Treatment of Acute Graft-Versus-Host Disease With Humanized Anti-Tac: An Antibody That Binds to the Interleukin-2 Receptor


Humanized anti-Tac is a genetically engineered human IgG1 monoclonal antibody specific for Tac, the α subunit of the interleukin-2 (IL-2) receptor, and blocks IL-2-dependent activation of human T lymphocytes. The safety, pharmacokinetics, and immunosuppressive activity of humanized anti-Tac were evaluated in 20 patients who developed acute graft-versus-host disease (GVHD) after allogeneic marrow transplantation. Patients had developed acute GVHD at 5 to 26 (median, 14) days after transplantation and had failed to respond to primary therapy with glucocorticoids. Sequential groups of 4 patients each received a single 1-hour infusion of antibody in escalating doses of 0.5, 1.0, or 1.5 mg/kg; 8 additional patients were then treated with 1.5 mg/kg. A second infusion of antibody was administered after 11 to 48 (median, 16) days in 8 patients who had transient improvement of GVHD after the first infusion. Acute side effects, limited to chills in 1 patient and diaphoresis in another, were observed during or shortly after the antibody infusion. Overall improvement of acute GVHD occurred in 8 patients, 6 of whom were treated with a single antibody infusion and 2 with two infusions. Four responses were complete and 4 were partial. Three additional patients had improvement in one organ but progression in another. Responses occurred in 9 of 16 cases with skin disease, 3 of 15 with liver disease, and 6 of 12 with gastrointestinal disease. Two patients survive at 529 and 645 days after antibody treatment. Two patients died after relapse of leukemia. Sixteen patients died of infection or organ failure between 5 and 211 (median, 55) days. The terminal elimination half-life of the antibody was 44 to 363 hours, with a harmonic mean of 79, 88, and 94 hours, respectively, for the three doses studied. Absolute peripheral blood T-lymphocyte counts remained unchanged during the 56 days after infusion of the antibody. A fraction of circulating T cells expressed the α chain of the IL-2 receptor that, in some patients, was bound by antibody in vivo.

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GRAFT-VERSUS-HOST disease (GVHD) is a frequent complication of allogeneic marrow transplantation caused by donor T lymphocytes reactive to histocompatibility antigens of the host. During early activation, T cells express a variety of surface molecules not found on resting cells including, among others, high-affinity receptors for interleukin-2 (IL-2). Binding of IL-2 to its receptor (IL-2R) is an essential requirement for T-cell proliferation. High-affinity IL-2Rs are complexes of at least three proteins, a p55 IL-2Ra, a p70 IL-2Rβ, and a p64 IL-2Rγ. IL-2Ra and IL-2Rβ, but not IL-2Rγ, are capable of binding IL-2 independently of the others, but with a much lower affinity than that of the multimeric complex. IL-2Rβ and IL-2Rγ are expressed constitutively on most lymphoid cells. IL-2Rβ plays a critical role in intracellular signaling induced by IL-2 binding. IL-2Rγ is required for normal T- and B-cell development, because its genetic mutation results in X-linked severe combined immunodeficiency disease in humans. IL-2Ra is expressed on T, B, and natural killer (NK) cells only after these cells have become activated by antigen or IL-2. Hence, an antibody that selectively destroys or inactivates IL-2Ra-bearing cells would specifically inhibit nascent or ongoing immune responses without suppressing natural immunity or the ability to mount a specific immune response to an antigen encountered once the immunosuppressive therapy has been terminated.

In rodents, monoclonal antibodies (MoAbs) specific to IL-2Ra are immunosuppressive in vivo in several models of transplantation and autoimmune diseases. Those antibodies can suppress delayed-type hypersensitivity responses, inhibit local or systemic graft-versus-host reactions, and prevent or reverse rejection of cardiac, skin, and pancreatic allografts. Possible mechanisms by which monoclonal anti–IL-2Ra antibodies exert their immunosuppressive activity include blocking IL-2 binding to its receptor and elimination of IL-2Ra-bearing lymphoid cells. In humans, efficacy of the murine monoclonal anti–IL-2Ra antibody anti-Tac was demonstrated in a randomized clinical trial of prevention of cadaveric kidney allograft rejection. Furthermore, three distinct rodent MoAbs were found to have some immunosuppressive activity in patients with acute GVHD resistant to treatment with cyclosporine and glucocorticoids.

