, one because of an apparent reaction to anti-TAC and four because of rejection during therapy.

The reaction to the antibody was the development of fever and pulmonary edema on the fifth day of treatment. The patient was not volume overloaded and there was no evidence of infection or rejection. Anti-TAC was discontinued and cyclosporine begun, with resolution of the symptoms and continued good graft function.

The four patients with rejection were managed by cessation of anti-TAC and initiation of cyclosporine. One of the rejection episodes was easily reversed with a single steroid pulse, while the others required more than one steroid pulse or OKT3. One of these patients subsequently died of disseminated CMV. Because this protocol did not appear to obviate the need for cyclosporine in the early posttransplant period, it was terminated.

Protocol 3

The current protocol was established to determine if anti-TAC will allow the use of a lower dose of cyclosporine in the early posttransplant period. Patients are being randomized to receive anti-TAC for ten days plus low dose cyclosporine or full dose cyclosporine. The cyclosporine dose in the experimental group is increased following conclusion of anti-TAC treatment; all patients in both groups receive azathioprine and prednisone. To date, nine patients have been entered in the treated group, ten in the control. There has been one rejection episode within ten days of transplantation in the treated patients, compared with eight in the control ($p < 0.05$). There have been no immunological graft losses in either group, but follow-up is less than four months in all cases. One patient who received anti-TAC, and who was not treated for rejection, died at four months from CMV and pneumocystis pneumonia. A single patient receiving anti-TAC developed pruritis, which was managed symptomatically and did not require cessation of therapy.

Anti-TAC and OKT3

As noted above, six patients treated with anti-TAC subsequently required therapy with OKT3. All six patients had rapid reversal of their rejection episodes, although one subsequently had a recurrent rejection and represents the sole graft loss from rejection in all three treatment groups. Details of the anti-mouse immunoglobulin response and clearance of circulating CD3 positive cells in these patients are described in a separate manuscript in this volume.[9]

Monitoring Studies

None of the monitoring studies performed revealed significant differences between the treated and control patients. Of particular note, treatment with anti-TAC did not prevent expression of the IL-2 receptor on the surface of circulating T-cells following transplantation.[10]

DISCUSSION

The data presented here demonstrate that anti-TAC, a Mab directed against the human IL-2R, will reduce the frequency of early rejection episodes following transplantation and delay the onset of those which do occur, when used in combination with cyclosporine. The early results with protocol 3 suggest that the dose of cyclosporine can be significantly reduced compared with standard immunosuppression regimens. This finding is in accord with animal models, in which significant synergy between anti-IL-2R Mab's and cyclosporine has been shown.[5] This reduced dose of cyclosporine simplifies the management of renal transplant recipients by decreasing the incidence of nephrotoxicity. When combined with the lower incidence of rejection, the early course of anti-TAC-treated patients is remarkably uncomplicated.

These results differ from those of Soulillou et al using another anti-IL-2R Mab, 33B3.1.[11] In that experience, excellent results were obtained without cyclosporine; but with a higher prednisone dose. Further experimentation with anti-IL-2R therapy will be required to determine optimal antibody characteristics and protocols.

A critical finding of the current study was the successful use of OKT3 to treat rejection in patients previously receiv-

ing anti-TAC. This observation permits the design of protocols employing sequential use of monoclonal antibodies of different idiotypes directed against the same or different targets. In particular, it allows the use of one or more Mab's for rejection prophylaxis without precluding use of other antibodies for rejection therapy. Moreover, when this finding is combined with the increasing variety of Mab's defining targets and subsets of immunologic interest, the opportunity for more detailed manipulation of the immune response is evident.

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