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## ***Brief Definitive Report***

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### **XENOGENEIC SKIN GRAFT REJECTION IS ESPECIALLY DEPENDENT ON CD4<sup>+</sup> T CELLS**

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The immune response to xenogeneic organ transplants (grafts from other species) is more potent than to allogeneic tissues (1). In many cases, this extra strength results from the existence of preformed "natural" antibodies that mediate hyperacute rejection. However, even in situations such as skin grafting, where antibody-mediated mechanisms are not thought to play a role, cell-mediated xenograft immunity is stronger than that to allografts (1, 2). To examine the cell-mediated response to xenogeneic compared with allogeneic antigens, we used anti-T cell antibodies *in vivo* in mice in an effort to prolong xenogeneic compared with allogeneic skin graft survival. The results of these studies show that *in vivo* treatment of mice with an anti-CD4 antibody prolonged survival of skin xenografts from rabbits or monkeys without prolonging survival of whole MHC-mismatched allografts from other mice.

These results represent the first achievement of better xenograft than allograft survival for such disparate species combinations. On the one hand, that achievement creates something of a paradox: why is cell-mediated xenogeneic immunity so powerful when it apparently lacks a CD4-independent pathway available in allogeneic responses? On the other hand, the prolongation of xenograft survival by anti-CD4 antibody is similar to the prolongation of minor antigen-disparate allograft survival by the same reagent. This similarity may provide a clue to understanding the mechanisms of xenograft rejection and may also suggest a possible approach for clinical xenogeneic transplantation.

#### **Materials and Methods**

**Animals.** C57BL/6J and BALB/c mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Cynomolgus monkeys and New Zealand White rabbits were obtained from Charles River Breeding Laboratories (Wilmington, MA). Skin from the monkeys and rabbits was procured after animals had been killed in the course of other experiments.

**In Vivo Procedures.** Thymectomies were performed on 6–8-wk-old mice using the suction pipet technique. Chloral hydrate anesthesia was used, supplemented by ether. Mice were rested at least 1 wk before further manipulation. Skin grafts were performed with the same anesthesia by preparing a bed on one or both lateral thoracic walls. Grafts were held in place by plaster bandages for 1 wk. Split thickness grafts (0.3–0.45 mm) were harvested from cynomolgus monkeys using a Padgett dermatome, stored overnight at 4°C in 20% FCS/RPMI with 10%

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gentamicin, and applied in 1.0–1.5-cm<sup>2</sup> rectangles to the lateral thorax. Rabbit skin was bluntly dissected from the cartilage of the concave aspect of the ear, stored overnight at 4°C in 20% FCS/RPMI with 10% gentamicin, and applied in 1.0–1.5-cm<sup>2</sup> rectangles. BALB/c trunk skin was harvested, cleaned of subcutaneous fat and vessels, and stored overnight as above. Complete epithelial destruction, "tanning" (loss of pliability with evolution of leathery appearance), and shrinkage to <10% of original surface area were used as independent endpoints of rejection. Allografts and some xenografts showed confluent erythema and edema followed promptly by epithelial loss (typical allogeneic rejection). Some rejecting xenografts, particularly in the GK1.5-treated group, exhibited a petechial or hemorrhagic pattern followed by tanning. Other xenografts, particularly those surviving for >20 d, underwent gradual shrinkage, with recurrent episodes of focal erythema preceding subtotal epithelial loss.

*mAbs.* Mice were treated intraperitoneally on the days indicated with 0.1 ml of ascites of GK1.5 anti-CD4 antibody (3), 2-43 anti-CD8 antibody (4), or both together as we have described before (5).

*Cyclosporine.* Oral CsA solution (Sandimmune; Sandoz, Basel, Switzerland) was used for in vivo treatment. CsA was diluted in olive oil to 5 mg/ml and then administered to mice by subcutaneous injection starting on the day before grafting.