The therapeutic efficacy of rodent MoAbs in humans is limited by their short half-life, by the immune response of

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the recipient to the heterotypic protein, and by the inefficient interaction of the marine antibody with host effector mechanisms. Chimeric MoAbs consisting of human constant regions and mouse variable regions were developed to circumvent these problems. Techniques were developed to more fully humanize mouse MoAbs by incorporating almost exclusively amino acids present in the complementary determining regions (CDRs) into human framework and C-region sequences. The humanized anti-Tac is a selectively modified human IgG1 MoAb that binds specifically to IL-2Ra. Murine sequences comprise only about 10% of the engineered molecule. The affinity of the humanized antibody for IL-2Ra is approximately threefold lower than that of the parent mouse antibody. However, the humanized antibody has acquired the ability to mediate antibody-dependent cell-mediated cytotoxicity of IL-2Ra cells in vitro, in contrast to the native murine IgG2a antibody, which does not possess this activity. Humanized anti-Tac does not activate complement-dependent lysis in vitro. Humanized anti-Tac was found to be less immunogenic, to have more favorable pharmacokinetics, and to be more immunosuppressive than native murine antibody in cynomolgus monkeys transplanted with cardiac allotrafts.

We report here results of a phase I-II study of humanized anti-Tac used in one or two doses for treatment of acute GVHD in patients refractory to therapy with cyclosporine and glucocorticoids. We found that treatment with humanized anti-Tac was not associated with appreciable side effects, that the terminal elimination half-life was higher than those reported for any rodent MoAb in humans, and that there was no detectable antibody formation to the humanized anti-Tac antibody in the patients studied. Improvement of GVHD was seen in approximately 40% of the patients treated, indicating that humanized anti-Tac has immunosuppressive activity in humans.

MATERIALS AND METHODS

Patients. Characteristics of the 20 patients enrolled in this study are summarized in Table 1. All patients received myeloablative treatment with cyclophosphamide plus total body irradiation or busulfan or both. Criteria for matching recipients and donors for human leukocyte antigens (HLA) have been described. GVHD prophylactic regimens included cyclosporine alone, cyclosporine and prednisone, or cyclosporine and methotrexate, as previously described. The clinical diagnosis of acute GVHD was confirmed by biopsy of skin, liver, or gastrointestinal tract in 16 of 20 patients. The onset of acute GVHD occurred at a median of 14 (range, 5 to 26) days after transplantation. Patients were treated with 6-methylprednisolone at a dose of 2 mg/kg/day intravenously (IV) for a median of 18 (range, 4 to 30) days. Two patients were enrolled on a randomized phase III primary GVHD therapy trial using 6-methylprednisolone in combination with either Xomazyme-CD5 plus or placebo administered at 0.1 mg/kg/d for 14 days. The study drug had been discontinued for at least 72 hours before treatment with humanized anti-Tac. One patient received humanized anti-Tac for overall grade I acute GVHD. In this patient, there was a relative contraindication to glucocorticoids because of prednisone-induced psychosis. The GVHD grades at time of antibody therapy are shown in Table 1.

Treatment protocol and definition of clinical response. Patients gave informed consent for antibody administration according to treatment protocol no. 638 approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. Hypersensitivity to humanized anti-Tac was tested in all patients by IV administration of 100 μg of antibody without premedication. Patients receiving prednisone or cyclosporine before humanized anti-Tac antibody continued treatment with these medications as tolerated by renal function. Antibody was administered as a 1-hour IV infusion with vital signs monitored every 15 minutes. Three groups each consisting of 4 patients received a single infusion of humanized anti-Tac at doses of 0.5, 1.0, or 1.5 mg/kg. The highest total single dose administered in any patient was 100 mg. Patients who showed transient improvement of GVHD were eligible for retreatment once at a subsequent time with an identical dose of antibody. After the first 12 patients were treated, the protocol was amended to allow enrollment of an additional 8 patients to be treated at the 1.5 mg/kg dose.

