Brief Definitive Report

ADMINISTRATION OF AN ANTI-INTERLEUKIN 2 RECEPTOR MONOCLONAL ANTIBODY PROLONGS CARDIAC ALLOGRAFT SURVIVAL IN MICE

By ROBERT L. KIRKMAN, LESLIE V. BARRETT, GLEN N. GAULTON, VICKI E. KELLEY, ARMELLE YTHIER, and TERRY B. STROM

From the Departments of Surgery, Medicine, and Pathology, Harvard Medical School, Brigham and Women's Hospital, and the Charles A. Dana Institute, The Harvard-Thorndike Laboratory of Beth Israel Hospital, Boston, Massachusetts 02115

The immune response to a vascularized allograft involves a complex series of events, including T cell activation. A critical marker of T cell activation is the acquisition of stereospecific membrane receptors for the lymphokine, interleukin 2 (IL-2) (1–4). While resting T cells lack IL-2 receptors, in vitro studies (5, 6) indicate that essentially all lectin- or alloantigen-stimulated proliferating T cells express the IL-2 receptor. Moreover, the interaction of IL-2 with receptor-bearing cells is required for the clonal expansion of activated T cells (1–13). Whereas engagement of the T cell receptor for antigen is the first step in T cell activation, the interaction of IL-2 with the newly expressed IL-2 receptor is a requisite step in the common pathway of activation of all T cells. The extension of these in vitro findings to the process of allograft rejection in vivo has not been extensively studied. IL-2 is necessary to reconstitute the acute rejection response in vivo in T cell–deprived rats (14). However, direct evidence in vivo that the IL-2 receptor is involved in allograft rejection has been lacking.

The rat monoclonal antibody (mAb) M7/20 binds to the murine IL-2 receptor, as demonstrated by its capacity to bind activated but not resting T cells in flow cytometry experiments, to prevent IL-2-mediated DNA synthesis in an IL-2-dependent cytotoxic T lymphocyte cell line, to precipitate an *N*-glycosylated 58 kilodalton glycoprotein, and to competitively inhibit binding of radiolabelled IL-2 (15). As such, it offers an opportunity to direct immunosuppressive therapy selectively against IL-2 receptor–bearing cells during allograft rejection. In this report, we examine the effect of administration of M7/20 on survival of cardiac allografts in mice.

Materials and Methods

Animals. Inbred male mice, weighing 20–25 gm, of strains C57BL/10, B10.BR, and B10.AKM (The Jackson Laboratory, Bar Harbor, ME) were used throughout. These strains are completely mismatched for the H-2 locus, but share the same genetic background.

Operative Technique. Vascularized, heterotopic heart allografts were performed as

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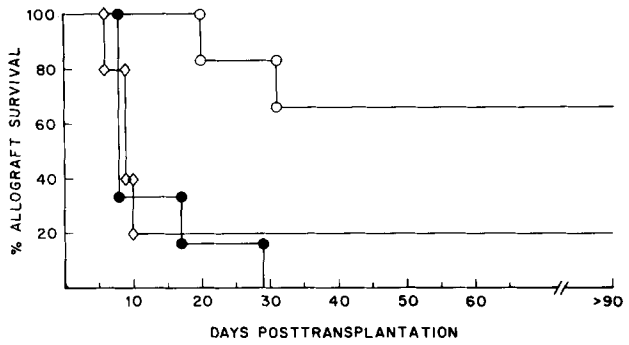

FIGURE 1. Survival of C57BL/10 heart allografts in B10.AKM recipients. The survival of grafts treated with M7/20 (open circles) was significantly greater than untreated controls (closed circles) ($p < 0.01$) or grafts treated with RA3-2C2 (diamonds) ($p < 0.05$).

originally described by Corry et al. (16). The aorta was anastomosed to the abdominal aorta, and the pulmonary artery to the adjacent vena cava using standard microvascular techniques with 10-0 nylon suture (Ethicon, Inc., Somerville, NJ) under 20× magnification. With completion of the anastomoses, and warming of the heart with Ringer's lactate at 37°C, normal sinus rhythm was resumed. Once proficiency with this technique was achieved, survival at 24 h routinely exceeded 90%.

Function of the transplanted heart was assessed by daily palpation of ventricular contractions through the abdominal wall. Rejection was defined as the cessation of all myocardial contractions, which was confirmed by laparotomy under ether anesthesia. Rejected hearts were removed, fixed in formalin, sectioned, and stained with hematoxylin and eosin.

Preparation and Administration of mAb. The production and characterization of mAb M7/20 has been previously described (15). M7/20, a rat u, k, Ig was purified from the culture supernatants of cells grown in serum-free medium (Hanna Labs, Berkeley, CA). Supernatants were precipitated with 40–50% saturated ammonium sulfate, dialyzed, passed over DEAE Affi-Gel Blue (Bio-Rad, Richmond, VA) in 20 mM Tris (pH 7.2) and 100 mM NaCl, and the eluate was fractionated on Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, NJ), run in 20 mM Tris (pH 7.2), 250 mM NaCl, and 0.5% *n*-butanol. Antibody purity was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The hybridoma producing mAb RA3-2C2, which binds to pre-B cells and to some mature B cells, was obtained from the American Type Culture Collection (Rockville, MD), and the mAb was purified by the same procedure (17).

