Therapy with monoclonal antibody to interleukin 2 receptor spares suppressor T cells and prevents or reverses acute allograft rejection in rats

(immunology/ART 18 monoclonal antibody/lymphokine)

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ABSTRACT The mouse hybridoma ART 18 monoclonal antibody (mAb), which binds to the rat interleukin 2 (IL-2) receptor, was studied for its effect on heterotopic cardiac allograft survival in two histoincompatible inbred rat strain combinations. Treatment with ART 18 mAb for 10 days after transplantation prolonged allograft survival in a dose-dependent fashion up to about 3 weeks (acute rejection normally occurs within 8 days). ART 18 mAb therapy started at 5 days after transplantation (the time of major rejection activity) abrogated acute rejection and extended the survival to about 18 days. The dense cellular infiltrate noted histologically in acute rejection had virtually disappeared after ART 18 mAb treatment. Thus, IL-2 receptor-targeted therapy can be successfully used to prevent and/or treat acute rejection. When spleen cells from antibody-treated recipients bearing well-functioning allografts were adoptively transferred to normal untreated rats that received cardiac allografts 24 hr later, the survival of donor-specific, but not third-party, test cardiac allografts was prolonged significantly; this supports the idea that ART 18 mAb induced "sparing" of suppressor T lymphocytes. Combining infusion of ART 18 mAb with exogenous IL-2-rich conditioned medium produced the same effect as if the mAb alone had been administered, suggesting that an excess of IL-2 does not prevent binding of ART 18 mAb to IL-2 receptor-bearing cells in vivo. These results support the important role of the IL-2 receptor-bearing cells in the mechanism of allograft rejection; they may represent an important target for immunosuppression in clinical organ transplantation.

The IL-2 receptor, the target for IL-2, is expressed transiently upon activated T cells (5–7). Resting T cells and memory T cells do not bear this receptor (5, 6, 8); however, de novo expression of IL-2 receptor is in the common pathway of T-cell activation (8). Interaction of IL-2 with receptor-bearing cells is a prerequisite for clonal expansion and continued viability of activated T lymphocytes. Because all proliferating T cells express IL-2 receptors (5–8), IL-2 receptor-targeted therapy delivered in the peritransplantation period should create a selective immune defect in the graft recipient. However, there is little information available on the use of anti-IL-2 receptor monoclonal antibodies in a setting of transplantation, with the exception of a study using a mouse allograft model (9).

The mouse ART 18 monoclonal antibody (ART 18 mAb), which reacts primarily with rat lymphoblasts, defines the rat IL-2 receptor (10–12). This antibody inhibits adsorption of IL-2 by IL-2 receptor-positive cells, thereby blocking IL-2-dependent T-lymphocyte proliferation. We have utilized ART 18 mAb in an attempt to combat rejection of heterotopic cardiac allografts in rats. ART 18 mAb therapy was found to prevent and/or treat acute rejection successfully in this model, and passive-transfer experiments revealed that the antibody regimen spares anti-donor suppressor T cells (Tα).

MATERIALS AND METHODS

Animals. Inbred male rats (200–250 g) were used throughout (Harlan Sprague-Dawley, Indianapolis, IN). Unmodified Lewis rats (RT1b) acted as organ recipients; (Lewis × BN)F1 hybrids (RT1a) served as heart donors. Wistar Furth rats (WF, RT1b) were used as heart donors for specificity studies.

Heart Grafting. Heterotopic cardiac grafts were anastomosed to the abdominal great vessels according to the method of Ono and Lindsey (13). The size and ventricular activity were assessed daily by palpation through the recipient flank. Rejection was taken as the time of complete cessation of myocardial contractions.

Preparation of Lymphocyte Suspensions. Single-cell suspensions were prepared by mincing and expressing spleens through a 60-gauge stainless-steel mesh into RPMI 1640 medium (M. A. Bioproducts, Walkersville, MD) supplemented with 5.0 mM Hepes buffer and 10% heat-inactivated fetal bovine serum (14). Erythrocytes were lysed by a brief treatment with 0.15 M NH₄Cl in Tris Cl buffer at pH 7.21. The cells then were filtered and were washed thrice.

