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TREATMENT OF ACUTE RENAL ALLOGRAFT REJECTION WITH OKT3 MONOCLONAL ANTIBODY

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Eight cadaver donor renal allograft recipients, who had received azathioprine and prednisone from the day of transplantation, were treated with OKT3 monoclonal antibody (reactive with all mature peripheral blood T cells) at the time of diagnosis of acute rejection. In all cases, loss of essentially all detectable peripheral blood OKT3-reactive cells was noted within minutes after the initial 1- to 5-mg i.v. infusion. Chills and fever invariably occurred following the first or second infusion of monoclonal antibody, but were not noted during the subsequent 10- to 20-day course of therapy, suggesting rapid cell lysis as the etiology of this toxicity.

The established rejection episode was reversed in all cases within 2 to 7 days without addition of any therapy other than OKT3 antibody and despite continued lowering of the steroid dosages. During the subsequent 3- to 12-month follow-up period, further rejection episodes occurred in five of these patients, two of these were irreversible with conventional therapy so that six of the eight allografts continue with excellent renal function.

These preliminary observations suggest that homogeneity, limited dosage requirements, and ease of in vitro monitoring of dosage effects should markedly simplify the use of monoclonal antibody to T cell populations in human allograft recipients. This second generation of antilymphocyte preparations offers the potential for not only increased effectiveness but also the possibility of manipulating specific T cell subsets.

Although some heterologous antisera to human lymphocytes have proved to be effective in delaying or reversing allograft rejection (1, 2), the preparation of those agents has been difficult using conventional immunization techniques. Even the purified IgG fraction from animals immunized with lymphocytes contains not only a heterogeneous group of antibodies to T lymphocytes, but also antibodies reactive with other normal cells as well as extraneous antibodies reflecting the animal's previous immunological activity (3). Therefore, techniques of developing more specific reagents have been sought.

Based upon the recent demonstration by Kohler and Milstein (4) that monoclonal antibody to a specific membrane determinant can be reliably produced using cell hybridization techniques, Kung et al. (5) have produced a panel of monoclonal antibodies specifically reactive with human lymphocyte subpopulations. A phylogenetic screen of these reagents in our laboratory revealed significant cross-reactivity of some of them with lymphocytes of subhuman primates. In order to evaluate the possible clinical role of monoclonal antibody as an immunosuppressive agent, we previously investigated in cynomolgus renal allograft recipients the effects of OKT4 antibody (6). This antibody is reactive with human T cells having major helper/inducer and T-T collaborative functions (7, 8). By using flow cytometry for monitoring of peripheral blood lymphocytes, we defined the dosage and timing of OKT4 antibody administration required to provide in vivo coating of this specific T cell population now in 10 cynomolgus recipients. With a dosage range of 0.5 to 1.0 mg/kg/day, coating of all reactive cells was observed and residual circulating Ab was usually detectable 24 hr after administration. When OKT4 therapy was started before transplantation, allograft survival was extended to as long as 7 weeks after a 1- to 2-week course of therapy (control survival 8 to 11 days).

Encouraged by the effectiveness, ease of administration, and lack of toxicity in this in vivo model, we have begun a trial of monoclonal antibody therapy in human renal allograft recipients. Although the ultimate goal is to evaluate only selected T cell subset suppression, in order to expose the patients in this initial study to the least risk of ineffective immunosuppression, we have tested OKT3 antibody which is reactive with all mature human T cells (9, 10).

MATERIALS AND METHODS

Eight cadaver donor renal recipients, who had received azathioprine and prednisone from the time of transplantation, were treated with OKT3 monoclonal antibody at the time of diagnosis of acute rejection. Allograft rejection was suggested in these patients by deterioration in renal function and was confirmed in all patients by histopathological evaluation of tissue obtained by percutaneous needle biopsy.

In the attempt to identify the most effective and least toxic combination of conventional and OKT3 therapy, several dosage schedules were pursued. In the first two patients, azathioprine was administered in a dosage of 10 mg/kg on the day of transplantation and then maintained at 1 to 2 mg/kg/day unless the white blood count fell below 3000/mm³. Prednisone was begun at a daily dosage of 2 mg/kg. Beginning on the 5th postoperative day, the dosage was decreased by 10 mg/day to 0.8 mg/kg/day, after which the dosage was more slowly tapered to the maintenance dosage of 0.25 mg/kg/day. Following confirmation of the diagnosis of rejection, OKT3 antibody was administered by bolus i.v. injection in a total daily dosage of 1 to 2 mg for 10 days. In the next two patients, the azathioprine was reduced to 0.75 mg/kg/day and prednisone dosage to 0.6 mg/kg day.
mg/kg/day during OKT3 therapy which was administered i.v. in a total daily dosage of 1 to 3 mg for 14 days. In the last four patients, the azathioprine and prednisone dosages were further reduced to 0.4 mg/kg/day during OKT3 therapy. In these patients OKT3 was administered daily for 14 to 20 days at a dosage of 4 to 5 mg/day (Fig. 1). After discontinuing OKT3 therapy, the azathioprine dosage was again increased to 1 to 2 mg/kg/day in the last six patients.