Patients were monitored for blood cell and platelet counts, serum chemistry, and renal function tests daily; for liver function every other day; and with a chest x-ray once weekly for 56 days. GVHD severity was graded as described. Measurements of involved skin surface area, serum bilirubin, stool volume (3-day average), visible blood in stool, and abdominal cramping were recorded weekly through day 56 after the administration of humanized anti-Tac or until death.

Responses were evaluated on day 29 after humanized anti-Tac antibody therapy, at initiation of alternative GVHD therapy, or at death, whichever occurred first. Response of skin disease was defined as resolution or decrease of rash involving the skin surface by ≥25%. Progressive skin disease was defined by an increase in involved skin area by ≥25%. Response of liver disease was defined by a decrease in serum bilirubin to less than 2 mg/dL for patients with baseline values of 2 to 4 mg/dL, a decrease of ≥2 mg/dL for patients with baseline values of 4 to 8 mg/dL, and a decrease of ≥25% for patients with baseline values greater than 8 mg/dL. Progressive liver disease was defined as an increase of serum bilirubin by ≥2 mg/dL for patients with baseline values less than 8 mg/dL or as an increase by ≥25% in serum bilirubin for patients with baseline values ≥8 mg/dL. Response of gut disease was defined by resolution of diarrhea or decrease in the 3-day average stool volume by ≥500 mL/day and with clearing of cramps and bleeding if present. Progressive gut disease was defined by an increase in the 3-day average stool volume by ≥500 mL, or development of new cramps or bleeding. Liver or gut complications other than GVHD were ignored while scoring response to humanized anti-Tac if the attending physician or the investigators believed that GVHD was the primary cause of organ dysfunction. Overall, complete response was defined as resolution of GVHD in all evaluable organs and no additional treatment administered for acute GVHD. Partial response was defined as improvement in at least one evaluable organ without deterioration in any other. Overall progression was defined as deterioration in at least one evaluable organ. No change was defined by the absence of any difference sufficient to meet minimal criteria for response or progression in any evaluable organ after treatment.

Lymphocyte studies. Blood samples were obtained just before treatment, and 18 hours, 7, 28, 56, and 360 days after each antibody infusion. Cell surface phenotype was evaluated as previously reported. IL-2Ra cells were monitored by staining with fluorescein isothiocyanate (FITC)-conjugated antibody 7G7-B6, specific for the CD25 antigen but binding to an epitope different from the one recognized by humanized anti-Tac. Cells coated by humanized anti-Tac were identified by staining with a phycoerythrin (PE)-labeled murine IgG2b antibody specific for human IgG1 (clone HP-6000; obtained from American Type Culture Collection, Rockville, MD). Saturation of humanized anti-Tac binding epitopes in vivo was evaluated by comparing the fluorescent intensity of cells incubated with PE-conjugated HP-6000 antoglobulin to that of cells incubated with excess exogenous humanized anti-Tac plus HP-6000. Free Tac-binding epitopes were also evaluated by comparing the fluorescent intensity of cells incubated with PE-conjugated humanized anti-Tac or
FITC-conjugated antibody 2A3 (murine IgG1 specific for Tac antigen; distributed by Becton Dickinson, San Jose, CA) before and after treatment. Surface expression of IL-2Rβ was identified by antibody Mikp3.23. The lower limit of detection for this assay was peroxidase-labeled humanized anti-Tac incubated simultaneously.

