Anti-CD3 Monoclonal Antibody Treatment of Patients with CD3-Negative Tumors: A Phase IA/B Study


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ABSTRACT

Anti-CD3 monoclonal antibodies induce the proliferation of human T-cells in vitro and activate specific and nonspecific cytolyis by human T-cell clones and human peripheral blood lymphocytes. In vivo administration of anti-CD3 prevents tumor growth of a UV-induced mouse fibrosarcoma. We conducted a phase I trial to determine the toxicity and immunomodulatory properties of low doses of anti-CD3 in 36 patients with cancer. In 23 patients, anti-CD3 was given i.v. over 3 h at 1, 10, 30, and 100 mg/ci7patient. Five other patients received anti-CD3 at 30 mg/ci7 by i.v. bolus. Patients were treated every 3 days for a total of four doses. An additional eight patients received anti-CD3 daily for 14 days at 3 mg/ci7 by i.v. bolus, 3-h infusion, or 24-h infusion. Dose-limiting toxicity was headache. Headache was often accompanied by signs and symptoms of meningeval irritation leading to performance of a lumbar puncture in nine patients. The opening pressure was usually elevated, and six patients had a cerebrospinal fluid lymphocytosis with an elevated protein. Increased levels of interleukin 6 were identified in the cerebrospinal fluid. The maximum tolerated dose by 3-h infusion was 30 mg/ci7. There were no objective tumor responses. There was a dose-related increase in the number of peripheral blood lymphocytes expressing the T-cell activation antigen CD69 (Leu 23), but no changes were seen in CD25 (interleukin 2 receptor) expression, and no changes were observed in the serum levels of the soluble interleukin 2 receptor. Even at these low doses of anti-CD3, 8 of 16 patients tested developed human anti-mouse antibodies.

INTRODUCTION

The T-cell antigen receptor is composed of two genetically and functionally distinct units: the variable ligand-binding heterodimer (a8 or y6) and the nonpolymorphic multichain CD3 complex. Antibodies specific for certain components of the CD3 complex are capable of either inhibition (1) or stimulation (2) of T-cell function, depending on the dose of antibody. Inhibition is seen with high doses of anti-CD3 and is associated with antigenic modulation of CD3 and the T-cell receptor. This strategy forms the basis for the Food and Drug Administration-approved use of anti-CD3 antibodies for the suppression of acute renal allograft rejection (3). Daily bolus administration of 5–10 mg of antibody for 10 or more days successfully aborts acute allograft rejection in a majority of patients. Lower doses of anti-CD3 are immunostimulatory and result in T-cell activation with proliferation, lymphokine production, and the induction of both antigen-specific (4, 5) and nonspecific cytotoxic T-lymphocytes (6). Thus, lower doses of anti-CD3 might actually augment immune responsiveness in vivo, a property that should be beneficial in the treatment of patients with cancer.

Murine studies have shown that low doses of the hamster anti-mouse CD3 monoclonal antibody 145-2C11 can exert antitumor effects (7, 8). Anti-CD3 treatment prevented the outgrowth of a weakly immunogenic UV-light-induced malignant fibrosarcoma that killed untreated control animals. Treatment also stimulated an in vivo immune response that provided lasting tumor immunity. Efficacy was dose dependent; lower doses effectively prevented tumor outgrowth, while higher doses led to enhanced tumor growth, presumably because of immune suppression. These antitumor effects appear to be the result of in vivo T-cell activation (9–11). Anti-CD3-treated mice exhibit increased cytokine production (TNFα,1 IFNγ, IL2, IL3, granulocyte-macrophage colony-stimulating factor, IL6), increased IL2R expression, increased responsiveness to IL2, enhanced reactivity in an allogeneic mixed lymphocyte reaction and mixed lymphocyte tumor culture, as well as enhanced colony-stimulating factor production with evidence of extramedullary hematopoiesis (7, 9–12). In vivo treatment with anti-CD3 resulted in the development of antigen nonspecific major histocompatibility complex-unrestricted cytotoxic cells as well as antigen-specific major histocompatibility complex-restricted cytotoxic T-cells. The latter were presumed to be responsible for the in vivo antitumor effects (13).