ART 18 mAb. Cloning, production, and purification of this mouse IgG1 anti-rat IL-2 receptor mAb, derived from the

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hybridoma clone ART 18, was performed according to the technique of Kohler and Milstein (15), as modified by Lemke et al. (16) and described previously in detail (10, 11). ART 18 mAb (protein concentration, 10 mg/ml; antibody, 5 mg/ml) was diluted in medium and administered to experimental animals intravenously at a dosage of 25–300 μg of antibody/kg of body weight per day for 5 or 10 consecutive days. Alzet osmotic pumps (mode 2 ML1, Alza, Palo Alto, CA) were inserted into the external jugular vein of some recipients to give a constant infusion of ART 18 mAb (10.5 μl/hr for 10 days).

IL-2-Rich Conditioned Medium (IL-2-CM). Lectin-free, partially purified, IL-2-rich supernatants from Con A-stimulated rat lymphocyte cultures were obtained commercially (Collaborative Research, Waltham, MA). The IL-2-CM used in the present studies supports the exponential growth of mouse cytotoxic T cells (Tc), so that 5 half-maximal units (u) promote growth from 2 × 10^4 to 2 × 10^5 cells per ml over a 4-day period in culture (17). IL-2-CM (100 μl/day) dissolved in medium was administered intravenously to the experimental animals for a period of 7 days.

Histologic Sections. The extirpated cardiac allografts from untreated or ART 18 mAb-treated recipients were fixed in buffered 10% (vol/vol) formalin. Paraffin sections were stained with hematoxylin/eosin and assessed by light microscopy.

Statistics. Statistical significance between experimental groups was ascertained using Student’s t-test.

RESULTS

ART 18 mAb Therapy Prolongs Cardiac Allograft Survival. (Lewis × BN)F1 hearts transplanted to untreated Lewis rats are rejected acutely in 8 ± 1 days (mean survival time ± standard deviation). In an attempt to prevent acute rejection, allograft recipients (4–7 in each group) received anti-IL-2 receptor mAb therapy from two different batches of antibody, according to arbitrarily chosen doses and durations of treatment. ART 18 mAb administered intravenously in doses of 25, 100, and 300 μg/kg each day for 10 consecutive days beginning on the day of grafting increased the mean allograft survival in a dose-dependent fashion to 13 ± 1 days, 14 ± 3 days, and 21 ± 1 days, respectively (Fig. 1A, P < 0.001). Limiting the period of treatment to the first 5 days after transplantation was less effective and resulted in a significant graft prolongation only when ART 18 mAb was given at a dose of 300 μg/kg per day (14 ± 2 days, P < 0.005).

The efficacy of anti-IL-2 receptor mAb therapy in reversing well-established allograft rejection was then tested. Treatment was initiated 5 days after transplantation, at which time the grafts were grossly enlarged and heavily infiltrated with lymphocytes (18). Interestingly, ART 18 mAb therapy started on day 5 after transplantation and continued for 5 days at a dose of 300 μg/kg per day improved allograft survival to 18 ± 4 days (Fig. 1B, P < 0.001), comparable to the effect produced by 10 consecutive injections. The dense cellular infiltrate, noted histologically in acute rejection at day 5, had virtually disappeared after ART 18 mAb treatment (Fig. 2A–C). Intermittent ART 18 mAb administration (days 5–9 and 15–19) extended graft survival even further, to 26–28 days; whereas lower doses of mAb were ineffectual in reversing ongoing rejection.

To confirm that these results were related to the specificity of ART 18 mAb for the IL-2 receptor, an additional control group of animals was treated with anti-asialo-GM1 antibody (ASGM-1, Wako Chemicals, Dallas, TX), recognizing selectively asialo-GM1 glycolipid antigenic structure on the surface of rat natural killer (NK) cells (19). A single, or repeated, intravenous administration of ASGM-1 following transplantation virtually eliminated NK activity in the spleen lymphocytes and peripheral blood mononuclear cells as tested in vitro with NK-sensitive 51Cr-labeled YAC-1 target cells. However, abrogation of NK activity in vivo did not significantly affect cardiac allograft survival in ASGM-1-treated rats, as compared to untreated controls. Mean survival time for the ASGM-1-treated rats was 9.4 ± 1.3 days, (n = 14), significantly shorter (P < 0.005) than in the recipients treated with ART 18 mAb.

