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Immunology

# Anti-Tac-H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival

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**ABSTRACT** **High-affinity interleukin 2 receptors (IL-2Rs) are expressed by T cells activated in response to foreign histocompatibility antigens but not by normal resting T cells. To exploit this difference in IL-2R expression, anti-Tac-M, a murine monoclonal antibody specific for the IL-2R$\alpha$ chain, was used to inhibit organ allograft rejection. However, the use of murine anti-Tac as an immunosuppressive agent was limited by neutralization by human anti-murine antibodies and by weak recruitment of effector functions. To circumvent these difficulties, a humanized antibody to the IL-2R, anti-Tac-H, was prepared. This molecule is human with the exception of the hypervariable segments, which are retained from the mouse. *In vivo* survival of anti-Tac-H is 2.5-fold longer than simultaneously administered anti-Tac-M (terminal $t_{1/2}$, 103 hr vs. 38 hr). In addition, anti-Tac-H is less immunogenic than anti-Tac-M when administered to cynomolgus monkeys undergoing heterotopic cardiac allografting. Specifically, all monkeys treated with anti-Tac-M developed measurable anti-anti-Tac-M levels by day 15 (mean onset, 11 days). In contrast, none of the animals receiving anti-Tac-H produced measurable antibodies to this monoclonal antibody before day 33. Finally, there was a prolongation of graft survival in the cynomolgus heterotopic cardiac allograft model in animals receiving anti-Tac. In animals that received anti-Tac-M, the allograft survival was prolonged compared to that of the control group (mean survival, 14 ± 1.98 days compared to 9.2 ± 0.48 days; $P < 0.025$). Graft survival was further prolonged by anti-Tac-H with a mean survival of 20.0 ± 0.55 days (compared to controls, $P < 0.001$; compared to anti-Tac-M, $P < 0.02$). There was no toxicity attributable to the administration of either form of anti-Tac. Thus, anti-Tac-H significantly prolonged allograft survival in primates, without toxic side effects, and may be of value as an adjunct to standard immunosuppressive therapy in humans.**

The development of specific, effective, and nontoxic immunosuppressive agents remains a major goal in the prevention of allograft rejection in humans. Many of the effective agents are associated with broad toxicity. Furthermore, all of the polyclonal, and most monoclonal, antibodies that have been clinically effective recognize the majority of circulating T cells, thus yielding broad nonspecific immunosuppression, leaving the transplant recipient susceptible to opportunistic infections.

An ideal immunosuppressive agent would selectively target only those T cells destined to participate in the immune rejection. Specifically, a strategy to achieve more specific immunosuppression would be to target antigens that are absent on resting lymphocytes but are expressed on lymphocytes responding to an allograft. We have chosen to target the interleukin 2 receptor $\alpha$ chain (IL-2R$\alpha$; p55, CD25, or Tac protein), as its expression marks a critical step in the activation of alloreactive T cells (1–3). The scientific basis for this approach is the observation that T cells in patients rejecting allografts express IL-2R identified by the anti-Tac monoclonal antibody, whereas normal resting cells and their precursors do not (1, 2). In individuals receiving organ allografts, the T lymphocytes of the host having appropriate T-cell antigen receptors that recognize the foreign histocompatibility antigens expressed on the donor organ become activated. These activated cells express the inducible IL-2R$\alpha$ and participate in the rejection of the allograft. In addition, the serum concentration of soluble IL-2R$\alpha$ released by activated cells is elevated before and during allograft rejection episodes (4, 5). Furthermore, antibodies to the IL-2R$\alpha$ inhibit the proliferation of T cells reacting to foreign histocompatibility antigens expressed on the donor organ and prevent the generation of cytotoxic T cells in allogeneic cocultures (6).