Thus, low doses of anti-CD3 can result in pan-stimulation of the T-cell population in vivo. This results in the generation of specific CD8 T-cells and the elimination of tumor cells. We wanted to see whether the murine monoclonal antibody OKT3 could induce in vivo T-cell activation in patients with CD3-negative tumors. Although this antibody had been used at high doses for immunosuppression, the dose of antibody required for the optimal activation of human T-cells in vivo was unknown. High doses, 1 mg or greater, activate T-cells (14–16) but have the undesirable effect of causing profound immunosuppression after multiple doses. The toxicity and immunomodulatory properties of lower doses of OKT3 were unknown. We therefore proposed a phase IA/IB study of OKT3 using doses ranging from 1 to 1000 mg/patient. The toxicity and immunomodulatory effects observed during this trial are reported below.
PATIENTS AND METHODS

Thirty-six consecutive patients with a variety of tumors (Table 1) were treated between March 1989 and April 1990.

Eligibility Requirements

Patients were eligible if they were over 18 years of age and if they had a histologically confirmed CD3-negative malignancy that had failed to respond to therapy or for which no effective therapy existed. Patients had to have evaluable disease with a Karnofsky performance status of 70 or greater, a 3-month life expectancy, adequate pulmonary function (FEV1 > 70% predicted and pO2 > 65 mmHg on room air) with no history of adult asthma or chronic obstructive pulmonary disease, no history of heart disease, adequate renal function (an estimated creatinine clearance of >55 cc/min), adequate hepatic function (bilirubin <1.8 mg/dl), a leukocyte count of >3,000/ml, granulocytes >1500/ul, lymphocytes >500/ul, and platelets >100,000/ul. The study was approved by the Institutional Review Boards of both the Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, and the Frederick Cancer Research and Development Center. All patients voluntarily gave their written informed consent before treatment. Pretreatment evaluation included history and physical examination, complete blood count, chemistry profile, coagulation profile (prothrombin time, partial thromboplastin time), human immunodeficiency virus antibody, hepatitis B surface antigen, pulmonary function tests, electrocardiogram, posterior-anterior and lateral chest X-rays, head CT scan in the 30 days before treatment, and other appropriate computed tomography and isotopic scans. Patients had a complete reevaluation 1 month after therapy. Responding patients were eligible for additional treatment at the same dose and schedule unless the patient developed HAMA.

Drug Supply and Preparation

The monoclonal antibody Orthoclone OKT3 was generously provided by the Biotech Division of the Ortho Pharmaceutical Corporation. The antibody was supplied in a buffered salt solution with polysorbate in water at 1 mg/ml in 5-ml ampules. The appropriate dose of antibody was diluted in 100 ml of normal saline with 5% human albumin for administration as a 3-h infusion and in 30 ml of normal saline with 5% human albumin for bolus injections.

Treatment Program

The patients are divided into two groups according to treatment schedule (Table 2). The initial 28 patients enrolled between March and August 1989 were included in group 1, while the seven patients treated during March and April 1990 constitute group 2. A single patient received 3 mcg anti-CD3 by 24-h i.v. infusion but is not included in the following analysis.

Group 1. Patients received the indicated dose of OKT3 in 100 cc of normal saline in the outpatient clinic through a peripheral vein over 3 h. Patients were monitored closely with frequent vital signs until discharge from the clinic 2 h after treatment was completed. Each patient was scheduled to receive four doses of anti-CD3 over 2 weeks on a Tuesday, Friday, Monday, Thursday schedule (Table 2). This schedule was chosen based on optimal tumor regression in our animal model and our desire to administer multiple treatments before the development of HAMA. Five patients were to be treated at each dose level until dose-limiting toxicity was observed. The next dose below that dose at which dose-limiting toxicity was observed in three of five patients was considered the MTD, and five additional patients were treated to quantify both the toxicity and the immunological effects at that dose. When 10 patients had been treated at the MTD for the 3-h infusion, 5 additional patients were treated with bolus infusion of OKT3 using the same schedule to determine the effect of method of administration on toxicity and immunomodulation.

Group 2. Because of the toxicity and the lack of significant immunomodulatory effects observed in patients treated in group 1, additional patients were treated with lower doses of OKT3 given by 3-h infusion or i.v. bolus on a different schedule, daily for 14 days. A single patient was treated with 3 mcg by 24-h continuous infusion.

Toxicity. Toxicity was graded according to the National Cancer Institute-Cancer Therapy Evaluation Program common toxicity scale. This toxicity scale does not grade headache separately. We graded the headaches as follows: Grade I, mild headache lasting less than 24 h that did not require narcotics; Grade II, headaches requiring narcotic administration; Grade III, headaches that were severe enough to require hospitalization for treatment or a lumbar puncture because of a concern for meningitis; and Grade IV, headaches accompanied by mental status changes.