To demonstrate that the results of anti-IL-2 receptor mAb treatment were not unique to one strain combination, we treated WF rat recipients of Lewis cardiac grafts with ART 18 mAb (300 μg/kg daily) for 10 days beginning the day of transplantation. Allograft survival was prolonged to 16 ± 1 days (n = 5, P < 0.001, as compared to untreated controls). Thus, ART 18 mAb therapy can be used to prevent or treat acute rejection in the rat cardiac allograft model. All the above experiments subsequently were repeated 3–5 times in comparable groups of recipients; the results were uniformly the same.

In the next series of experiments, ART 18 mAb was administered (300 μg/kg per day for 10 days) in a constant intravenous infusion of 10.5 μl/hr using an Alzet osmotic pump. Such treatment was significantly less effective than mAb “pulses” in preventing rejection (graft survival 12–13 days, n = 3, P < 0.005).

The potentially offsetting effects of exogenously supplied ART 18 mAb and IL-2-CM were studied in vivo within the microenvironment of unmodified graft recipients. As shown in the present studies, therapy with ART 18 mAb, directed at the rat IL-2 receptor, can increase cardiac allograft survival to about 3 weeks. In contrast, previous studies showed that a course of IL-2-CM accelerates immune responsiveness (14, 20).
17). Thus, the optimal doses (300 μg of ART 18 mAb/kg and 100 μg IL-2-CM) were mixed and delivered in daily intravenous injections for a period of 10 days. This combined treatment produced an effect the same as if ART 18 mAb but not IL-2-CM had been administered (graft survival 20 ± 2 days, n = 4), suggesting that IL-2 does not prevent binding of ART 18 mAb to IL-2 receptor-bearing cells in vivo when the mAb and IL-2 are administered simultaneously. Moreover, these results suggest that ART 18 mAb treatment prolongs engraftment by destroying IL-2 receptor-positive cells rather than by pharmacological blocking of the receptor.

**ART 18 mAb Therapy Spares Tc**. Spleen cells were harvested at day 10 from heart-grafted hosts, after the dose regimen of ART 18 mAb had been completed, and were transferred intravenously (40–50 × 10^6 cells) into normal recipients that received test cardiac allografts 24 hr later. Such adoptive transfer prolonged donor-specific (Lewis × BN)F₁, but not third-party (WF) test-graft survival (15 ± 1 days and 8 ± 1 days, respectively; n = 5, P < 0.001).

In contrast, adoptive transfer of unseparated spleen cells from untreated recipients undergoing acute rejection accelerated donor-specific test-graft rejection in a second-order manner (mean survival time 6.5 ± 0.6 days, n = 5). Thus, potent, antigen-specific suppressor activity but not alloaggressive activity can be demonstrated in animals maintaining well-functioning cardiac allografts following ART 18 mAb therapy.

**DISCUSSION**

Much attention has recently been focused on the characterization of cell-surface activation markers in an effort to delineate better both the functional significance of the marker proteins themselves and the spectrum of immunological activities exerted by the cells expressing these distinctive phenotypic markers. Cells bearing the phenotype of activated T lymphocytes are of particular interest in transplantation immunobiology because they represent a functionally diverse ensemble engaged in the allograft response (3, 4). Unlike their resting counterparts, activated T cells bear insulin receptors (20), transferrin receptors (21), and IL-2 receptors (5–8).

The IL-2 receptor is the only well-studied early, lymphocyte-specific activation antigen that appears on all activated, proliferating T lymphocytes (5–8) as well as on certain activated B cells (22). The isolation and preliminary characterization of monoclonal antibodies to human (23), murine (9, 24), and rat (10–12) IL-2 receptors have been reported. One of these antibodies, ART 18 IgG1 mAb was raised in mice primed with phorbol 12-myristate 13-acetate-treated rat T lymphoblasts (10–12). This antibody recognizes the rat 50-kDa glycoprotein molecule of the IL-2 receptor and binds to rat T lymphoblasts (7.5 × 10^8 binding sites per cell) but not to mature, resting T cells; ART 18 mAb does not affect the function of mouse T cells. ART 18 mAb inhibits the capacity of rat T lymphoblasts to bind IL-2, thereby blocking IL-2-dependent proliferation in a species-specific and dose-dependent manner. The time course of the acquisition by mitogen-stimulated spleen cells of the capacity to bind IL-2 is paralleled by that of their capacity to bind 125I-labeled ART 18 mAb.