Prior to transplantation, during azathioprine and prednisone therapy, and at frequent intervals after institution of OKT3 antibody therapy, peripheral blood lymphocytes fromuffy coat preparations were analyzed for OKT3-reactive cells using flow cytometry (11). Recipient serum was monitored by incubating sequentially diluted sera with normal human peripheral blood lymphocytes, followed by staining with fluoresceinated goat anti-mouse antibody, in order to detect and maintain a circulating level of OKT3 antibody. Effectiveness of therapy was judged by reversal of rejection defined as the day after which consistent improvement in renal function occurred. Percutaneous renal biopsies were performed on all patients after OKT3 therapy.

Toxicity was studied by daily monitoring of recipient complete blood count, blood urea nitrogen, and creatinine, weekly assays of hepatic function and urine protein excretion, and careful observation for any clinical evidence of serum sickness. Serial urine, salivary, anduffy coat specimens were cultured for viral activity as previously described (12).

RESULTS

The clinical course of a representative patient treated with OKT3 antibody is depicted in Figure 1. Following cadaver donor renal transplantation in this 41-year-old male, the serum creatinine level fell to normal levels by the 4th post-transplant day. Subsequently, the onset of rejection was suggested by the rising serum creatinine level which occurred in conjunction with decreased urinary output, weight gain, hypertension, and low-grade fever. Allograft biopsy confirmed the diagnosis on the 7th post-transplant day and the initial 5-mg dose of OKT3 antibody was infused i.v. after an i.d. skin challenge was observed to produce no reaction. Approximately 45 min later, an episode of shaking chills with fever to 101 C occurred. In addition, the patient complained of shortness of breath and diffuse wheezes were noted over the lung fields. These symptoms rapidly responded to acetaminophen and antihistamine therapy. The patient had no further chills, fever, or other adverse reactions with subsequent OKT3 infusions.

Sequential monitoring of peripheral blood lymphocytes was begun 15 min after the initial injection. As noted in Figure 1, there was essentially complete loss of OKT3-reactive cells from the peripheral circulation, a condition which persisted throughout the 14-day course of therapy. That this was not attributable to masking or modulation of OKT3 antigen was indicated by the failure of fluorescein conjugates of OKT4 and OKT8 monoclonal antibodies, which bind to other T cell antigens (11), to react with the residual cells. In addition, detectable antibody excess was present throughout the course of therapy during which a total dosage of 70 mg of OKT3 antibody was administered.

The serum creatinine level continued to rise for several days after institution of therapy but the patient's clinical condition rapidly improved with diuresis, weight loss, and improved control of blood pressure being noted within 36 hr of initiation of treatment. Continuous improvement in renal function began 72 hr after OKT3 treatment was initiated with the serum creatinine eventually stabilizing at 1.3 mg/100 ml. As depicted in Figure 1, the azathioprine and steroid dosages were rapidly tapered during this period. A second allograft biopsy performed on the last day of therapy showed essentially complete resolution of the histopathological findings of rejection (Fig. 2).

The initial results of treatment of the eight patients studied are summarized in Tables 1 and 2. In every instance, the rejection episode for which OKT3 therapy was instituted was reversed with steady improvement in allograft function beginning after 2 to 7 days of therapy. In the first three patients and the last patient treated, a subsequent rejection episode occurred beginning 2 to 6 weeks after cessation of OKT3 therapy while the patients were being maintained on azathioprine and prednisone. These episodes were easily reversed in three of these patients with increased steroids. The second rejection episode in patient 3, however, could not be reversed despite increased steroids, local irradiation, and actinomycin D therapy. She
returned to dialysis 2 months after transplantation. She subsequently developed severe cytomegalovirus infection and expired 3 months after transplantation.