In light of these studies, the use of monoclonal antibodies directed toward the IL-2R$\alpha$ chain represents a rational approach that may allow the development of donor-specific immunological unresponsiveness, since only activated cells express this receptor subunit. In studies reported by Kirkman *et al.* (7), the survival of allografts was prolonged in rodent recipients treated with anti-IL-2R monoclonal antibodies. In collaborative studies with that group, treatment with our murine IgG2a monoclonal antibody to the primate IL-2R$\alpha$ (anti-Tac-M) resulted in a significant increase in both renal allograft and recipient survival in a renal allograft model performed in *Macaca fascicularis* (cynomolgus) monkeys (8). Due to these encouraging results, human recipients of cadaveric-donor renal allografts were treated with various anti-IL-2R$\alpha$ monoclonal antibodies as adjunctive immunotherapy. In studies performed by Soulillou *et al.* (9) and Cantarovich *et al.* (10), prophylactic use of a monoclonal antibody (33B3.1) directed against the IL-2R was shown to be highly efficient in preventing renal allograft rejection in the first weeks after human transplantation. Similarly, in a collaborative study with Kirkman and coworkers (11), a randomized prospective trial of anti-Tac-M in human renal transplantation was undertaken. There was a significant reduction in early rejection episodes in the anti-Tac-treated patients and the time to first rejection was delayed. Despite these effects, anti-Tac-M administration did not affect actual or actuarial graft or patient survival.

Therapy with murine monoclonal antibodies such as anti-Tac-M has been hampered by two major problems that may contribute to their relatively limited effectiveness in preven-

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Abbreviations: IL-2R, interleukin 2 receptor; CDR, complementarity-determining region.

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tion of allograft rejection. First, murine monoclonal antibodies are foreign proteins that are neutralized when patients develop antibodies to them. Second, most murine monoclonal antibodies are less effective than human antibodies at recruiting human host effector functions. To circumvent these difficulties, we used genetic engineering to prepare a humanized anti-Tac monoclonal antibody (anti-Tac-H) by combining the complementarity-determining regions (CDRs) of the murine anti-Tac antibody with human IgG1 $\kappa$ framework and constant regions (12, 13). This hyperchimeric anti-Tac antibody maintains high binding affinity ($3 \times 10^9$ $M^{-1}$) for Tac-expressing cells and preserves the ability to inhibit antigen and mixed leukocyte-induced T-cell proliferation (12, 13). Furthermore, anti-Tac-H manifests a new activity of antibody-dependent cellular cytotoxicity with human mononuclear cells that was absent in the parental murine anti-Tac.

In this report, we compare the *in vivo* characteristics of unmodified murine anti-Tac and humanized anti-Tac antibody in cynomolgus monkeys. Anti-Tac-H manifested longer *in vivo* survival and reduced immunogenicity when compared to anti-Tac-M. Furthermore, cardiac allograft survival was prolonged in the group treated with anti-Tac-H as compared to the untreated group as well as the group treated with murine anti-Tac.

## MATERIALS AND METHODS

**Monoclonal Antibody Production.** Anti-Tac, a murine-derived monoclonal antibody (anti-Tac-M) that binds to the IL-2R$\alpha$ subunit, was produced and characterized as described (14). Humanized anti-Tac antibody (anti-Tac-H) was constructed as described by combining the CDRs of the anti-Tac antibody with human IgG1 $\kappa$ framework and constant regions (12). A computer model of murine anti-Tac was used to identify several amino acids that, while outside the CDRs, were likely to interact with the CDRs or antigen. These murine amino acids were also retained in the humanized antibody. Anti-Tac-H was produced in a continuous perfusion bioreactor from SP2/0 cells transfected with the genes encoding the heavy and light chains of the hyperchimeric antibody and purified on an IL-2R affinity column. The eluted antibodies were further purified on two serially linked Sephacryl S-300 columns. The final products were sterilized through a 0.2-$\mu$m Corning filter and were shown to contain $<10$ endotoxin units/mg. Anti-Tac-H was shown to be $>99\%$ pure as determined by SDS/polyacrylamide gel electrophoresis under reducing and nonreducing conditions.

