

# Immunologically Nonspecific Mechanisms of Tissue Destruction in the Rejection of Skin Grafts

By Daniel P. Doody, Karla S. Stenger, and Henry J. Winn

---

*From the Transplantation Unit, General Surgical Services, and the Department of Surgery, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts 02114*

## Summary

When mice are lethally irradiated and reconstituted with allogeneic bone marrow cells, their skin is repopulated over a period of several months with Langerhans cells (LC) of marrow donor origin. Skin from such mice, when transplanted to unirradiated syngeneic recipients, became in many cases the sites of intense inflammatory responses that led to varying degrees of destruction of the transplanted skin and in some instances, to rejection of the entire graft. The frequency and intensity of these responses were influenced by the nature of the immunogenetic disparity between the donors and recipients of the marrow cells. Chimeric skin placed on hybrid mice derived from crosses between the marrow donors and recipients behaved in all respects as syngeneic grafts or autografts. When the recipients of the chimeric skin were presensitized to the antigens of the marrow donor, the responses were especially intense, and resulted in all cases in complete rejection. Thus the immunologically mediated attack on the allogeneic LCs was accompanied by widespread and nonspecific destruction of bystander cells. In all cases, the inflammation and tissue damage were confined sharply to the grafted skin, showing clearly that nonspecific or indirect tissue destruction is entirely consistent with highly selective destruction of grafted tissues. This finding removes a major objection to postulated mechanisms of rejection that involve indirect destruction of grafted tissues.

A remarkable and consistent feature of the rejection of allografts is the absence of significant damage to host tissues that are close to or even in contact with elements of the grafts. In the case of skin, for example, microscopic examination of fixed sections of a rejecting graft reveals an intense inflammatory response that is confined almost exclusively to the graft and its adjacent bed, and cell damage is found to stop abruptly at the plane that separates graft and host tissues. Moreover, even gross inspection of the rejecting graft conveys a sense of strict selectivity. Similar or analogous observations have been made for grafts of other tissues and organs, and for transplanted tumors as well. This selectivity has long been recognized, and attempts to delineate mechanisms of graft rejection have had to take it into account. It is not surprising, therefore, that the striking specificity of killing exhibited by some T cells *in vitro* has led to the widely held view that this activity reflects the role of these cells in the rejection of grafts *in vivo* (1). In particular, the absence of detectable effects on bystander cells *in vitro* has fostered the notion that cells of the graft are likewise killed individually by cytotoxic T cells. This is an attractive view insofar as it accounts nicely for the selective destruction of donor cells, but it is not in accord with some important features of rejection. We have reported, for example, that skin grafts undergoing acute rejection may con-

tain only small numbers of T cells among the invading mononuclear cells, and the distribution of these T cells bears no obvious relationship to the pattern of damage observed in the grafts (2). The paucity of T cells is particularly striking in the case of donor-recipient combinations that involve MHC class I disparity only, but destruction of donor cells is found to occur in the absence of contact with host T cells even when there is disparity with respect to all of the MHC antigens. This may, of course, reflect ischemic death occurring secondarily to specific killing of endothelial cells, and indeed, we and others (3-6) have stressed the importance of the vascular bed as the major interface between the graft and its host, and as a crucial target for cellular as well as humoral mediators of rejection. Thus, it is possible that there is specific destruction of endothelial cells and that the selective destruction of graft elements reflects the anatomic distribution of donor vessels. Still, the strict selectivity of destruction is observed even in the case of grafts of pure epidermis, or full thickness grafts that are known to contain few, if any, donor endothelial cells, suggesting the possibility of an indirect attack on the vascular bed of the graft. Moreover, it has been observed that there are alterations in the vessels of host origin found in the graft bed that are indistinguishable from those seen in the vessels of grafts undergoing active rejection (3), and similar vascular responses are observed in delayed type

hypersensitivity (DTH)<sup>1</sup> reactions where they can be viewed only as indirect consequences of immune reactions (7).

We have sought evidence in favor of indirect mechanisms of damage through the use of an experimental system in which skin from bone marrow chimeric mice was transplanted to mice syngeneic to the marrow recipients. These grafts are immunogenetically compatible with their hosts except for bone marrow-derived cells, principally, if not solely, Langerhans cells (LC). We report here that the immune responses engendered by these allogeneic cells lead to the induction of intense inflammatory responses that are strictly confined to, and frequently result in widespread or total destruction of the grafts. Thus, destruction of bystander cells is observed in a manner that is entirely consistent with the selectivity of graft rejection.