6605 31F MDS/RAEB TR Unrelated Identical CsA/MTX 16 3 2 3 III
6606 38F CML/CP Unrelated Identical CsA/MTX 10 3 0 3 III
6607 36F CML/AP Unrelated Identical CsA/MTX 16 3 3 0 III
6608 51M CML/AP Unrelated Identical CsA/MTX 14 3 3 0 III
6609 36M CML/B C Unrelated Identical CsA/MTX 10 3 0 3 III
6610 38M CML/CP Unrelated Identical CsA/MTX 10 3 3 0 III
6611 36F CML/AP Unrelated Identical CsA/MTX 10 3 3 0 III
6612 52F NHL/Rel Sibling Identical CsA/MTX 23 2 4 0 IV
6613 32F MDS/RAEB TR Unrelated Identical CsA/MTX 21 4 0 3 IV
6614 28F CML/AP Unrelated Identical CsA/MTX 16 3 2 0 III
6615 24/M RA Unrelated Identical CsA/MTX 6 4 0 0 III
6616 45/M CML/AP Unrelated Identical CsA/MTX 14 0 3 0 III
6617 32F CML/AP Unrelated Identical CsA/MTX 26 3 1 0 III
6618 40/M CML/3rd CP Unrelated Identical CsA/MTX 21 0 3 3 III
6619 28/M CML/AP Unrelated Identical CsA/MTX 5 3 3 0 III
6620 25/M ALL/Rel Unrelated Identical CsA/MTX 21 4 2 0 III
6621 51M CML/AP Unrelated Identical CsA/MTX 21 0 1 3 III

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; AP, accelerated phase; BC, blast crisis; CML, chronic myelogenous leukemia; CP, chronic phase; CsA, cyclosporine; GI, gastrointestinal tract; loci, HLA A, B, D loci; MDS, myelodysplastic syndrome; MTX, methotrexate; NHL, non-Hodgkin’s lymphoma; Pred, 6-methylprednisolone; RA, refractory anemia; RAEB(TR), refractory anemia with excess blasts (in transformation); Rel, relapse.

Pharmacokinetics. Serum concentrations of humanized anti-Tac were measured before and at various intervals through 56 days after administration. Antibody was measured by competitive enzyme-linked immunosorbent assay (ELISA) using purified soluble IL-2Ra protein as the capture reagent, with the test serum competing with peroxidase-labeled humanized anti-Tac incubated simultaneously. The lower limit of detection for this assay was 25 pg/L. Model independent pharmacokinetic analysis was performed. Values for terminal elimination half-life (t1/2β), volume of distribution (Vdβ), systemic clearance (CLs), and area under the curve (AUC o → ∞) were determined. Pharmacokinetic parameters are listed in the table accompanying Fig 1.

Circulating soluble IL-2Ra. Unbound soluble IL-2Ra protein circulating in serum was measured by a sandwich ELISA assay. Solid-phase–bound 7G7/B6 antibody was used as the capture reagent. Plates were then incubated with serum containing soluble IL-2Ra peptide and then washed. The detecting antibody was peroxidase-labeled humanized anti-Tac. The normal level of soluble IL-2Ra in healthy volunteers is 200 to 500 U/mL, where 1 U equals 0.2 pg.

Human antibody against humanized anti-Tac. Formation of human antibodies specific for humanized anti-Tac was evaluated by two assays. The first assay was a sandwich ELISA using humanized anti-Tac bound to a microtiter plate as the capture reagent and peroxidase-conjugated humanized anti-Tac as the detecting reagent. The concentration of antibodies to humanized anti-Tac could be determined from a standard curve of purified goat antibodies specific to humanized anti-Tac. The lower limit of detection was 7.8 pg/L. The second assay used was a dot blot assay. Complexes of specific antibodies and humanized anti-Tac were captured by protein G Sepharose and then dissociated from protein G Sepharose and from each other by treatment with pH 2.5 buffer. Dissociated IgGs were immobilized on a polyvinylidene fluoride (PVDF) membrane. Humanized anti-Tac was detected by peroxidase-conjugated IL-2Ra. Antibodies specific for humanized anti-Tac were detected by peroxidase-conjugated humanized anti-Tac. The concentration of each antibody was determined from standard curves of mixtures of humanized anti-Tac and goat antibodies to humanized anti-Tac. The lower limit of sensitivity of the dot blot assay for antibodies to humanized anti-Tac and for humanized anti-Tac were 312 and 80 pg/L, respectively.