Both mAb were diluted to a final concentration of 25 μg/ml in phosphate-buffered saline. Treated allograft recipients received 0.2 ml (5 μg) by intraperitoneal injection, beginning on the day of transplant and continuing for a total of 10 daily doses.

Statistical Analysis. Survival times were compared using Mann-Whitney rank-sum analysis.

Results

Untreated B10.AKM recipients of C57BL/10 heart allografts rejected their grafts on days 8 (four subjects), 16, and 29 (Fig. 1). In contrast, treatment with M7/20 at a dose of 5 μg/mouse/d for 10 d in this strain combination caused indefinite survival (>90 d) of four of six grafts, with two rejecting at 20 and 31 d (Fig. 1), a highly significant prolongation ($p < 0.01$).

To confirm that these results were related to the specificity of M7/20 for the IL-2 receptor, an additional control group of recipients was treated with RA3-

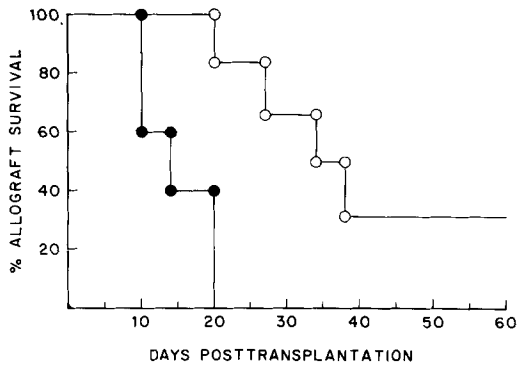

FIGURE 2. Survival of B10.BR heart allografts in C57BL/10 recipients. The survival of grafts treated with M7/20 (open circles) was significantly greater than untreated controls (closed circles) ($p < 0.01$).

2C2, a rat mAb of the same class as M7/20, which binds pre-B cells, but not T cells. Four B10.AKM recipients of C57BL/10 grafts rejected their grafts on days 6, 9 (two mice), and 10, with a fifth graft surviving for >90 d (Fig. 1). These survival times were not different from the untreated controls, but were significantly shorter than those in animals treated with M7/20 ($p < 0.05$).

To demonstrate that the results were not unique to one strain combination, a second set of experiments was performed using C57BL/10 recipients of B10.BR grafts. Untreated control recipients rejected their grafts at 10 (two animals), 14, and 20 d (two animals). Treatment with M7/20 prolonged survival to 20, 27, 34, and 38 d, with two grafts still functioning at >60 d ($p < 0.01$) (Fig. 2).

Discussion

The precise mechanism by which a vascularized allograft is rejected remains a subject of intense investigation, but the participation of T cells in the process is unquestioned. Moreover, the process of T cell activation has been well studied in vitro (1–13). Alloantigenic activation of T cells in vitro leads to the induction of IL-2 receptors on T cell surface membranes, and the interaction of IL-2 with these receptors is required for T cell proliferation. Whereas the receptor for antigen is required for the first stage of T cell activation, in which preprogrammed, antigen-specific cells are stimulated, induction of the IL-2 receptor is part of a common pathway that all T cells must follow to produce immunity.

The results presented in this report provide important new in vivo evidence that IL-2 receptor–bearing cells are required for allograft rejection. Administration of M7/20, an mAb known to bind to the mouse IL-2 receptor, significantly prolonged vascularized heart allograft survival in two separate H-2-incompatible strain combinations in mice. Indeed, several grafts survived indefinitely, although the antibody was administered only for the first 10 d posttransplant. Such long-term engraftment following cessation of therapy makes it unlikely that M7/20 prolongs graft survival by pharmacologic blockade of the IL-2 receptor. Whether or not such prolonged graft survival represents deletion of the responding T cell clones is a subject of current investigation.

In addition to supporting the role of IL-2 receptor–dependent mechanisms in

graft rejection, these experiments suggest that the IL-2 receptor is an important new target for immunosuppression in clinical transplantation. Since all recently activated T cells express IL-2 receptor, all relevant clones can be targeted, regardless of their antigen specificity. Moreover, as resting and memory T cells do not express significant quantities of the receptor (18), specific immunosuppression may be possible.

Summary

Administration of the monoclonal antibody M7/20, which binds to the murine interleukin-2 (IL-2) receptor, significantly prolongs cardiac allograft survival in two H-2-incompatible strain combinations of inbred mice. The results support the important role of the IL-2 receptor in the mechanism of graft rejection, and suggest its suitability as a target for immunosuppressive therapy.

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