### Materials and Methods

**Animals.** C3H/fSed mice were obtained from the Edwin L. Steele Laboratory of Radiation Biology, the Department of Radiation Oncology, Massachusetts General Hospital, and all F<sub>1</sub> mice of which they were parents were raised in our laboratories. All other mice were purchased from The Jackson Laboratory (Bar Harbor, ME). CD rats were purchased from the Charles River Breeding Laboratories (Wilmington, MA).

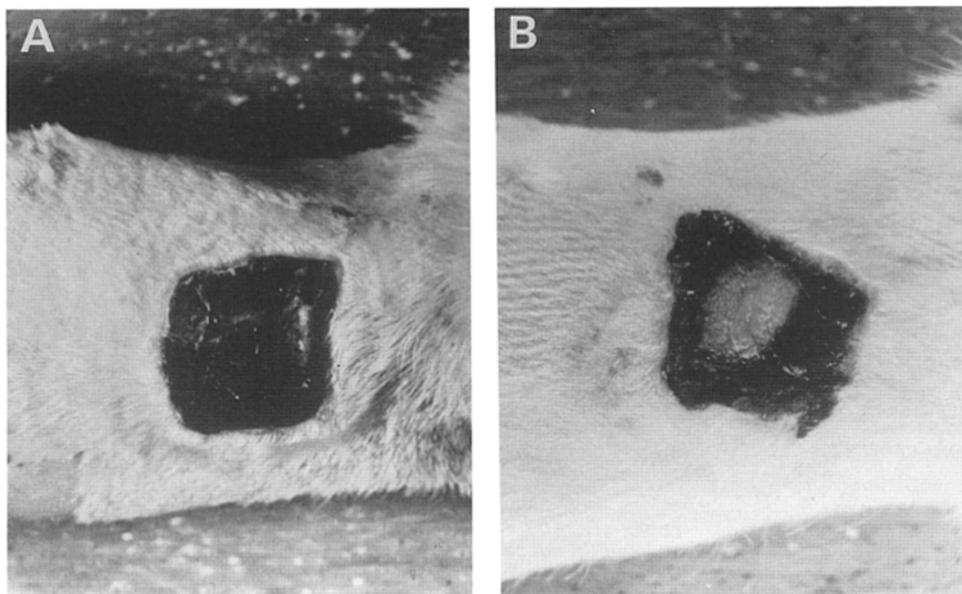
**Preparation of Bone Marrow Chimeric Mice.** C3H/fSed mice received 900–950 rad total body irradiation from <sup>137</sup>Cs sources (Gamma Cell 40, AECL Technologies Inc., Rockville, MD) at a rate of 80 rad/min, or (Mark I; JL Shepherd and Associates, San Francisco, CA) at 290 rad/min. 20 h after irradiation, the mice were given 15–20 × 10<sup>6</sup> allogeneic bone marrow cells intravenously. They were housed in microisolator cages under sterile conditions for 14 d during which time they received tetracycline in

their drinking water. Thereafter they were kept in microisolator cages under conventional conditions with acidified drinking water.

**Skin Grafts.** Mouse trunk skin was transplanted as described (8) using 1.5 cm<sup>2</sup> full thickness grafts applied to the dorsolateral thoracic wall. The dressings were removed 7 d after transplantation and the grafts were inspected daily for signs of inflammation (erythema and edema) and necrosis. Rat ear skin was prepared and grafted to the dorsolateral thoracic wall of recipient mice as described (9). Briefly, the intended recipients were thymectomized 2–3 wk before receiving the skin grafts. Their immune responses were further suppressed by the administration of rabbit anti-mouse lymphocyte serum in doses of 0.25 ml 2 d before grafting, on the day of grafting, and on the second and fourth days after placement of the grafts.