Patient 4 is of particular interest because of the development during therapy of an antibody response to the OKT3 reagent. During the initial 10 days of therapy, peripheral blood T cell monitoring of this patient revealed essentially complete loss of cells reactive with OKT3 antibody, and allograft function steadily improved from a peak serum creatinine of 8.1 to 2.9 mg/100 ml. During the final 4 days of OKT3 therapy, however, large numbers of OKT3-reactive cells were repeatedly demonstrable in the patient’s peripheral blood and no serum excess of OKT3 could be achieved even after the dosage of monoclonal antibody had been increased from 2 to 5 mg/day. At the time, the steadily falling serum creatinine again began to rise and allograft biopsy revealed extensive evidence of acute rejection. The immunosuppressive protocol was immediately changed to conventional therapy with high-dose steroids, local irradiation, and actinomycin D; but the rejection process continued, necessitating allograft nephrectomy 31 days after transplantation. Evaluation of serial serum samples from this patient by flow cytometry documented the appearance of anti-OKT3 antibody initially on day 10 of therapy with the titer peaking 5 days after therapy was discontinued. Characterization of these antibodies will be reported in detail elsewhere. No evidence of serum sickness or anaphylaxis was noted at any time in this patient.

Therefore, six of the eight allografts continue with excellent renal function 3 to 12 months after OKT3 treatment. All of the patients treated with OKT3 antibody developed chills and fever

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**TABLE 1. Cadaver donor renal allograft recipients treated for acute rejection with OKT3 monoclonal antibody**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Etiology of renal disease</th>
<th>HLA antigens matched</th>
<th>Follow-up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>Female</td>
<td>55</td>
<td>IgA nephropathy</td>
<td>0</td>
<td>12</td>
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<tr>
<td>2</td>
<td>52</td>
<td>Male</td>
<td>76</td>
<td>Nephrosclerosis</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>Female</td>
<td>50</td>
<td>Interstitial nephritis</td>
<td>1</td>
<td>3</td>
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<tr>
<td>4</td>
<td>41</td>
<td>Male</td>
<td>70</td>
<td>Chronic glomerulonephritis</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>Male</td>
<td>78</td>
<td>Chronic glomerulonephritis</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>Female</td>
<td>55</td>
<td>Dysplasia + focal sclerosing glomerulonephritis</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>52</td>
<td>Male</td>
<td>56</td>
<td>Chronic glomerulonephritis + diabetes</td>
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<td>4</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>Female</td>
<td>51</td>
<td>Polycystic renal disease</td>
<td>1</td>
<td>3</td>
</tr>
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</table>

* Expired with cytomegalovirus infection after second rejection episode.
  * Maintained on dialysis after loss of allograft during second rejection episode.

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**TABLE 2. Results of treatment of acute rejection with OKT3 monoclonal antibody**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Post-transplant day of rejection</th>
<th>Prerejection creatinine (mg/100 ml)</th>
<th>Peak creatinine</th>
<th>Days to reversal</th>
<th>Post-therapy creatinine</th>
<th>Total OKT3</th>
<th>Subsequent rejection episode</th>
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<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>1.6</td>
<td>5.4</td>
<td>4</td>
<td>1.3</td>
<td>13</td>
<td>10</td>
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<td>2</td>
<td>16</td>
<td>1.9</td>
<td>4.8</td>
<td>2</td>
<td>1.6</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>1.6</td>
<td>4.3</td>
<td>2</td>
<td>0.9</td>
<td>18</td>
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<td>9</td>
<td>5.0</td>
<td>8.1</td>
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<td>2.9</td>
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</tr>
<tr>
<td>6</td>
<td>6</td>
<td>2.9</td>
<td>8.5</td>
<td>5</td>
<td>0.9</td>
<td>57</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>3.0</td>
<td>8.1</td>
<td>4</td>
<td>1.2</td>
<td>90</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>10.4†</td>
<td>11.3†</td>
<td>7</td>
<td>1.1</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>

* Reversed with conventional immunosuppression.
  * Irreversible using conventional immunosuppression.
  * On hemodialysis.
to as high as 102 C within 1 hr of the first injection, and several
reactions were noted to have diffuse wheezing during this period. No
reactions to subsequent injections were noted and no other
evidence of toxicity could be identified.

The effective removal of OKT3-reactive cells (mature T
lymphocytes) from the circulation within minutes after infusion
of OKT3 antibody was clearly documented by flow cytometry
analysis in each case. This dramatic lysis of cells occurred only
with the first injection, the OKT3-reactive population then
remaining depressed (except in patient 4) throughout the course
of therapy.

DISCUSSION

A central problem in transplantation remains that of incompletery controlled rejection. Intense, nonspecific suppression of
the recipient’s immune system is produced with currently used
agents but the level of clinical success and toxicity has changed
little over the past decade. The only significant addition to this
regimen has been the gradual acceptance of heterologous anti­
lymphocyte preparations such as antilymphocyte globulin, anti­
thymocyte globulin (ATG), etc. (13), with which a more
selective suppression of the recipient’s cellular immune re­
sponses is anticipated. However, with currently available
agents, prepared by routine immunization techniques, only 5 to
10% of the total dose administered represents the actual ther­
apic product. As hybridoma technology has developed, the
possibility of producing anti-T cell monoclonal antibodies with
effectiveness similar to currently used ATG preparations but
which are active in much smaller quantities has been realized.
Furthermore, the feasibility of using antibodies to suppress
selected T cell subsets rather than the entire population can
now be tested.