Immunological Monitoring. Immunological studies were performed on serum and peripheral blood mononuclear cells obtained from patients before treatment (2-3 samples) and at various time points during therapy. During part 1, blood was drawn just before (0 h) to 2 h and 24 h after the first, third, and fourth (last) doses of anti-CD3 in all patients and at 1, 4, 6, and 12 h after the first dose in selected patients. In group 2, samples were obtained on days 0, 1, 7, 8, 13, and 14 with early time points (1, 3, and 4 h) drawn on treatment days 0, 7, and 13.

Whole blood was collected in preservative-free heparin, and following centrifugation through FicolI-Hypaque gradients, peripheral blood mononuclear cells were cryopreserved in RPMI 1640 (Gibco) supplemented with 10% fetal calf serum (Whittaker Bioproducts, Walkersville, MD) and 7.5% dimethyl sulfoxide (Fisher, Silver Spring, MD) using a Cryo-Med controlled-rate freezer (Mt. Clemens, MI) and stored in liquid nitrogen. Serum was separated and frozen at −70°C.

Proliferation Assay. Proliferative responses were determined by [3H] thymidine uptake and run in triplicate for each sample. Cryopreserved cells from all time points on a single patient were run on the same day to minimize variability. Cells (1 x 10^5/well) were plated into 96-well round-bottomed microtiter plates (Costar, Cambridge, MA) and stimulated with 10 mcg/ml phytohemagglutinin (Sigma Chemical Co.), 1000 units/ml IL2 (Hoffmann-LaRoche, Nutley, NJ), 100 ng/ml OKT3 (Ortho Pharmaceutical Corporation), or 50,000 irradiated allogeneic cells. Plates were pulsed with 0.44 μCi [3H]thymidine (6.7 Ci/mmol;
New England Nuclear, Boston, MA), and proliferative responses were measured after 3 days (phytohemagglutinin and IL2), 4 days (alloantigen), or 6 days (OKT3).

Neopterin. Serum levels of neopterin were measured using a radioimmunoassay kit (Neopterin-RIA; Henning Berlin GmbH, Berlin, Germany) according to the manufacturer’s instructions. The upper limit of normal is approximately 10 nmol/l.

Interferon. IFNγ was assayed by a sandwich radioimmunoassay kit (Gamma Interferon Radioimmunoassay; Centocor, Inc., Malvern, PA) according to the manufacturer’s instructions. Normal serum does not contain detectable levels of IFNγ.

Interleukin 2 Receptor. Serum IL2R levels were determined using a commercially available enzyme immunoassay (Cellfree Interleukin 2 Receptor Test Kit; T Cell Sciences, Cambridge, MA). The upper limit of IL2R values for normal individuals is reported by the manufacturer as 477 units/ml.

Human Anti-Mouse Antibody. Human anti-mouse IgG responses were measured in patient serum samples using a commercial enzyme-linked immunosorbent assay (Immustrip HAMA; Immunomedics, Warren, NJ). The test depends on the HAMA bridging between mouse IgG bound to a plastic plate and soluble, enzyme-conjugated mouse IgG. Values up to 200 ng/ml are considered to be within the normal range (product insert, Immunostrip HAMA).

Flow Cytometry. Phenotyping of leukocyte subpopulations was performed by flow cytometry using a panel of monoclonal antibodies directed against cell surface antigens. Cryopreserved mononuclear cells from an individual patient were examined on a single day to minimize variability in staining and instrument calibration. The following monoclonal antibodies were obtained from Becton-Dickinson Immunocytometry Systems (Mountain View, CA): anti-Leu 4 (CD3); anti-Leu 1 (CD5); anti-Leu 5b (CD2); anti-TCR-1 (aß T-cell receptor); anti-Leu 3a (CD4); anti-Leu 2 (CD8); anti-Leu 11 (CD16); anti-Leu 12 (CD19); anti-Leu M3 (CD14); anti-Leu 23 (CD69); anti-Leu 17 (CD38); anti-IL2R (CD25); and anti-HLA-DR. All antibodies were directly conjugated with FITC or phycoerythrin. Analyses were performed with single-color FITC-conjugated antibodies or as two-color samples in which cells were stained with a mixture of monoclonal antibodies directly conjugated with FITC or phycoerythrin. The cells were analyzed on a Coulter Profile flow cytometer with 4 decade log amplification of fluorescent signals (Coulter Cytometry, Hialeah, Florida). Lymphocyte and monocyte populations were bitmap gated based on low angle forward and right angle light scatter properties. The percentage of cells reactive with each monoclonal antibody was determined by comparison of fluorescently labeled cells with cells that had been incubated with labeled immunoglobulin subclass controls. Detection of OKT3 on the surface of lymphocytes was performed by staining the cells with fluorescein-conjugated goat anti-mouse IgG F(ab')2 (Tago, Inc., Burlingame, CA) and comparing fluorescence to samples stained with FITC-conjugated mouse subclass controls. On-treatment samples were also compared to baseline samples stained identically.

