Evidence that the effector mechanism of skin allograft rejection is antigen-specific
(transplantation/allophenic mice/alloreactivity)

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Communicated by David W. Talmage, June 30, 1988

ABSTRACT In vivo rejection responses are initiated by specific T-cell recognition of foreign histocompatibility antigens expressed by tissue allografts, but it is not certain if the effector mechanism mediating the actual tissue injury is also antigen-specific. To directly assess the specificity of the effector phase of in vivo rejection responses, we constructed B6-A/J allophenic mice that are genetic mosaics whose individual cells express either H-2b or H-2a histocompatibility antigens but not both. Trunk skin from B6-A/J allophenic mice was grafted onto immunoincompetent H-2b nude mice and allowed to heal and regrow hair that was both black and white, reflecting the genetic mosaicism of the allophenic grafts. One month after engraftment, the H-2b nude animals were reconstituted with syngeneic H-2b T cells reactive against H-2a allografts. An obvious rejection response ensued involving antigen-nonspecific inflammatory destruction of the epidermis and complete hair loss. Despite the intensity of the nonspecific inflammatory response, the allophenic skin grafts survived. Importantly, the allophenic grafts regrew hair and the predominant color of that hair was black, providing visual proof that syngeneic B6 melanocytes and hair follicle cells had not been destroyed. Thus, these results demonstrate that although the intense inflammatory component of skin graft rejection responses is capable of damaging superficial epidermal cells nonspecifically, it does not cause rejection of skin allografts. Rather, rejection of skin allografts is mediated by antigen-specific effector T cells that assess individual cells within the dermis of the graft for expression of foreign histocompatibility antigens.

The immunologic response to skin allografts is initiated by T-cell recognition of foreign histocompatibility antigens (1). However, it is uncertain if, once triggered, the effector mechanisms that actually destroy the engrafted tissue do so by assessing individual cells for expression of foreign histocompatibility antigens or, alternatively, if they nonspecifically destroy cells within the graft regardless of their expression of foreign antigens (2–4). Previously, we reported that skin graft rejection required the presence in vivo of antigen-specific T-helper cells (TH cells) and antigen-specific T-killer cells (TK cells) (5), providing indirect support for the hypothesis that in vivo rejection responses are initiated by TH cells and are effected by antigen-specific TK cells. However, these studies did not preclude the possibility that in vivo rejection responses were actually effected by antigen-nonspecific soluble factors secreted by antigen-specific TH cells (6) or delayed-type hypersensitivity T cells (T DTH cells) (7, 8) that either recruit inflammatory effector cells or cause intense vasospasm leading to ischemic necrosis of the graft. Consistent with the possibility that skin allograft rejection might be effected by antigen-nonspecific soluble factors is the relative paucity of antigen-specific T cells found in skin allografts undergoing rejection (17).

To assess the specificity of the effector mechanisms resulting in skin allograft rejection, we adapted the elegant approach of Mintz and Silvers (9, 10) of assessing rejection of allophenic skin grafts to an adoptive transfer model of skin allograft rejection. Skin from B6-A/J allophenic mice, composed of cells expressing H-2a determinants or H-2b determinants but not both, was engrafted onto immunoincompetent nude mice that cannot reject skin grafts. Several weeks following engraftment, these grafts grew hair that was both black and white, confirming the genetic mosaicism of the cells within the graft. The mice were subsequently reconstituted with unFractionated H-2b T cells and the ensuing rejection response was observed. We found that the graft rejection response had two components, an antigen-nonspecific inflammatory response primarily causing destruction of epidermal cellular elements and an antigen-specific destruction of dermal cellular structures. Despite the intensity of the nonspecific inflammatory component of the response, the allophenic grafts survived and regrew black hair, indicating survival of syngeneic H-2b cellular structures.