Fig 1. Mean ± SEM serum concentrations of humanized anti-Tac in patients treated at doses of 0.5 (n = 4), 1.0 (n = 4), or 1.5 mg/kg (n = 12).
RESULTS

Clinical safety and efficacy. Treatment with humanized anti-Tac was administered at a median of 32 (range, 16 to 50) days after transplantation (Table 2). Humanized anti-Tac was administered IV as a single infusion over 1 hour at 0.5 mg/kg in 4 patients, at 1.0 mg/kg in 4 patients, and at 1.5 mg/kg in 12 patients. Eight patients who manifested transient improvement after the first antibody infusion received a second infusion at the same dose, a median of 16 (range, 11 to 48) days after the first infusion. Two of 28 antibody infusions (7%) were followed by side effects possibly related to the infusion of the antibody: chills in 1 patient and diaphoresis in another. There were no consistent changes in serum chemistries after treatment with humanized anti-Tac. There was no evidence of other toxicities.

Four patients had complete resolution of acute GVHD. 4 had a partial overall improvement, 3 had improvement in one organ but deterioration in another, 3 had no changes, and 6 had progression. Six responses were achieved after one infusion of humanized anti-Tac, and 2 responses were achieved after the second infusion. Nine of 16 patients (56%) had response to treatment of skin GVHD; complete response in 5 and partial response in 4. Three of 15 patients (20%) responded to treatment of liver GVHD; complete response in 1 and partial response in 2. Six of 12 patients (50%) responded to treatment of gastrointestinal GVHD; complete response in 4 and partial response in 2. One patient with a complete overall response did not develop chronic GVHD and is alive on day 645, off immunosuppression. Seven patients with complete or partial overall responses later developed chronic GVHD. One is alive on day 529, 2 died after relapse of leukemia, and 4 died with pneumonia on days 100, 112, 170, and 211 after treatment with humanized anti-Tac. Twelve patients who failed to respond to humanized anti-Tac died of organ failure, infection, or bleeding at a median of 40 (range, 5 to 98) days after treatment with humanized anti-Tac. Median patient survival was 76 days after treatment with humanized anti-Tac. Causes of death are listed in Table 2. Among the 4 patients who did not have a biopsy performed to confirm the clinical diagnosis of acute GVHD, 2 patients achieved a complete response and survived at least 1 year, and the other 2 patients had progression of GVHD and died.

Pharmacokinetics of humanized anti-Tac. Mean serum concentration/time curves for patient groups treated at different dose levels and results of noncompartmental pharmacokinetic analysis are shown in Fig 1. Observed maximum serum concentration (Cmax) values were predictable for each dose of humanized anti-Tac administered. AUC o → ∞ values showed a dose-proportional increase with increasing doses. Terminal elimination half-life values showed high intersubject variability (44 to 360 hours), with the overall harmonic mean half-life being 88 hours. The mean Vdβ was 83 (range, 50 to 167) mL/kg, greater than the plasma volume, indicating tissue penetration. Systemic serum clearance values were variable among patients, and the mean values were low (CL, 0.6 [range, 0.21 to 1.25] mL/kg/h). Values obtained from patients after a first or a second infusion of antibody had similar clearance values (Fig 2).

Human antibody formation against humanized anti-Tac. No human antibody responses against humanized anti-Tac were detected by either ELISA or dot blot assays in any of the 15 patients who survived at least 28 days after therapy and were tested weekly through day 56 after infusion of the antibody.

Circulating soluble IL-2Rα. Levels of soluble IL-2Rα antigen circulating free in the serum varied widely patient to patient, from 170 to 23,328 (median, 3,116) U/mL, and did not correlate with severity of acute GVHD at the time of treatment with humanized anti-Tac (data not shown). By day 28, soluble IL-2Rα levels decreased in 4 of 8 patients who showed improvement of GVHD and increased 6 of 7 patients who did not show improvement of GVHD.