The functions of in vivo-activated IL-2 receptor-positive cells during periods of immunological responsiveness have not been well defined. Administration of rat anti-mouse M7/20, a mAb that binds to the murine IL-2 receptor, has been shown to prolong, often indefinitely, mouse cardiac allograft survival (9). In the present studies, we have attempted to combat acute rejection of heterotopic cardiac allografts in otherwise unmodified rat recipients, focusing therapy selectively at the IL-2 receptor-bearing target cells. We showed that ART 18 mAb treatment can be used in a dose-dependent fashion not only to prevent acute rejection but also to reverse ongoing rejection mounted against strongly histoincompatible grafts in otherwise untreated hosts. We detected potent donor-specific suppressor activity following mAb treatment of grafted hosts in vivo, an observation that supports the idea that the anti-IL-2 receptor regimen may eliminate alloaggressive T cells and spare donor-specific Tc. The spared suppressor clones may subsequently proliferate.
and be responsible for prolonged graft survival following cessation of ART 18 mAb treatment.

That T	extsubscript{h} play a regulatory role in the induction and maintenance of transplantation unresponsiveness has been supported by results in a variety of experimental models. Suppressor effects have been documented in hosts immunologically enhanced (25), treated with cyclosporine (26), and undergoing total lymphoid irradiation (27) or blood transfusion (28).

ART 18 mAb may spare T	extsubscript{h} because they may lack either IL-2 receptor sites or an IL-2 receptor epitope that is recognized by ART 18 mAb or because IL-2 receptors may be expressed on T	extsubscript{h} and T	extsubscript{c} prior to expression upon T	extsubscript{c}. As a consequence of dysynchronous IL-2 receptor expression, early ART 18 mAb therapy may preferentially destroy T	extsubscript{c} and T	extsubscript{c} but not T	extsubscript{c} clones. The IL-2 receptor densities on various T-cell populations may differ (29). Additionally, recent adoptive-transfer studies showed that hosts treated with ART 18 mAb and maintaining well-functioning cardiac allografts exhibited low-to-undetectable levels of endogenous IL-2 production at the peak of suppressor activity (J.W.K.-W., unpublished data). The present studies suggest that a peak of ART 18 mAb activity, rather than a steady but low level of antibody in the serum delivered through an osmotic pump, is necessary to obtain an optimal biological effect. In the rat, permanent tolerance is not achieved and the graft is eventually rejected.

Incubation of glutaraldehyde-fixed T lymphoblasts with excess IL-2-CM did not affect their ability to bind 125I-labeled ART 18 mAb (10). This demonstrates that IL-2 molecules do not compete with the mAb for occupation of the same binding site on the IL-2 receptor molecule. The present studies addressed the issue of possible competition between exogenously supplied ART 18 mAb and IL-2-CM in vivo. The biological effect produced by concomitant administration of ART 18 mAb and IL-2-CM caused effects identical to those produced by ART 18 mAb given alone.

In summary, the present studies suggest that IL-2 receptor-bearing cells are required for acute allograft rejection and, therefore, that these cells may represent an important target for immunosuppression in clinical organ transplantation. As anti-IL-2 receptor mAb should react with a highly select population of activated lymphocytes, this antibody may prove to cause a specific form of immunosuppression. Other schemes to produce antigen-specific T-cell clonal deletion have proposed use of anti-idiotypic antibodies (30). While a myriad of such anti-idiotypic antibodies would be required in clinical transplantation owing to the intense polymorphism of human lymphocyte antigen (HLA) molecules, a single anti-IL-2 receptor antibody may cause selective immunosuppression in all graft recipients. Moreover, ART 18 mAb treatment selectively spared suppressor activity. A paramount role of T	extsubscript{c} in interrupting the cellular rejection cascade and prolonging survival of histoincompatible vascularized organ allografts is thereby supported.

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