**Cardiac Allograft Model.** Cardiac allografts between outbred cynomolgus (*M. fascicularis*) monkeys were performed by a modification of the operative technique of Michler *et al.* (15) as described (16). The donor aorta was anastomosed end to side to the infrarenal abdominal aorta of the recipient and the donor pulmonary artery was anastomosed to the adjacent vena cava. All procedures were carried out under standard aseptic conditions and were approved by the Animal Research Committee of the National Heart, Lung, and Blood Institute according to the guidelines of the National Institutes of Health for humane use and care of laboratory animals (17). Palpation and inspection were the methods used to determine graft function and to diagnose rejection. A swollen, warm, or boggy graft with decreased systolic function was indicative of impending rejection. Graft loss was determined by cessation of systolic function.

**Experimental Groups.** There were three experimental groups of five animals each. The animals in group I underwent heterotopic, cardiac allograft transplantation as described above but did not receive any form of immunosuppression. The animals in group II received 1 mg of anti-Tac-M per kg of body weight intravenously on the day before the operation and then every other day until the day of graft rejection. The animals in group III were treated identically to those in group II except that anti-Tac-H (1 mg/kg) was substituted for anti-Tac-M.

**Statistical Analysis.** Analysis of variance was used to determine statistical significance. Significance was considered to occur at the $P \leq 0.05$ level. All values reported are reported as mean ± SEM.

**Immunogenicity Assay.** Monkey antibodies to anti-Tac-M and anti-Tac-H were evaluated by an antigen-bridging ELISA. Dynatech Immulon II wells were coated with 50 ng of either anti-Tac-M or anti-Tac-H in 50 $\mu$l of carbonate/bicarbonate buffer for 3 hr at 37°C. Wells were washed with PBS containing 0.05% Tween 20 (PBST), blocked for 1 hr at 37°C with bovine serum albumin/PBST, and washed again prior to addition of test samples. Wells were incubated with either 50 $\mu$l of affinity-purified goat anti-anti-Tac standards or test samples for 18 hr at 4°C. The unbound proteins were washed from the wells and 50 $\mu$l of biotinylated anti-Tac-M (or -H) was added to each well for 2 hr at 37°C, washed, and then incubated with 50 $\mu$l of alkaline phosphatase-conjugated streptavidin for 2 hr at 37°C. After washing off excess streptavidin, the quantity of biotin-labeled anti-Tac bound by monkey antibodies to the monoclonal antibodies was determined colorimetrically by the addition of 50 $\mu$l of *p*-nitrophenyl phosphate in diethanolamine buffer (pH 9.8) with incubation at 37°C for 1 hr (Sigma). The absorbance at 405 nm was determined with a Titertek microplate reader. Since the color intensity developed is directly proportional to the antibody concentration in the serum samples, the concentrations of antibodies to anti-Tac-H and anti-Tac-M in the monkey samples were calculated from a standard curve developed with the titered purified goat antibodies directed to the monoclonal antibodies.

**Serum Concentrations of Anti-Tac-H and Anti-Tac-M.** The serum concentrations of anti-Tac-H and anti-Tac-M were determined by a solid-phase IL-2R inhibition assay (18). Plates were coated with 16 ng of the soluble form of IL-2R$\alpha$ (sIL-2R$\alpha$) overnight at 4°C and then blocked with 1% bovine serum albumin. Wells were washed and incubated with 200 $\mu$l of serum sample overnight at 4°C. Without washing the samples from the wells, 50 $\mu$l of horseradish peroxidase-labeled IL-2 was added to a final concentration of 1:2000 for 3 hr. During this reaction, IL-2 binds to the immobilized sIL-2R$\alpha$ that was not previously occupied by anti-Tac. The unbound proteins were washed from the wells and the quantity of bound horseradish peroxidase-labeled IL-2 was determined in a colorimetric enzymatic assay with 2,2′-azinobis(3-ethybenzthiazoline sulfonate) (Sigma) in 0.1 M citrate buffer/0.03% $H_2O_2$, pH 4.2, for 30 min. The color intensity in this reaction was inversely proportional to the antibody concentration in the serum samples. The concentrations of anti-Tac-H and anti-Tac-M were calculated from standard curves developed with purified anti-Tac-H and anti-Tac-M titrated on each plate.