Statistical Analysis

Repeated measures ANOVA, profile analysis, and post hoc tests were performed to analyze hematological data. Following the global analyses, further investigation was performed on each dose group separately. Changes in peripheral white blood cell counts were also analyzed by one-sample t tests on paired comparisons.

RESULTS

Clinical Effects

Among the 28 patients in group 1, only 20 patients received all four intended doses of antibody. Seven patients had therapy discontinued because of toxicity and one patient for progressive disease (acute obstruction of the biliary tree in a patient with metastatic colon cancer). In group 2, two of seven patients completed all 14 doses of therapy, both at 3 mcg by bolus. The other five patients failed to complete therapy because of toxicity. The one patient who received anti-CD3 at 3 mcg by 24-h infusion received all 14 doses. There were no objective tumor responses in any of the 36 treated patients.

The toxicities associated with anti-CD3 treatment are listed in Table 3. The majority of patients at all dose levels experienced fever with or without chills. The temperature elevation was usually low grade and never went above 40°C. Peak temperature appeared to be dose related. The chills were mild and only occasionally required treatment with meperidine. Occasional patients experienced myalgias and arthralgias during therapy; patients treated by bolus injection had more complaints than patients receiving antibody by 3-h infusion.

The most significant toxicity observed in our patients was the development of headache. Headache was the reason for removal of 12 patients from the study. The character of the headache did not depend on the dose, method, or schedule of administration. Headaches were typically described as throbbing, bifrontal, or global with a prominent occipital component. They were poorly controlled by acetaminophen or aspirin, and in many cases oral narcotics were unable to control the pain and parenteral narcotics were required. Eight patients required hospitalization for treatment of their headaches, which usually responded slowly even to parenteral narcotics, with complete resolution requiring up to a week in some patients. Premedication with nonsteroidal antiinflammatory agents and narcotics did not prevent the development of the headaches. Patients often complained of light sensitivity, and the majority, particularly those with severe (Grade 3) headaches, also had accompanying symptoms of nausea and vomiting. In 10 patients the headache was accompanied by a stiff neck and photophobia; two patients also had confusion and disorientation consistent with a meningoencephalitis.

The presence and severity of headache was dose related and depended on both the method and frequency of administration (Table 3). No headaches were seen in five patients receiving 1 mcg of anti-CD3 by 3-h infusion, but headache was a nearly

<table>
<thead>
<tr>
<th>Table 3 Toxicity of anti-CD3</th>
</tr>
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<tbody>
<tr>
<td>Dose level (mcg/ patient)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>&lt;38°C</td>
</tr>
<tr>
<td>38-40°C</td>
</tr>
<tr>
<td>Chills</td>
</tr>
<tr>
<td>Myalgia</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
<tr>
<td>Dyspnea</td>
</tr>
<tr>
<td>Confusion</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
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</tbody>
</table>

* Anti-CD3 administered by bolus infusion.
universal complaint in patients treated at ≥ 3 mcg. At 10 mcg, all five patients had headaches, but they were usually graded as mild to moderate and were never felt to be significant enough to perform a lumbar puncture. The time to development of the headache also depended on the dose of OKT3. At 10 mcg, patients developed headaches after the first (2), second (2), or third (1) dose of antibody, while five of seven patients at 30 mcg developed headache after the first dose, with one each after the second and third doses. At 100 mcg all three patients had headaches after the first dose. At the lower doses headache occurred later in the evening or the morning following a dose, whereas with progressively higher doses the headaches occurred sooner and were seen within 2–6 h of antibody administration in patients receiving 100 mcg of anti-CD3. The headaches also lasted longer following higher doses of anti-CD3; headaches at 10 mcg tended to last less than 24 h and resolved before the next dose, while headaches at 30 mcg and 100 mcg often lasted more than 48 h. The method of administration also affected the incidence of headache. All five patients receiving 30 mcg by bolus injection had severe headaches after the first dose, while only 5 of 10 receiving the same dose by 3-h infusion had headache. Thus, bolus administration may increase the incidence and severity of headache. In part two, all five patients receiving 3 mcg by bolus infusion had a headache after a median of 2 doses (range, 1 to 5) but headaches reached grade 3 in only two patients. Both patients receiving 3 mcg of antibody by 3-h infusion developed severe headaches requiring cessation of treatment; no further dose escalation was attempted.