Circulating peripheral blood lymphocytes. Absolute counts of CD3+, CD4+, CD8+, and CD16+CD56+ lymphocytes were low before treatment, but did not decrease significantly after the infusion of humanized anti-Tac. Cell counts remained low for at least 56 days after treatment. Three patients studied between 300 and 360 days after treatment showed increasing counts of each lymphocyte subset (Fig 3). Expression of IL-2Rα or β on the surface of circulating lymphocytes was not affected by treatment with humanized anti-Tac (Fig 4). The IL-2Rα epitope recognized by humanized anti-Tac was saturated on the cells of all patients who were studied on day 7 after treatment and on the cells of most patients studied on day 28 (Fig 4, □). Staining of freshly isolated lymphocytes with an antihuman globulin showed that cells from patients isolated 7 through 28 days after treatment were coated with humanized anti-Tac antibody (Fig 5). Lymphocyte-bound antibody was no longer detectable in most patients studied on day 56 after treatment with humanized anti-Tac.

DISCUSSION

Clinical improvement of GVHD was documented in 40% of the patients treated with one or two infusions of humanized anti-Tac. This rate of response is similar to the rate of 41% previously reported by our group in 420 patients after second-line therapy of GVHD using multiple doses of glucocorticoids, cyclosporine, or polyclonal anti-thymocyte globulin, and appears to be better than the 9% response rate observed in 11 patients treated with seven infusions of an IL-2Rα–specific murine antibody. Administration of humanized anti-Tac appeared to be safe, and adverse patient outcome was caused by limited efficacy and progression of GVHD rather than to therapy-related toxicity. Median patient survival was 76 days in this study, compared with 27 and 29 days in two preceding studies of glucocorticoid-refractory GVHD at our institution in which patients were treated with murine monoclonal anti–T-cell antibodies specific for IL-2Rα or CD3ε. Four of 20 patients (20%) in the current study recovered completely from acute GVHD, 2 are alive after more than 1 year, and 2 died of leukemia relapse. In published reports of phase I/II studies of various anti–T-cell murine MoAbs tested for anti-GVHD activity (ie, anti-CD3 antibodies OKT3, 64.1, or BC3; IL-2Rα–specific antibodies 2A31 or BT563; or anti-CD5 immuno- toxin H65-RTA), 18 of 108 patients (17%) with glucocorticoid-resistant GVHD survived a minimum of 4 months.
Table 2. Results of Acute GVH Treatment With Humanized Anti-Tac

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<th>Time From Transplant to Antibody Therapy (d)</th>
<th>Antibody Dose (mg/kg)</th>
<th>Response to Antibody Therapy</th>
<th>Time From Antibody Therapy to GVHD Progression (d)</th>
<th>Time to Second HAT Infusion (d)</th>
<th>Time to Alternative GVHD Therapy (d)</th>
<th>Time From Alternative GVHD Onset (d)</th>
<th>Chronic GVHD</th>
<th>Survival From Start of Antibody Therapy (d)</th>
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Abbreviations: ARDS, adult respiratory distress syndrome; ATG, ATG (Ujohn) administered at 10 mg/kg/dose >6 doses q.d.; BC, oral Beclomethasone for GI GVHD; CR, complete response; EBV-LPS, Epstein-Barr virus associated lymphoproliferative syndrome; GI, gastrointestinal tract; HAT, humanized anti-Tac; HSV, Herpes simplex virus; IP, interstitial pneumonia; NC, no change; ND, not done; NE, not evaluable; NR, no response; P, progression; PR, partial response; Pred, 6-methylprednisolone used at 2 mg/kg/day; PUVA, Psoralen and ultraviolet-A light therapy for skin GVHD; RSV, respiratory syncytial virus; SD, study drug, either Xamozyme or placebo.