MATERIALS AND METHODS

Mice. C57BL/10Scn nu/nu (B10 nude) mice were obtained from the Small Animal Section, National Institutes of Health (Bethesda, MD). C57BL/6 (B6), A/J, and B6AF1 mice were obtained from The Jackson Laboratory. B6-A/J allophenic mice were constructed by the fusion of B6 embryos with A/J embryos (11). Immature females of donor strains C57BL/6J and A/J were superovulated with pregnant mare serum (5 international units) and human chorionic gonadotropin (5 international units) and mated. Donors were sacrificed by cervical dislocation day 2 after mating and eight-cell-stage embryos were flushed from the oviduct. The zona pellucida was removed and embryo pairs were pipetted together to establish broad contact between blastomeres and incubated overnight at 37°C. Amalgamated embryos were then checked and those that had completely integrated and reached the blastocyst stage were implanted into 2½-day pseudopregnant recipients, who had been mated to vasectomized males. Five to 10 embryos were implanted into the uterine horns of each recipient.

Skin Engraftment and Adoptive Transfer of Nude Mice. B10 nude mice were engrafted with trunk skin grafts from donor B6, B6AF1, or B6-A/J mice according to an adaptation of the method of Billingham and Medawar (12). The mice were allowed to rest for 31 days to allow the grafts to heal and regrow hair and then injected intravenously with 4.5 × 10⁷ unseparated spleen cells from naive B6 normal mice.

Abbreviations: T DTH cell, delayed-type hypersensitivity T cell; TH cell, helper T cell; TK cell, killer T cell.

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Mice reconstituted with B6 spleen cells failed to reject control B6 trunk skin grafts over the time course of the assay (0/10 rejections) (Fig. 2). In contrast, mice reconstituted with B6 spleen cells rejected B6AF1 grafts completely (10/10), with contraction of the graft and scar formation (Fig. 2). Thus, expression of allogeneic A/J histocompatibility antigens by the B6AF1 cells comprising these grafts led to complete eradication of the F1 grafts, without regeneration of grafted tissue or regrowth of hair.

In marked contrast to their rejection of B6AF1 skin grafts, mice reconstituted with B6 spleen cells and engrafted with skin from B6<i>→</i>A/J allophenic mice containing both black and white hairs underwent unique graft rejection responses. In 17 mice, the allophenic grafts were enveloped by an intense inflammatory response with loss of all hair, followed by scab formation (Fig. 2). Yet, with healing, these allophenic grafts failed to shrink down and scar as did the F1 grafts but instead appeared to maintain much of their original volume (Fig. 2, day 21). Remarkably, by day 35, 14 of 17 of these grafts regrew hair, and that hair was overwhelmingly black, the color of the syngeneic B6 parent (Fig. 2). Thus, despite the massive nonspecific inflammatory response resulting from recognition of allogeneic A/J cellular elements in the B6<i>→</i>A/J skin allografts, B6 hair follicle cells and melanocytes survived and were able to regrow hair. It might be noted that the black hair color in the renascent grafts was due to surviving B6 melanocytes and was not due to melanocytes migrating from the nude host into the graft, as such melanocytic migration does not influence hair color (13).

Though the allophenic grafts regrew predominantly black hair, inspection of the renascent grafts revealed the presence of some white hairs. The white hairs in these renascent grafts presumably resulted from B6 hair follicle cells giving rise to a hair in the absence of an associated melanocyte, as opposed to the white hairs in the original grafts that were associated with A/J melanocytes. To document that the white hairs of these renascent grafts did not reflect the persistence of A/J cells, we examined the skin cells present in the renascent grafts by fluorescence microscopy and confirmed that none detectably expressed either I<sub>K</sub> or I<sub>A</sub> determinants of the allogeneic A/J parent (data not shown).

It should be noted that in addition to the 17 allophenic skin grafts that underwent rejection responses, there were 9 H-2<i>b</i> nude mice engrafted with B6<i>→</i>A/J allophenic skin grafts and reconstituted with H-2<i>b</i> T cells that failed to undergo any visible rejection response. As the degree of genetic mosaicism is random from one allophenic animal to another and from one patch of skin to another, we understand this alternative response pattern to represent a situation in which the cellular elements of the graft were, in fact, not mosaic, but overwhelmingly H-2<i>b</i>. Reciprocally, hair follicle cells in the 3 of 17 grafts that triggered a rejection response but failed to regrow hair might have all been of H-2<i>b</i> origin.