Maximum Tolerated Dose

The MTD for a 3-h continuous infusion given four times 3 days apart was 30 mcg. The MTD for bolus administration by this same schedule was less than 30 mcg. Changing to daily administration for 14 days decreased the tolerable dose; the MTD by this method of administration was less than 3 mcg for either bolus or 3-h infusion. Dose-limiting toxicity in all cases was severe headache.

Lumbar Puncture Results

Lumbar puncture was performed in 7 of 28 group 1 patients, and in 2 of 8 patients in group 2 (Table 4). The patients undergoing lumbar puncture generally had the most severe headaches and usually had associated symptoms of stiff neck, photophobia, and/or altered mental status. The opening pressure was elevated in all patients in whom it was measured, and two patients experienced marked relief of their headache following removal of some CSF. Only two patients had normal CSF, one patient had an increased CSF protein as the only abnormality, and the remainder had increased protein levels associated with a pleocytosis. In two cases this was solely a lymphocytosis; in four cases it was primarily a lymphocytosis, and in one case there was a preponderance of neutrophils. All CSF was tested routinely for Venereal Disease Research Laboratory test (VDRL) and cryptococcal antigen and cultured for bacterial growth, Mycobacteria, and viruses. All cytological examinations were negative. None of the patients had infections of the central nervous system, and all headaches eventually resolved with return of neurological status to baseline after discontinuation of therapy. Only one patient underwent a repeat lumbar puncture. Repeat examination 4 days after treatment revealed a persistently abnormal CSF at a time when the headache had almost completely resolved (Table 4).

All patients had had a CT scan of the brain performed in the 30 days before starting treatment, and in all cases the scan was negative for intracranial metastases. The first few patients who developed a severe headache underwent repeat CT scan and magnetic resonance imaging, but no abnormalities were detected. Subsequent patients did not routinely undergo reevaluation with CT or magnetic resonance imaging. One patient with melanoma treated at the 100-mcg dose had recurrent headache upon returning home and was found by magnetic resonance imaging to have evidence of meningeal metastases 1 month after her last dose of anti-CD3. The cytology of her CSF during anti-CD3 treatment was negative even upon review. Cell surface marker analysis was performed by flow cytometry on the cells from the CSF of six patients. The results are provided in Table 5. The majority of cells were T-cells with a small number of B-cells and natural killer cells in percentages similar to those found in peripheral blood. The cells did not appear to be activated, since few cells expressed the p55 IL2 receptor (CD25), HLA-DR, or the transferrin receptor (CD71). CSF from three patients was assayed for IL1α, IL2, TNFα, and granulocyte-macrophage colony-stimulating factor. No detectable levels were found in any patient. Six patients had detectable levels of soluble IL2 receptor (from 100 to 290 units/ml), but these levels were below the level normally seen in serum. Five patients had levels of IL6 in their CSF that exceeded those found in paired serum samples (Table 6). We did not have baseline CSF samples, and we did not follow serial IL6 CSF levels, so we cannot comment on whether these represent significant increases within an individual patient. No anti-CD3 antibodies were detected in the CSF (data not shown).

Table 4 Cerebrospinal fluid analysis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose</th>
<th>Opening pressure</th>
<th>Protein (mg/dl)</th>
<th>Glucose (mg/dl)</th>
<th>WBC (cumulative)</th>
<th>Differential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>30 (1)</td>
<td>3</td>
<td>290</td>
<td>176</td>
<td>47</td>
<td>2000</td>
</tr>
<tr>
<td>(4 days later)</td>
<td>250</td>
<td>56</td>
<td>46</td>
<td>1100</td>
<td>9812P</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>30 (1)</td>
<td>ND*</td>
<td>96</td>
<td>63</td>
<td>148</td>
<td>9812P</td>
</tr>
<tr>
<td>16</td>
<td>100 (1)</td>
<td>1</td>
<td>370</td>
<td>49</td>
<td>118</td>
<td>45</td>
</tr>
<tr>
<td>17</td>
<td>100 (1)</td>
<td>1</td>
<td>ND</td>
<td>64</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>100 (1)</td>
<td>2</td>
<td>ND</td>
<td>78</td>
<td>58</td>
<td>1300</td>
</tr>
<tr>
<td>24</td>
<td>30 (B)*</td>
<td>1</td>
<td>245</td>
<td>28</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>27</td>
<td>30 (B)</td>
<td>1</td>
<td>190</td>
<td>98</td>
<td>80</td>
<td>400</td>
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* Anti-CD3 administered by 3-h infusion (l).a
ND, not determined; L, lymphocytes; P, polymorphonuclear leukocytes.