**Pharmacokinetics.** The pharmacokinetics of anti-Tac-H and anti-Tac-M were assessed by using $^{125}$I-labeled humanized anti-Tac ($^{125}$I-anti-Tac-H) and $^{131}$I-labeled murine anti-Tac ($^{131}$I-anti-Tac-M). Iodination of each of the above preparations was performed by the iodine monochloride technique of McFarlane (19). Five to 20 $\mu$Ci (1 Ci = 37 GBq) of $^{131}$I-anti-Tac-M and $^{125}$I-anti-Tac-H was administered in a single syringe to five cynomolgus monkeys. A 10-min blood sample was obtained for plasma volume determination. Additional blood samples were collected for counting at 2-hr intervals and then daily for 10 days after the administration of the labeled protein. The time course of decline of radioactivity from the plasma was plotted semilogarithmically. The terminal biological half-life ($t_{1/2}$) of each labeled protein was determined graphically. The fraction of the intravascular

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pool catabolized per day (fractional catabolic rate) was determined according to the method of Matthews (20) as discussed (21).

**Function and Assay.** The ability of the monoclonal antibodies to function in antibody-dependent cellular cytotoxicity was assessed with human and cynomolgus mononuclear cells as described (13).

## RESULTS

**Pharmacokinetics.** To determine their pharmacokinetic properties $^{131}$I-anti-Tac-M and $^{125}$I-anti-Tac-H were administered simultaneously in a single syringe to cynomolgus monkeys. These animals also received either (*i*) unlabeled anti-Tac [0.5 mg of anti-Tac-M per kg of body weight and 0.5 mg of anti-Tac-H per kg of body weight (three animals)] in association with the radiolabeled monoclonal antibodies or (*ii*) 2 mg of anti-Tac-H per kg (two animals) on the day prior to the radiolabeled anti-Tac injection. The unlabeled anti-Tac was administered to saturate the antigenic target of the monoclonal antibodies—that is, the Tac protein soluble in the circulation as well as that on the surface of T cells. Initially, there was a rapid decline in the fraction of the administered radioactivity remaining intravascular, predominantly reflecting distribution of the radiolabeled antibody from the intravascular to the extravascular compartments (Fig. 1 and Table 1). This phase was followed by a slower terminal exponential decline in plasma radioactivity that predominantly reflects catabolism of the monoclonal antibody. In the five animals studied, the $t_{1/2}$ of the terminal exponential of $^{125}$I-anti-Tac-M decline was 38 ± 3 hr with a mean fraction of the intravascular pool of anti-Tac-M catabolized per day of 0.58 ± 0.07. The survival $t_{1/2}$ of $^{125}$I-anti-Tac-H was prolonged when compared to radiolabeled anti-Tac-M. The mean $t_{1/2}$ of the terminal exponential of the serum $^{125}$I-anti-Tac-H die-away curve was 103 ± 9 hr with a fraction of the intravascular pool catabolized per day of 0.25 ± 0.03. Thus, anti-Tac-H had an