### Results

C57BL/6J (B6) mice were thymectomized and treated with anti-CD4, anti-CD8, or both antibodies together. Previous studies in our laboratory and by others have shown that treatment with these antibodies can deplete their respective T cell subpopulations and achieve in vivo immunosuppression (5–9). The performance of a thymectomy in the recipient prolongs this immunosuppression for many weeks (5). Mice treated in this manner received monkey skin grafts. The results shown in Fig. 1 reveal that xenogeneic skin grafts were rapidly rejected in untreated recipients. Anti-CD8 antibody also failed to prolong graft survival. On the other hand, most xenogeneic skin grafts were maintained for 30 d on mice treated with both anti-CD8 and anti-CD4 antibodies together. Anti-CD4 antibody alone also caused significant prolongation of graft survival. Over the course of many experiments some xenogeneic skin grafts have survived as long as 1 mo after anti-CD4 treatment.

These results were in contrast to those previously obtained and reported by us for whole MHC-disparate allogeneic skin grafts since anti-CD4 antibody alone had

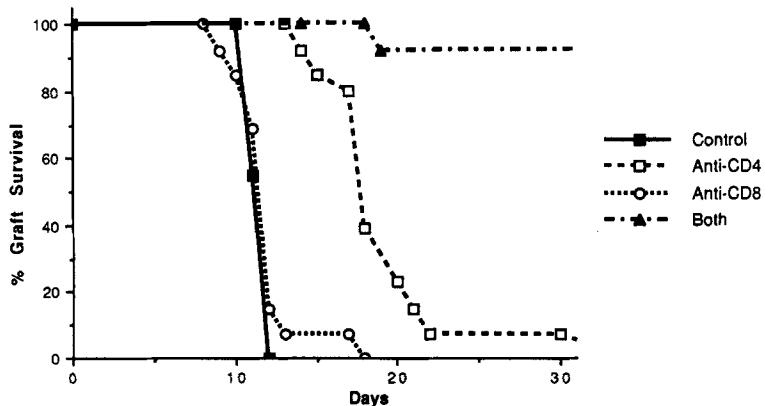


FIGURE 1. Survival of monkey skin grafts on B6 mice treated with anti-T cell mAbs. Data are combined from two experiments with identical protocols. Each group had between 9 and 13 mice that were treated with antibody on days -3, -1, +4, +8, and +18 with respect to grafting.

not been very successful in prolonging survival of these grafts (5). In our experiments, CD4<sup>+</sup> lymphocyte depletion has only prolonged survival of class II-disparate skin grafts and those differing only in their minor histocompatibility antigens (6-8, 10, 11). Others, however, have shown some prolongation of whole MHC-mismatched allografts by anti-CD4 antibody treatment (7-9). Therefore, to compare the survival of allografts and xenografts directly, thymectomized mice were treated with anti-CD4 antibody and then given grafts from both allogeneic and xenogeneic donors, one on each thoracic wall. Typical graft survival in such an experiment (using rabbit skin) is shown in Fig. 2. Most animals treated with the anti-CD4 antibody maintained intact xenografts after they had rejected the allogeneic skin. Fig. 3 shows photographs of typical graft appearances in untreated and anti-CD4-treated mice. The viability of the xenogeneic skin grafts was demonstrated by their uptake of fluorescein revealed under the Woods lamp and by pathologic examination. The results were further confirmed in multiple experiments using rabbit or monkey skin and using BALB/c recipients in addition to B6 mice.

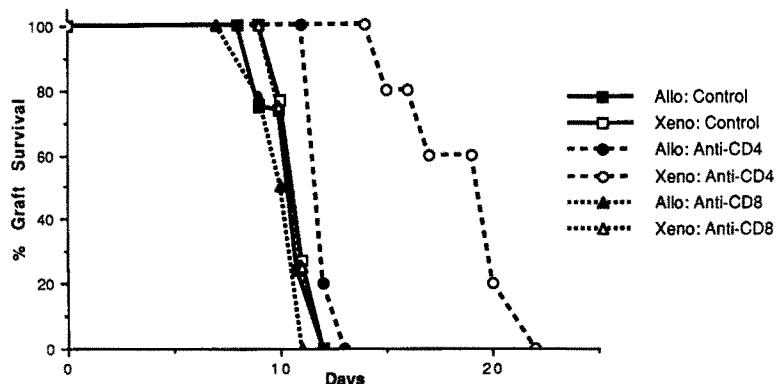


FIGURE 2. Survival of xenogeneic rabbit skin and allogeneic BALB/c skin on B6 mice. Groups had between four and five mice. Treated animals received 0.1 ml of ascites intraperitoneally on days -2 and -1 before grafting.