Hematological Effects

There was a dose-dependent decrease in lymphocyte count 2–24 h following treatment in patients receiving antibody by 3-h infusion (Fig. 1). This decrease was transient in all cases and had returned to baseline by the next dose 72 h later. All three patients treated at 100 mcg and 6 of 10, 2 of 5, and 1 of 5 patients treated at 30, 10, or 1 mcg, respectively, had decreases in peripheral blood lymphocyte counts after injection of anti-CD3. One sample repeated-measures ANOVA showed that lymphocyte counts obtained 2 and 24 h after treatment with OKT3 from patients in the 1 or 10 mcg/m2 dose groups did not have a significant trend from baseline. Follow-up one-sample t tests showed no significant change from baseline in either of these groups. For patients treated at 30 mcg/m2, ANOVA showed a borderline significant downward linear trend in lymphocyte counts from baseline after therapy (P = 0.0111).
Table 5 Flow cytometric analysis of CSF

<table>
<thead>
<tr>
<th>Patient</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD4 + 8</th>
<th>CD19</th>
<th>CD56</th>
<th>CD25</th>
<th>HLA-DR</th>
<th>CD38</th>
<th>CD69</th>
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<tbody>
<tr>
<td>11</td>
<td>91*</td>
<td>ND</td>
<td>ND</td>
<td>5</td>
<td>2</td>
<td>ND</td>
<td>5</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>16</td>
<td>74</td>
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<td>85</td>
<td>59</td>
<td>23</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>23</td>
<td>1</td>
</tr>
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<td>22</td>
<td>78</td>
<td>71</td>
<td>18</td>
<td>1</td>
<td>4</td>
<td>17</td>
<td>8</td>
<td>6</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>27</td>
<td>60</td>
<td>36</td>
<td>28</td>
<td>1</td>
<td>9</td>
<td>26</td>
<td>13</td>
<td>9</td>
<td>43</td>
<td>4</td>
</tr>
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* Percentage of cells that were positive.
+ Not done.

Table 6 CSF IL-6 levels

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<thead>
<tr>
<th>Patient</th>
<th>Serum*</th>
<th>CSF</th>
</tr>
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<tbody>
<tr>
<td>16</td>
<td>0.16</td>
<td>7.0</td>
</tr>
<tr>
<td>22</td>
<td>2.6</td>
<td>3.8</td>
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<tr>
<td>24</td>
<td>0.0</td>
<td>6.0</td>
</tr>
<tr>
<td>27</td>
<td>0.1</td>
<td>11.0</td>
</tr>
<tr>
<td>2*</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

* ng/ml measured by enzyme-linked immunosorbent assay.
+ Patient no., 2 from part 2.

Follow-up one-sample t tests on the individual time points showed no significant change at 2 h after therapy (P = 0.0679) and borderline significant decreases 24 h after treatment (P = 0.0382). In patients treated at 100 mcg/m², significant downward linear (P = 0.001) and quadratic (P = 0.0035) trends in lymphocyte counts were detected by ANOVA. In spite of the small number of patients treated at this dose, one-sample t tests for differences from baseline showed borderline significant decreases at 2 h (P = 0.0194) and significant decreases at 24 h after therapy (P = 0.0043). Patients receiving 30 mcg by bolus were comparable to patients receiving the same dose by 3-h infusion, with 4 of 5 patients having decreases in their lymphocyte count.

Pharmacokinetics

OKT3 could be detected on lymphocytes in a time- and dose-dependent manner; positive staining was noted in 10 of 11 evaluable patients treated with ≥30 mcg by infusion and in all five patients treated with 30 mcg by bolus. In contrast, only 3 of 10 patients treated with <30 mcg exhibited goat anti-mouse immunoglobulin-FITC staining above background. There was a wide variation in the percentage of positively stained lymphocytes even among patients treated at the same dose, but peak levels for 3-h infusion of 30 mcg ranged from 6 to 54% of all lymphocytes. Bolus administration appeared to result in similar levels, with a range of 22 to 46% positivity for 3 mcg and 19 to 46% positivity at 30 mcg. The peak staining occurred 1–2 h after antibody administration and returned toward baseline levels within 24 h (Fig. 2). Blood samples were not obtained at the same time points for patients in Groups 1 and 2; no samples were obtained during the 3-h infusion, so all samples were taken at least 4 hours after the initiation of antibody administration, conditions that are much different from those of patients receiving bolus therapy.