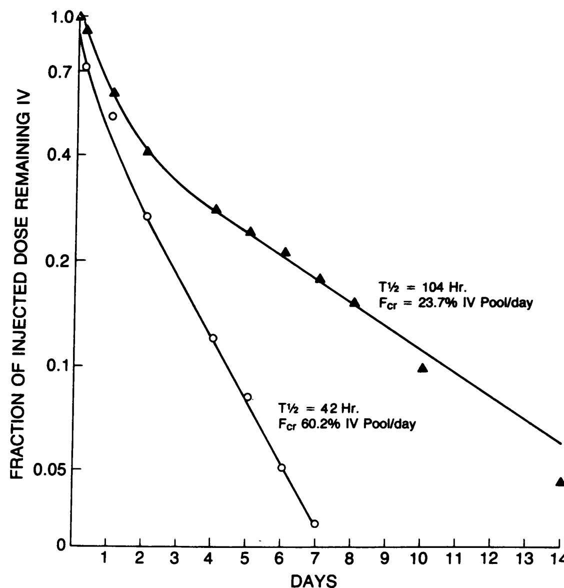


FIG. 1. The metabolism of $^{125}$I-anti-Tac-H and $^{131}$I-anti-Tac-M (each at 0.5 mg/kg) in a single cynomolgus monkey. The fractions of the injected dose remaining in the serum for $^{125}$I-anti-Tac-H (▲) and for $^{131}$I-anti-Tac-M (○) are indicated. The $t_{1/2}$ of the terminal exponential with anti-Tac-H ($t_{1/2}$, 104 hr) was 2.5-fold longer than that observed with anti-Tac-M ($t_{1/2}$, 42 hr).

Table 1. Metabolism of $^{125}$I-anti-Tac-H and $^{131}$I-anti-Tac-M in cynomolgus monkeys

| | $^{125}$I-anti-Tac-H | | $^{131}$I-anti-Tac-M | |
|---|---|---|---|---|
| Animal | Survival $t_{1/2}$, hr | Fraction of group IV pool catabolized per day | Survival $t_{1/2}$, hr | Fraction of group IV pool catabolized per day |
| 1 | 108 | 0.25 | 41 | 0.49 |
| 2 | 84 | 0.35 | 25 | 0.85 |
| 3 | 104 | 0.24 | 42 | 0.60 |
| 4 | 85 | 0.22 | 43 | 0.48 |
| 5 | 133 | 0.18 | 41 | 0.46 |
| Mean ± SEM | 103 ± 9 | 0.25 ± 0.03 | 38 ± 3 | 0.58 ± 0.07 |

Animals 1–3 received 0.5 mg of anti-Tac-H per kg and 0.5 mg of anti-Tac-M per kg in association with the radiolabeled monoclonal antibodies, whereas animals 4 and 5 received 2 mg of anti-Tac-H per kg on the day prior to the radiolabeled anti-Tac turnover studies.

≈2.5-fold longer survival in cynomolgus monkeys than did the simultaneously administered anti-Tac-M.

**Function.** In functional studies, anti-Tac-M was inactive in antibody-dependent cellular cytotoxicity assays with either human or cynomolgus mononuclear cells. In contrast, anti-Tac-H was able to support antibody-dependent cellular cytotoxic activity with either human or cynomolgus monkey mononuclear cells (data not shown).

**Efficacy of Anti-Tac Monoclonal Antibodies in Prolonging Cardiac Allograft Survival.** The efficacy of the two forms of the anti-Tac monoclonal antibody in prolonging allograft survival was assessed by using a primate heterotopic cardiac allograft model. The animals were either untreated (group I; $n = 5$) or treated with 1 mg of anti-Tac-M per kg (group II; $n = 5$) or the same dose of anti-Tac-H (group III; $n = 5$) by bolus intravenous infusion every other day until graft rejection. The five animals that received no immunosuppression (group I) rejected their grafts on or about day 9 posttransplant (mean survival, 9.20 ± 0.48 days; Table 2). In the animals that received unmodified murine anti-Tac (group II), graft survival was prolonged when compared to that of the control group: mean survival in anti-Tac-M group 14 ± 1.98 days ($P < 0.025$ compared to the untreated group). Graft survival was further prolonged with anti-Tac-H with a mean survival of 20.0 ± 0.55 days ($P < 0.001$ compared to control). Graft survival after treatment with anti-Tac-H was also prolonged over that observed in the group treated with anti-Tac-M ($P < 0.02$). There was no evidence of toxicity attributable to the administration of either form of anti-Tac.