* Response was evaluated on day 29 after antibody therapy, or at time of alternative therapy or death if before day 29.
† Survival, as of the last date of contact, is current as of October 21, 1993.
‡ Transient improvement, followed by progression.
§ Venoocclusive disease of the liver was present.
¶ Actual dose administered was 1.16 mg/kg.
GVHD TREATMENT WITH HUMANIZED ANTI-TAC

Better survival might be expected in patients treated earlier with less advanced GVHD, as suggested by a study of IL-2Ra-specific antibody BB-10 in which 14 of 32 patients (44%) were surviving at the time of the report with a follow-up between 2 and 14 months after treatment. Incomplete therapeutic success of humanized anti-Tac in this first trial might be related to the study design that limited the use of the antibody to one or two infusions to carefully evaluate acute safety and terminal half-life of the antibody. Because neutralizing antibodies were not developed by any patient, there is a rationale to believe that administration of multiple doses may achieve a better therapeutic effect. Another major factor that might have limited the therapeutic efficacy of humanized anti-Tac is the advanced stage of GVHD in most patients. IL-2Ra-specific antibodies can efficiently block generation of alloreactive cytotoxic T cells, the putative effectors in GVHD. However, once GVHD is established, one would expect that mature effector T cells lose expression of IL-2Ra and become less susceptible to the effect of IL-2Ra-specific agents. Humanized anti-Tac may be more effectively used at onset of GVHD, and perhaps even better if used for prevention rather than treatment of established GVHD.

Experiments in nonhuman primates have suggested that humanized anti-Tac might be more immunosuppressive than murine antibodies directed towards the same specificity. Humanized anti-Tac has a half-life of 94 hours at the highest dose studied, as opposed to approximately 40 hours for murine antibodies. In this study, there was no antibody formation against humanized anti-Tac, in contrast to 4 of 8 patients treated with murine anti-IL-2Ra antibody 2A3. The observation that the rates of antibody elimination were similar for the first and the second dose is consistent with the concept that neutralizing antibodies were not formed after the first infusion. The lack of immunogenicity of humanized anti-Tac in this study cannot be generalized with confidence in view of the profound immunodeficiency of patients early after allogeneic marrow transplantation.

Whether humanized anti-Tac destroys IL-2Ra+ cells in vivo could not be determined by our study. Administration of humanized anti-Tac induced a small, insignificant decrease in the number of circulating T cells. In contrast, administration of either CD3-specific murine antibody OKT3, CD5-specific immunotoxin H65-RTA, or CD52-specific humanized antibody Campath-1H is associated with profound and long-lasting lymphopenia. Circulating IL-2Ra+...
cells are bound by humanized anti-Tac up to 4 weeks after a single infusion, indicating that binding activity of the antibody is long lasting in vivo. This observation does not exclude the possibility that humanized anti-Tac might induce killing of IL-2Rα+ cells, because newly activated T cells may be generated and enter circulation at the same rate that others are destroyed.

Pharmacokinetic studies demonstrated that the terminal elimination half-life of humanized anti-Tac had a harmonic mean value of approximately 4 days. This value appears similar to those reported for another humanized antibody specific for a tumor antigen (100 hours) or for human cytomegalovirus (CMV)-specific IgG administered in the posttransplant period (5 to 10 days), but is lower than values reported in patients receiving CMV-specific human MoAbs (13 to 17 days) or other human IgG (20 days). Antibody clearance is determined by two distinct processes: binding to the specific antigen and antigen-independent breakdown of the antibody molecule. High intersubject variability makes comparison of half-life values difficult between dose groups. The AUC of humanized anti-Tac increased in proportion to the dose administered, and it is possible that not all antigen binding sites were saturated even at the highest dose tested, and that administration of a higher dose of antibody may be required to achieve saturation. However, it remains to be tested whether therapeutic efficacy requires a saturating dose of antibody.

The data presented here provide the basis for initiating clinical trials investigating the effectiveness of multiple doses of humanized anti-Tac for prevention or treatment of GVHD and for exploring the activity of this agent in other disorders that might benefit from selective T-cell immunosuppression.

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