Immunological Effects

Cell Surface Markers. There were transient decreases in the percentages of CD3* cells of some patients treated at the 30- and 100-mcg doses that were accompanied by modest increases in CD4/CD8 ratios and relative increases in the percentages of CD19* and/or CD56* cells. There were no predictable dose-related increases in surface IL2 receptor (CD25) expression or the activation antigens CD38 and HLA-DR on lymphocytes, but there were transient increases in the intensity of HLA-DR expression on some patients’ monocytes 2 h after treatment (data not shown). There were increases in the percentage of lymphocytes expressing the early activation marker CD69. The best example of this was in a patient treated with 100 mcg by 3-h infusion (Fig. 3). This event appeared to be dose related because none of the 6 patients evaluated at 1 or 10 mcg exhibited changes, whereas 3 of 8 at 30 mcg by 3-h infusion also showed an increase. Two of these 3 patients were evaluated for CD69 expression after OKT3 treatments on days 0, 6, and...
or mitogen-stimulated proliferation of peripheral blood mononuclear cells. Enhanced CD69 expression was noted on both CD3+ and CD3- populations and was usually observed in blood samples drawn 24-48 h after treatment. With the exception of the patient treated with 100 mcg, CD69 increases were modest (<10%) when compared to expression by lymphocytes cultured overnight with 10 ng/ml OKT3 (data not shown).

Cytokine and Cytokine Receptor Induction. It is possible that T-cells activated by anti-CD3 would leave the circulation. Studies in mice have shown that anti-CD3-activated cells produce soluble IL2R and IFNγ, among other cytokines (10-12). Therefore, we reasoned that even if the activated cells left the circulation, we could assess their state of activation indirectly by measuring serum levels of their soluble products that were secreted as a result of their activation. Neopterin is produced with anti-CD3 (data not shown). Functional Assays. There were no significant dose-related detectable differences in the spontaneous or IL2-, anti-CD3-, or mitogen-stimulated proliferation of peripheral blood mononuclear cells obtained from patients during or after treatment with anti-CD3 (data not shown).

DISCUSSION

This is the first dose escalation trial of anti-CD3 performed in patients with cancer. To our surprise, we observed dose-limiting toxicity in the form of severe headache at low doses of antibody, which led to our conclusion that the MTD for anti-CD3 by 3-h infusion was 30 mcg. This is more than 100-fold lower than the dose that is currently recommended for the treatment of renal allograft rejection (5 mg). Perhaps this observation should not have been so unexpected because there are data to indicate that lower doses of anti-CD3 are more immunostimulatory than higher doses (1, 13). Since much of the toxicity observed after anti-CD3 is thought to result from massive cytokine release (18), lower, potentially more immunomodulatory doses could be more toxic. The systemic reaction in transplant patients consists of fever, chills, headache, vomiting, diarrhea, and tachycardia and is usually observed only after the first dose of anti-CD3. This syndrome is thought to be the result of T-cell activation with secondary induction of the cytokines IFNγ and TNFα (19, 20). Elevated levels of IL2 and IL6 have also been described (21). Our patients experienced a similar, although not identical symptom complex after anti-CD3. However, these symptoms were not accompanied by measurable cytokine release except in a small minority of patients. The headache, if anything, was more severe in our patients and did not resolve after multiple doses. The symptoms in renal transplant patients get better and do not recur after multiple doses, probably because high doses of anti-CD3 lead to down-regulation of cell surface CD3, and daily administration leads to measurable circulating levels of anti-CD3 that prevent the reappearance of CD3+ cells. In the absence of CD3 on the T-cell surface, there can be no triggering of cells by anti-CD3, so there is no cytokine induction and therefore no symptoms. In our patients, low doses of anti-CD3 never led to the total disappearance of CD3-positive cells, and there was no clear evidence from flow cytometry of down-regulated CD3. This could explain headaches that occurred after multiple doses of anti-CD3. Our data are not consistent with IFNγ or TNFα as the cause for the anti-CD3-induced headache. Perhaps the mechanism of headache induction is different at higher doses, or there may be an additional, as yet unidentified cytokine that is responsible for symptoms at both levels. The syndrome of aseptic meningitis has been described previously with high doses of anti-CD3, but in a much lower percentage (<10%) of treated patients (22, 23). These patients are receiving anti-CD3 for immunosuppression and are often on other immunosuppressive drugs like steroids, azathioprine, and cyclosporin that might block induction of the factor inducing headaches. Thus, the more infrequent occurrence of this dose-limiting toxicity in patients receiving high dose anti-CD3 could be related to a decrease in cells expressing the target (CD3) or to the concomitant therapy.