### Results

We have described in detail our observations on the acute destruction, by humoral antibody, of rat skin that had been transplanted to immunosuppressed mice (5, 6). It was clear from these studies that the reaction of antibodies with antigens on endothelial cells was sufficient to lead to the selective destruction of the entire graft. In the course of these studies we found that grafts that had been in place for long periods of time on the suppressed mice lost their sensitivity to humoral antibodies, and this loss was associated with the replacement of donor (rat) endothelium by cells derived from the recipient mice. Thus, the rat endothelial cells seemed to be the principal, and perhaps sole target of the antibodies. Moreover, when these longstanding grafts of rat skin were removed and regrafted onto rats of the donor strain, they were without exception rejected acutely, whereas rat skin that had been in place on mice for only 2–4 wk was accepted indefinitely when returned to the rat donors. The rejection of the long-term grafts was attributed to a cell-mediated attack on the mouse endothelial cells. An example of this selec-



**Figure 1.** Rat skin was transplanted to immunosuppressed mice, and at various intervals thereafter the grafts were removed with a surrounding cuff of recipient mouse skin and returned to the original donor rats. (A) Graft retransplanted to original donor after a 92-d residence on a suppressed mouse. Both rat and mouse skin are completely rejected (9 d after regrafting). (B) Rat skin returned to donor after 14 d residence on mouse. Note rejection of surrounding cuff of mouse skin in the absence of damage to the central patch of rat skin (9 d after regrafting).

<sup>1</sup> Abbreviations used in this paper: DTH, delayed type hypersensitivity; LC, Langerhans cell.

**Table 1.** Fate of Skin Taken from Bone Marrow Chimeras and Transplanted to Mice of the Marrow Donor and Recipient Strains

Chimeric donor	Recipient	Disparity	Inflammation	Rejection	
				Partial	Complete
C3H.SW → C3H	C3H.SW	K I <sub>A</sub> I <sub>E</sub> D	5/5	–	5/5
	C3H	K I <sub>A</sub> -D*	5/5	3/5	2/5
	(C3H × C3H.SW) <sub>F1</sub>	None	0/5	–	–

\* Disparity with respect to donor LC only.

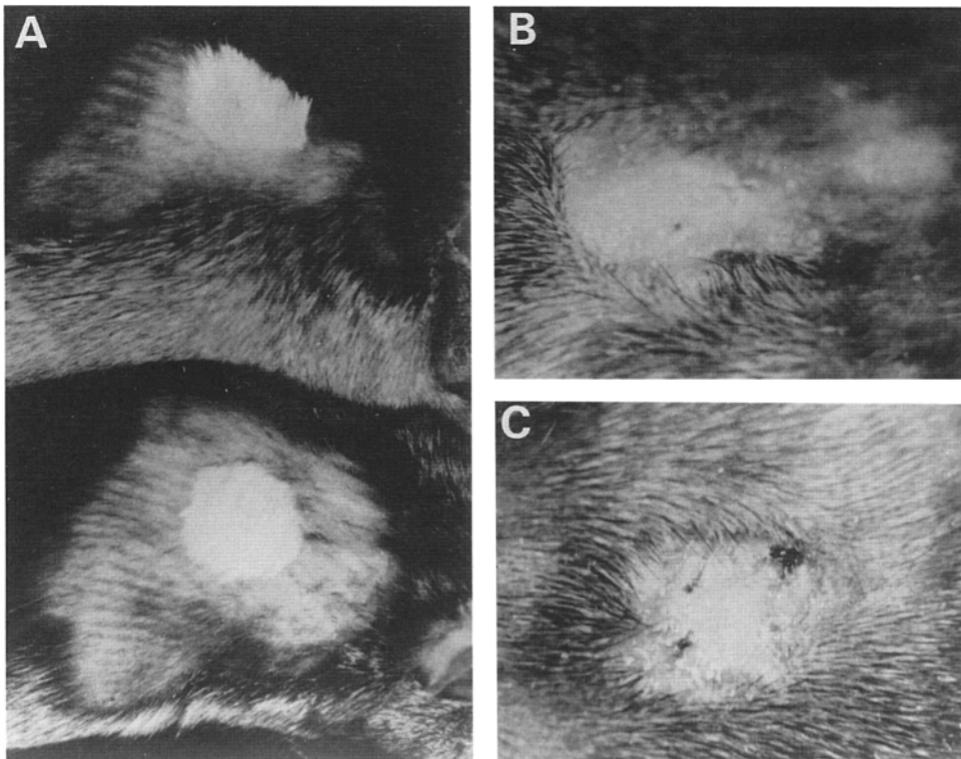
tive, though nonspecific, form of rejection is shown in Fig. 1 A. In this case, we used rat ear skin so that we could return the grafts to the actual donors, avoiding, thereby, problems associated with genetic segregation within the strain of rats. Thus, the animal depicted here has, like all of 11 others so treated, acutely rejected his own ear skin in the course of attacking the mouse endothelial cells. In removing the rat skin grafts from the mice we included small surrounding cuffs of the host skin that were grafted along with the rat skin. When 2–4-wk-old grafts were returned to the donors, there was complete destruction of the cuff of mouse skin with no detectable damage to the rat skin, an observation that underscores the selectivity of the rejection process (Fig. 1 B). When longstanding grafts were transferred to mice syngeneic to the primary recipients, the surrounding cuffs of mouse skin were maintained in perfect condition whereas the rat skin was selectively rejected. The endothelium of these grafts was derived entirely, or almost entirely, from mouse cells. Thus, selective