**Immunogenicity of Anti-Tac-M and Anti-Tac-H.** The immune response to anti-Tac-M and anti-Tac-H was assessed by determining the serum concentrations of monkey antibodies to murine and humanized anti-Tac. All five animals receiving anti-Tac-M developed measurable antibody levels directed toward the murine monoclonal antibody. In the four animals with a graft survival of <9 days, monkey anti-anti-Tac-M antibodies were demonstrable 1–10 days (mean, 4

Table 2. Efficacy of anti-Tac monoclonal antibodies in prolonging cardiac allograft survival

| Group | Antibody (dose)* | Graft survival, days | Mean survival ± SEM, days | *P* vs. control |
|---|---|---|---|---|
| I | None | 8, 9, 9, 9, 11 | 9.2 ± 0.48 | |
| II | Anti-Tac-M (1 mg/kg) | 9, 12, 12, 17, 20 | 14 ± 1.98 | <0.025 |
| III | Anti-Tac-H (1 mg/kg) | 19, 19, 20 20, 22 | 20.0 ± 0.55 | <0.001 |

*Given every other day.

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days) before allograft rejection. In these four animals, antibodies first appeared on days 6–15 (mean, day 11) after initiation of anti-Tac-M administration. Exceedingly high levels of anti-anti-Tac-M antibodies were still present 17 months after anti-Tac-M therapy.

Anti-Tac-H was much less immunogenic when administered to cynomolgus monkeys. None of the animals produced antibodies to anti-Tac-H during the 19- to 22-day period between initiation of anti-Tac administration and the time of allograft rejection. In two animals, no anti-anti-Tac-H antibodies were demonstrable during the 21 and 26 days of observation. The remaining three animals developed anti-anti-Tac-H antibodies on days 33, 33, and 42 after initiation of anti-Tac-H administration. Furthermore, the highest concentrations of anti-Tac-H antibodies observed 4 months after initiation of therapy (0.47, 3.6, and 23 μg/ml) in three animals producing antibodies were ≈100-fold lower than the highest concentrations of monkey anti-anti-Tac-M antibodies (300, 331, 1675, 2510, and 14,000 μg/ml) observed in animals receiving the murine antibody. During the first week of therapy, all 10 animals receiving either anti-Tac-M or anti-Tac-H had measurable serum levels of the monoclonal antibody both immediately after administration as well as 48 hr afterwards—that is, just prior to the subsequent dose of antibody—thus indicating that adequate anti-Tac antibody had been administered to saturate the receptors and to leave residual circulating antibody (Fig. 2). Specifically, the geometric mean peak and trough levels for anti-Tac-M during the initial week of study were 1.03 and 3.9 μg/ml, respectively. However, after this initial period of administration but prior to graft rejection, the animals receiving anti-Tac-M demonstrated rapid clearance of the administered monoclonal antibody so that anti-Tac-M was no longer demonstrable (i.e., <40 ng/ml) in the circulation prior to the subsequent administration of the antibody. This precipitous fall in measurable anti-Tac-M was associated with the development of monkey antibodies directed toward anti-Tac-M. Such antibodies were demonstrable within 2 days after the first demonstration of rapid clearance of anti-Tac-M. In contrast, the animals receiving anti-Tac-H generally maintained measurable levels of this monoclonal antibody throughout the period of administration of the monoclonal antibody—that is, from initiation of therapy until after graft rejection (Fig. 2*B*). In all cases, anti-Tac-H was demonstrable in the trough sample obtained within 48 hr of graft rejection. Taken together, these results indicate that anti-Tac-H is less immunogenic in cynomolgus monkeys than is the anti-Tac-M monoclonal antibody.