Another possible mechanism of headache could be enhanced local production of nitric oxide leading to high levels of nitrates that could cause vasodilatation and headache. Enhanced nitric oxide has been observed after the activation of T-cells (24). Analysis of the CSF revealed a nonspecific inflammatory reaction that does not help us determine the etiology. Extensive preclinical testing failed to show cross-reactivity between brain tissue and CD3 (Ortho Pharmaceutical Corporation), and OKT3 was not detected in the CSF; however, two previous studies have shown a cross-reactivity between brain tissue and peripheral blood T-cells. Garson et al. (25) showed that the monoclonal antibodies UCHT1 and anti-Leu 4 reacted with a cytoplasmic protein found in Purkinje fibers in addition to CD3. Jingwu et al. (26) showed that a monoclonal antibody to human myelin basic protein reacts with the CD3 complex and actually is mitogenic for resting T-cells. However, neither study was able to demonstrate that OKT3 antibody bound to the
neural tissue in question. Therefore, we think that it is unlikely that cross-reactivity explains the side effects observed.

The major biological effect observed was a dose-related leukopenia and lymphocytopenia following anti-CD3 administration. This was so pronounced in some patients that they were insufficient peripheral blood cells to examine cell surface marker expression and perform functional analysis. Phenotypic analysis of peripheral blood in patients with adequate cell numbers failed to detect evidence of a dose-related lymphocyte activation. IL2 receptor, transferrin receptor, and HLA-DR, which are up-regulated in activated or proliferating lymphocytes, did not change during anti-CD3 treatment. Similarly, none of the functional assays performed indicated that we had activated peripheral blood lymphocytes. This is in contrast to what has been observed in anti-CD3-treated mice in which IL2 receptor expression on lymphocytes is increased and lymphocytes exhibit enhanced spontaneous proliferation and enhanced proliferation to IL2 and alloantigens (10). However, these observations were made in mice with lymphocytes obtained from lymph nodes, not from the peripheral blood. Our inability to detect activated lymphocytes may merely reflect their preference for lymphoid tissues once they have been activated. The insignificant increases in soluble IL2 receptor expression argue against this, because if large numbers of T-cells were activated, we would expect to be able to detect increased levels of their soluble products in the serum even if the activated cells had become sequestered in the lymph nodes or the spleen.

Our data are most consistent with the interpretation that we were using doses of anti-CD3 that were suboptimal for in vivo T-cell activation. We appeared to be approaching more optimal doses at 100 mcg, where we saw dramatic decreases in lymphocyte number and significant increases in CD69 expression. CD69 is a phosphorylated M, 28,000-32,000 disulfide-linked homodimer that is rapidly induced after lymphocyte activation (27). It has been found on T-cells, B-cells, and natural killer cells (28). Since its up-regulation is one of the earliest cell surface events detectable following triggering of the CD3/T-cell receptor complex, we know that there has been some in vivo T-cell activation in our patients. There may be insufficient stimulus to trigger the entire cascade of events, including anti-CD3-activated IL2R expression; TNF, IFN, and IL2 secretion; and enhanced proliferation seen in vitro and at higher doses of anti-CD3 in humans in vivo. It may simply be that higher doses of anti-CD3 are required to achieve significant in vivo T-cell activation. Preliminary data from a trial being performed at the University of Chicago indicate that this is indeed the case because higher doses of anti-CD3 have been shown to produce in vivo T-cell activation. Giving anti-CD3 according to our schedule resulted in excessive toxicity without sufficient T-cell activation.

Other innovative strategies will be required to take advantage of the favorable immunomodulatory properties of anti-CD3 without interference from its undesirable toxic ones. A current approach following adoptive transfer and treatment of the patient with IL2. This approach has led to substantial in vivo proliferation of activated T-cells without excessive toxicity.

* J. Richards, personal communication.

REFERENCES

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Anti-CD3 Monoclonal Antibody Treatment of Patients with CD3-Negative Tumors: A Phase IA/B Study


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