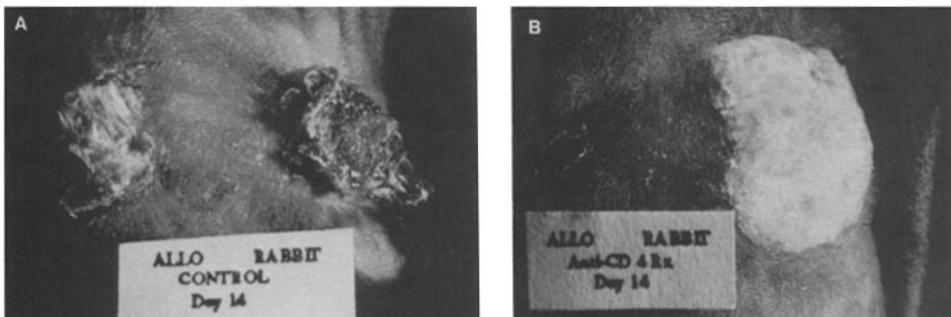


FIGURE 3. Typical appearance of skin grafts in the experiment shown in Fig. 2. (A) An untreated control mouse on day 14 with rejected BALB/c skin on the left and rejected rabbit skin on the right. (B) A mouse treated with anti-CD4 antibody with rejected BALB/c skin on the left on day 14 but intact rabbit skin on the right.

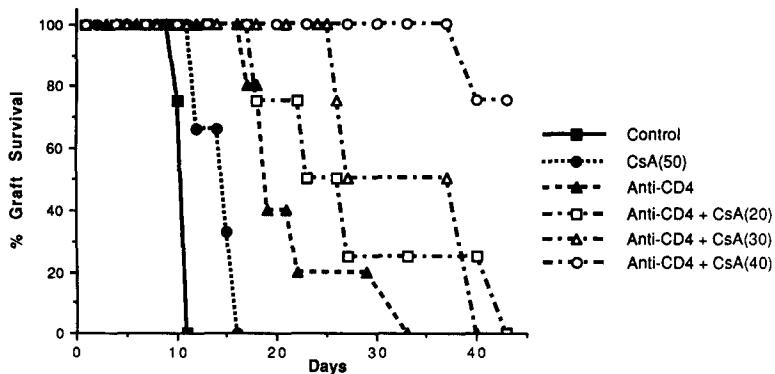


FIGURE 4. Survival of monkey skin on B6 mice treated with anti-CD4 antibody plus cyclosporine. Three to five mice were in each group. Mice were thymectomized, treated with antibody, and given monkey skin grafts as in Fig. 1. CsA oral solution was administered subcutaneously starting the day before grafting. The number after CsA in parentheses indicates the dose in mg/kg/d based on 25 g/mouse administered to each group.

Anti-CD4 treatment generally prolonged xenograft survival in our experiments by 2–3 wk. In an effort to achieve even longer graft survival, we tested other forms of immunosuppression alone and in combination with the anti-CD4 antibody. CsA alone had little effect on xenogeneic skin graft survival even when used at 50 mg/kg/d, a dose that we have found prolongs graft survival in many allogeneic combinations. Experiments combining CsA with anti-CD4 treatment, however, revealed a marked synergy between them. The results in Fig. 4 show that even 20 mg/kg/d of CsA was synergistic with anti-CD4 treatment and that 40 mg/kg/d plus antibody achieved >30-d graft survival in all mice tested. This synergy is not specific to xenografts since we have observed it for allografts as well. These results, however, suggest that CsA might augment the immunosuppression of anti-CD4 antibodies for xenogeneic transplantation.