The results of our studies have begun to delineate the in vivo
effects of such reagents. We have observed significant immu­
nosuppression in subhuman primate renal allograft recipients
receiving relatively minute quantities of monoclonal antibody
(total recipient dosage: 17 to 56 mg compared with 50 to 100
mg/kg/day (14) when using heterologous ATG preparations).
Moreover, the OKT4 antibody administered to these recipients
has been shown to be directed only to the helper/inducer T cell
subset, reacting with approximately 50 to 60% of human pe­
ripheral blood T cells and 45 ± 9% of cynomolgus T cells. These
observations demonstrate not only the immunosuppressive po­
tency of monoclonal antibody but also that effective protocols
may be developed in which the requirement for agents, which
produce indiscriminate T cell depression, might be markedly
reduced.

In our previous clinical evaluation of equine ATG, we have
found the most definitive means of demonstrating effectiveness
was in studies in which ATG alone was added to the immuno­
suppressive protocol at the time of diagnosis of acute rejection
(2). Since only a single agent is being used to reverse a readily
defined event, it is possible to elucidate immediately the effect
of the added therapy without the need for long-term random­
ized trials. Thus, we have pursued a similar model for the
evaluation of monoclonal antibody. We have selected OKT3
antibody rather than antibody to a T cell subpopulation in
order to reduce the likelihood of inadequate suppression in this
initial trial. Our most important clinical observation in the
patients treated to date has been the initial reversal in all cases
of the established rejection episode without addition of any
therapy other than OKT3 antibody and despite continued
lowering of the steroid dosage.

Whether improved long-term allograft survival can be
achieved with such therapy remains to be established; however,
some observations regarding the addition of monoclonal anti­
body to conventional therapy can be made. In the first four
patients, the attempt was made to use the minimum total
immunosuppression which would produce reversal of the rejec­tion
episode. Thus, the dosage of OKT3 antibody administered
was limited to that (1 to 2 mg/day) which maintained a barely
detectable serum antibody level. In each case, except patient 4
who developed anti-OKT3 antibodies, all clinical manifestations
of rejection were abolished. However, post-therapy allograft
biopsies showed persistent cellular infiltration and recurrent
rejection requiring conventional high-dose steroid therapy ap­
peared within 2 to 6 weeks. All of these patients subsequently
suffered significant oral infection secondary to herpes simplex
virus and cytomegalovirus pneumonitis was documented after
the second rejection episode in two of the patients, one of whom
subsequently died. These observations appear to emphasize
the previously proposed concept that a modestly high-dose “net
state of immunosuppression” over a prolonged period of time is
probably of greater risk to patients than a limited, more inten­sive
course of therapy (12). In the next four patients, therefore,
the dosage of OKT3 antibody was sharply increased in an
attempt to reverse more definitively the rejection activity dur­ing
a limited period of therapy. In addition, as in our studies
with ATG (14), we have continued to try to limit the dosages of
azathioprine and prednisone administered during the period in
which the patients received OKT3 antibody. To date, the
clinical course in these patients has been much more satisfac­
tory.

Unacceptable in vivo toxicity of monoclonal antibody admin­
istration has not been observed. The chills, febrile response,
and occasional wheezing noted on the first day of treatment
have been found to be readily controlled with antihistamine
and acetaminophen therapy. These symptoms have been noted
with ATG treatment as well (15) and have been thought to be
secondary to release of endogenous pyrogens following exten­sive
lysis of peripheral blood lymphocytes. The observations in
the patients treated with OKT3 antibody would tend to support
such an explanation, for the chills and fever were noted only
after the first infusion concomitant with the dramatic drop in
the number of circulating T lymphocytes. Subsequent infusions
were tolerated without similar incidents. It is hoped that later
morbidity, primarily infection, can be minimized if an appro­
priate dosage of monoclonal antibody can be defined which will
maximize control of rejection without the addition of other
agents. It might then be anticipated that long-term morbidity
would be less than with conventional therapy, since it is gen­
erally accepted that serious complications are most likely to
occur when large doses of steroids are required to reverse
rejection activity.

In conclusion, these observations suggest that the homoge­
nity, limited dosage requirements, and ease of in vitro moni­
toring of the effects of monoclonal antibody upon T cell popu­
lations in the peripheral blood will simplify their application
to patient management. Although, to date, only a reagent
reactive with all peripheral blood T cells has been evaluated in patients,
the possibility of manipulating selected T cell subsets is now at
hand.

LITERATURE CITED

HM. Effect of antilymphocyte-globulin potency on survival of

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