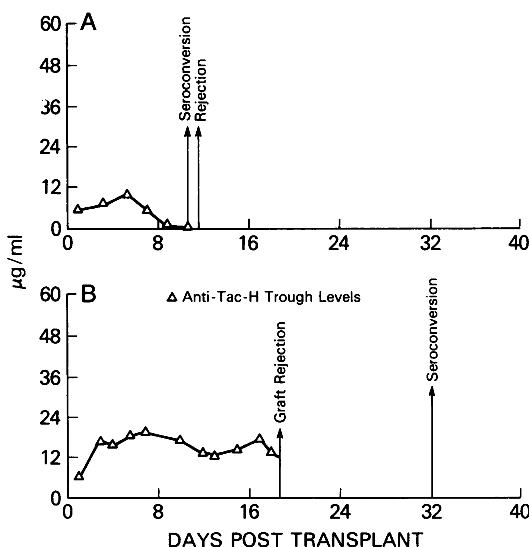


FIG. 2. Anti-Tac-M and anti-Tac-H antiserum concentration profile in individual monkeys receiving anti-Tac-M (*A*) and anti-Tac-H (*B*). The samples were obtained at the times indicated (Δ) immediately before the antibody infusion. The last infusion of anti-Tac-M was on day 11 after transplantation (12 days after first infusion) and that of anti-Tac-H was on day 17 after transplantation (18 days after first infusion), since the animals rejected their allografts on days 12 and 19, respectively. Seroconversion is defined as the first time that antibodies to the monoclonal antibodies were detected.

## DISCUSSION

The IL-2Rα chain has been used as a target for relatively specific immunosuppression to exploit the difference in receptor expression between resting T cells that do not display IL-2Rα and T cells that express this receptor when stimulated by the specific interaction with foreign histocompatibility antigens on an allograft. Anti-IL-2R-directed therapy has been incorporated into combination immunosuppression protocols involving human recipients of cadaveric renal allografts. In each of these studies, there was a reduction in the number of early rejection episodes without evident toxicity. These prior attempts to use the murine anti-Tac monoclonal antibodies in allograft transplantation protocols were limited by the relatively short survival of the murine monoclonal antibody, weak recruitment of effector functions, and neutralization by human antibodies directed to the mouse monoclonal antibodies. To circumvent these difficulties, humanized anti-Tac-H was constructed by combining the CDRs of the murine anti-Tac antibody with human γ1 heavy and κ light-chain framework and constant regions (12). In the present study, we demonstrate that these humanized antibodies have a longer *in vivo* survival in primates compared to the murine antibody, are more effective in recruiting effector functions, have reduced immunogenicity, and display greater effectiveness in preventing allograft rejection in the primate heterotopic cardiac allograft model used.

The pharmacokinetics of radiolabeled anti-Tac-H differ substantially from those of radiolabeled anti-Tac-M when administered to normal cynomolgus monkeys. In animals receiving unlabeled anti-Tac to block the antigenic target, the $t_{1/2}$ of the terminal exponential of humanized anti-Tac was 103 hr compared to 38 hr for anti-Tac-M. Furthermore, the percentage of the intravascular pool of radiolabeled antibody catabolized per day was 25% with radiolabeled anti-Tac-H, markedly lower than the 58% observed with anti-Tac-M. The observations of prolonged survival of a humanized monoclonal antibody in the present study are in accord with the observations of LoBuglio and coworkers (22), who noted longer survivals of murine/human chimeric monoclonal antibodies in humans when compared to the parent murine monoclonal antibody. In studies defining the metabolism of immunoglobulin subunits, it was shown that the rate of catabolism of an immunoglobulin is controlled by the Fc region of the immunoglobulin, specifically the $C_H2$ domain (C, constant region; H, heavy chain) (21, 23). Thus, the longer survival of anti-Tac-H when compared to anti-Tac-M probably reflects the replacement of the murine IgG2a $C_H2$ domain with the IgG1 human $C_H2$ domain. The survival $t_{1/2}$ of the IgG1 humanized anti-Tac molecule in cynomolgus monkeys noted in the present study (103 hr) is shorter than the mean $t_{1/2}$ of >20 days we reported previously for human IgG1 immunoglobulins administered to humans (24). This disparity may reflect the differences in the recipient species used in the pharmacokinetic studies since polyclonal human IgG was shown to have a $t_{1/2}$ of survival of >20 days in humans, 12 days in baboons, and only 6.6 days in rhesus monkeys (21). In light of these observations in subhuman

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primates and humans, anti-Tac-H may have a much longer survival in humans than the 103 hr observed for this monoclonal antibody in cynomolgus monkeys.