### Discussion

Only once before have experiments revealed more prolonged survival of xenogeneic than allogeneic skin on individual recipients. Those studies involved a closely related rat-mouse combination under treatment with antilymphocyte serum (12). The present report, therefore, describes a striking outcome in such disparate species as the mouse and monkey.

The basic observation in these studies is that CD8<sup>+</sup> T cells alone are less able to mediate xenograft rejection than MHC-disparate allograft rejection. In the case of MHC-mismatched allografts, that CD4-independent pathway has been attributed to the function of IL-2-producing CD8<sup>+</sup> lymphocytes, which can provide their own "help" in generating cytotoxic effector cells (13). If so, then in the case of xenografts, such IL-2 producing CD8<sup>+</sup> cells do not appear to function. In vitro investigation of helper-independent CD8<sup>+</sup> cells has found that they do not function in response to class II MHC antigens or in response to self class I MHC antigens when modified as if presenting the peptides of nominal antigens (14). Thus, the question emerges whether the absence of CD4-independent xenograft rejection indicates that xeno-

MHC antigens, unlike allo-MHC antigens, must undergo antigen processing and presentation (perhaps entirely in association with class II antigens) in order to elicit an immune response. Minor histocompatibility antigens, like nominal antigens, also require antigen processing and presentation on MHC molecules (15). As for xenografts, anti-CD4 antibody treatment has also been found to prolong the survival of minor antigen-disparate, MHC-matched allografts (6-8). Thus, again there is cause to consider whether xeno-MHC antigens may require antigen processing.

The notion that xeno-MHC antigens might not be recognized in the same manner as allo-MHC antigens has been discussed before. Some have speculated that the T cell repertoire, biased toward recognition of self-like MHC antigens, might find xenogeneic MHC antigens too dissimilar from self in their critical epitopes (reviewed in reference 1). Alternatively, recognition of xenogeneic MHC molecules on APCs of another species might involve accessory molecules or a requirement for lymphokines that cannot function across species barriers (16). In either case, the immune response to the xenogeneic MHC antigens would require the processing and presentation of peptides of these antigens on MHC molecules on APCs of the recipient species.

The hypothesis that xenogeneic MHC antigens function as minor histocompatibility antigens would explain the absence of a CD4-independent pathway in xenograft rejection. In addition, the hypothesis is not incompatible with the observed strength of cell-mediated xenograft destruction. Minor histocompatibility antigens do not necessarily cause weak graft rejection especially when multiple minor disparities exist together (17). The many disparities of xenogeneic grafting would elicit an extreme expression of the cell-mediated mechanisms of minor antigen rejection.

While cell-mediated xenograft rejection is a powerful event, the observations reported here provide evidence that well-selected immunosuppression can overcome this response even while leaving other elements of the immune system intact. Although successful performance of some forms of clinical xenogeneic transplantation must await improvements in our ability to control preformed antibody, successful achievement of others (such as liver or pancreatic islet transplantation, which may not be as susceptible to antibody-mediated rejection) might be accomplished in the near future using anti-CD4 plus CsA treatment.

### Summary

B6 mice were treated *in vivo* with anti-CD4, anti-CD8, or both anti-T cell antibodies together in an effort to prolong xenogeneic compared with allogeneic skin graft survival. Mice treated with anti-CD4 antibody showed prolonged survival of xenogeneic monkey or rabbit skin even after they had rejected whole MHC-disparate allogeneic mouse skin. Furthermore, the addition of cyclosporine was synergistic with the anti-CD4 antibody in prolonging graft survival. These results suggest that the cell-mediated response to xenogeneic antigens is especially dependent on CD4<sup>+</sup> lymphocytes, a feature shared by the response to allogeneic minor histocompatibility antigens. In addition, the results suggest a possible approach to clinical immunosuppression for some forms of xenogeneic transplantation.

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