**DISCUSSION**

In their original studies assessing rejection of allophenic skin grafts, Mintz and Silvers (9, 10) emphasized the specificity of
FIG. 2. (Legend appears at the bottom of the preceding page.)
the in vivo rejection response, as most allophenic skin grafts persisted indefinitely. Together with experiments demonstrating the specificity of in vivo antitumor responses (14), their studies have importantly influenced our concepts of transplantation immunity. However, Mintz and Silvers (10) also observed that in certain circumstances allophenic skin grafts appeared to be rejected in an antigen-nonspecific fashion, raising the possibility that inflammatory cells might be able to nonspecifically effect the rejection of skin allografts. The relative importance of specific and nonspecific effector mechanisms in rejecting skin allografts was difficult to ascertain from their experiments because the allophenic skin grafts were placed on immunocompetent animals that initiated their rejection responses immediately at the time of engraftment. Thus, the relative importance of antigen-specific and nonspecific effector mechanisms in skin allograft rejection has remained uncertain and controversial. The present study has utilized an adoptive transfer system in which initiation of the rejection response could be postponed until the allophenic skin grafts had healed and regrown hair, permitting the rejection response to be clearly observed and followed.

The present study demonstrates that the immunologic response to skin allografts consists of two components: (i) an antigen-nonspecific inflammatory destruction of the epidermis and (ii) an antigen-specific destruction of dermal cellular elements. Since the viability of skin depends on the regenerative structures located within the dermis, the survival versus rejection of these grafts is determined by the response to the cellular elements within the dermis. As can be seen, the specific survival of syngeneic hair follicles and melanocytes in the dermis of the allophenic grafts indicates that the effector mechanism causing tissue destruction in this critical layer is antigen-specific, sparing cells not expressing allogeneic histocompatibility antigens. Histologic examination of these grafts during the rejection response revealed that some hair follicles (presumably H-2b) in the dermis of these grafts were infiltrated by inflammatory cells, whereas other hair follicles (presumably H-2k) in the same grafts were devoid of any inflammatory infiltrate (data not shown). Thus, despite the intense inflammatory response that accompanies in vivo rejection responses, it is the antigen-specific effector mechanism that determines whether skin allografts are retained or rejected.

The susceptibility of skin epidermal cells to damage by the nonspecific inflammatory component is graphically illustrated by the destruction and scabbing of the epidermis of the allophenic skin grafts (Fig. 2). Although it is possible that the nonspecific inflammatory response results from the recruitment of inflammatory cells by lymphokines secreted by activated TH or TdTH cells, we do not think this is the case. In previous experiments (5), we found that reconstitution of engrafted nude mice with isolated antigen-specific TH and TdTH cells failed to elicit a visible inflammatory response in the allografts. Thus, we think the inflammation may represent a nonimmune response to inflammatory mediators secreted by TH cells (15, 16) or to cellular debris created by FC-cell destruction of allogenic epidermal cells. The reason why epidermal cellular structures are relatively susceptible to destruction by antigen-nonspecific inflammatory mechanisms compared to dermal cellular structures is not known. Factors relating to the rapidity of cell cycling or cellular density may be important. In any event, it is conceivable that some organ allografts might resemble epidermal tissue more than dermal tissue in their susceptibility to destruction by inflammatory mediators. Thus, it is possible that the relative importance of antigen-specific versus antigen-nonspecific effector mechanisms may vary in rejection responses to different types of organ allografts.

In conclusion, the present study documents that skin allograft rejection requires immune destruction of the dermal cellular elements and is mediated by antigen-specific T-effector cells, probably possessing cytolitic capabilities, which destroy cells in the dermis expressing allogeneic histocompatibility antigens and spare cells expressing only syngeneic histocompatibility antigens.

We thank Dr. Willys Silvers and Dr. Stephen Katz for helpful discussions; Ms. Pamela Rose and Mr. Brian Weatherley for technical expertise and Dr. Bonnie Mathieson for helpful advice in the construction of the allophenic animals; Dr. Stephen Katz and Mr. Jay Linton for their aid in immunofluorescence microscopy of skin epidermal cells; Mr. David Stephany for flow cytometry; Ms. Shuna Johnson for expert photography; Ms. Linda Brown and Dodge Color, Inc., for the composite photograph; and Drs. Stephen Katz, Susan McCarthy, Dinah Singer, and David Sachs for critical readings of the manuscript. A.S.R. is the 1986 recipient of the American Society of Transplant Physicians–Sandoz Fellowship in Transplantation.