A major goal in the generation of humanized antibodies is the production of agents that lack the epitopes recognized by T-helper cells that may be required for the effective production of anti-monoclonal antibody responses. The chimeric antibodies (variable region retained from the mouse and constant region derived from human) to different antigens studied by Hale and LoBuglio and their coworkers (22, 25) elicited modest antiglobulin responses. In the present study, humanized anti-Tac-H was less immunogenic than the parent murine monoclonal anti-Tac-M. With anti-Tac-M, all animals produced antibodies to the monoclonal antibody with antibodies evident prior to allograft rejection in all animals with an allograft survival of >9 days. Antibodies directed toward the murine monoclonal antibody were detectable in the circulation by the 15th day in all animals studied. In contrast, only three of the five animals receiving anti-Tac-H produced anti-monoclonal antibody responses during the study period. Furthermore, in the remaining three animals, antibody was first detected on days 33–42 after initiation of therapy, in all cases after allograft rejection had occurred. In more extensive studies, this lowered immunogenicity of the humanized anti-Tac was demonstrated in groups of cynomolgus monkeys injected with 0.05, 0.5, and 5 mg/kg doses of anti-Tac-H or anti-Tac-M daily for 14 days (J.H., unpublished observations).

The development of antibodies to the monoclonal antibody drastically altered the pharmacokinetics of the radiolabeled monoclonal antibody. For example, the $t_{1/2}$ of radiolabeled anti-Tac-M was reduced from the normal value of 38 hr to 9 hr and to <10 min in the two animals studied that manifested high-titer antibodies to this monoclonal antibody 17 months after initial administration of anti-Tac-M (T.A.W., unpublished observations). In the present therapeutic trial, the development of antibodies to the anti-Tac-M also led to accelerated catabolism of the antibody and thus reduced the amount of anti-Tac-M available for blocking IL-2 binding to Tac-expressing cells. Specifically, after the development of antibodies to the murine monoclonal antibody, anti-Tac-M was no longer demonstrable in the plasma 2 days after its infusion—that is, in the plasma sample obtained immediately before the antibody infusion. Thus, the development of antibodies to anti-Tac-M may be one of the factors leading to the failure of this monoclonal antibody preparation to prevent allograft rejection beyond day 14 of the study.

A major goal in the production of humanized antibodies is to increase effector functions by recruiting immune effector cells. Mouse monoclonal antibodies are frequently unable to promote antibody-dependent cellular cytotoxicity in assays with human effectors and nucleated target cells. In some, but not all, cases, murine/human chimeric antibodies mediated antibody-dependent cellular cytotoxicity with human effector cells more efficiently than murine antibodies (22). In previous studies, we demonstrated that anti-Tac-H manifested antibody-dependent cellular cytotoxicity with human mononuclear cells, a function absent in the parental murine anti-Tac (13). In the present study, we extend these observations by indicating that anti-Tac-H can mediate antibody-dependent cellular cytotoxicity with cynomolgus monkey mononuclear cells. Thus, anti-Tac-H manifests new capabilities to mediate cytotoxicity with primate mononuclear cells absent with the murine monoclonal antibody.

In the present study, cardiac allograft survival was modestly but significantly increased in the group of animals treated with anti-Tac-M, with an increase of mean survival from 9.2 to 14 days. As noted above, the development of antibodies to anti-Tac-M in monkeys may have contributed to the modest efficacy of anti-Tac-M in preventing allograft rejection. Allograft survival in anti-Tac-H-treated animals was further prolonged to 20.0 days, a survival significantly ($P < 0.001$) longer than that observed with anti-Tac-M. In contrast to the situation with anti-Tac-M, the rejection of the cardiac allografts by day 20 in animals receiving anti-Tac-H does not appear to be due to the development of antibodies to the infused monoclonal antibody since such antibodies were not demonstrable prior to graft rejection. Therefore, it would appear that, although anti-Tac-H can significantly prolong graft survival in primates without toxic side effects, it is not sufficient as a single agent to prevent allograft rejection. Nevertheless, it may complement the efficacy of other immunosuppressive agents in allograft protocols. Thus, our developing understanding of structure and function of the IL-2R on the surface of activated T lymphocytes taken together with our capacity to produce humanized forms of IL-2R-directed monoclonal antibodies provides an additional perspective for the prevention of allograft rejection.

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