damage in this case might be attributed to an indirect attack on the vessels of the graft. To look for evidence of such indirect effects, we carried out the following experiments in which chimeric skin was grafted to syngeneic recipients who could respond only to the allogeneic dendritic cells of the grafts.

*The Fate of C3H.SW → C3H Chimeric Skin Grafted onto C3H Recipients.* When mice are lethally irradiated and reconstituted with allogeneic bone marrow, their skin is repopulated over a period of several months with LC of marrow donor origin (10). LC are important contributors to effector responses in skin, and they are also known to serve as stimulators of intense immune responses when placed in allogeneic recipients. We wished to determine whether they could serve as prime targets in the rejection of skin as well as initiators of the response to the presence of allogeneic tissue. Accordingly, we lethally irradiated C3H mice and reconstituted them with C3H.SW bone marrow. 4 mo later, these mice were used as donors of skin grafts for C3H, C3H.SW, and (C3H



**Figure 2.** C3H → C3H skin was taken at 4 mo after irradiation and reconstitution with C3H.SW bone marrow, and grafted onto a C3H mouse. Picture taken 12 d after grafting shows selective inflammation of the graft. Subsequent fate of the graft is shown in Fig. 3 C.



**Figure 3.** C3H.SW  $\rightarrow$  C3H chimeric skin was taken at 4 mo after irradiation and reconstitution with C3H.SW bone marrow, and grafted onto (A) (C3H  $\times$  C3H.SW) $F_1$  mice. (B) C3H mice. Pictures were taken 30 d after transplantation. (A) Grafts on  $F_1$  mice are fully intact and are indistinguishable from syngeneic grafts. (B and C) Responses of C3H mice to chimeric skin. (B) Complete rejection with scarring; (C) partial rejection with about 15% of donor skin surviving.

$\times$  C3H.SW) $F_1$  recipients. The results of this experiment are summarized in Table 1, and Figs. 2 and 3. As expected, all of five C3H.SW mice promptly rejected their grafts, and five of 5 $F_1$  mice retained the chimeric skin without signs of rejection. All of the grafts placed on C3H mice were severely inflamed within 24 h of removing the dressings, the inflammation showing the selectivity characteristic of rejection (Fig. 2). Two of the grafts were rejected acutely. The other three were extensively damaged, though small portions of them survived and became normal in appearance after the inflammatory process had subsided. (Fig. 3). The survival of a small amount of donor skin could be detected on the basis of the direction of hair growth. The grafts were positioned in the beds such that the hair would grow in a direction opposite to that of recipient hair, and the loss of pigment in donor skin was due to radiation damage. Evidently, some of the C3H.SW LC induced an allogeneic response in the draining lymph nodes, and the T cells generated in the response entered the graft and reacted with the remaining C3H.SW cells, resulting in the induction of intense inflammatory responses that lead to tissue damage. The destruction of the C3H.SW LC removed the inciting antigen, and the inflammation abated allowing those C3H cells that escaped destruction to persist indefinitely. It is important to note that the grafts on the (C3H  $\times$  C3H.SW) $F_1$  behaved essentially as syngeneic grafts with only very mild and transient signs of inflammation at the time of removal of the dressings. Furthermore, the intensity of the responses in the grafts on the C3H mice was at least as great during the first 2 d after removal of the dressings

as those observed in the grafts on the C3H.SW recipients. Evidently, allogeneic LC not only induced a rapid response but also played an important role in the rejection of the grafts.

*The Fate of Chimeric Grafts in other Donor-Recipient Combinations.* We have repeated this experiment using donor-recipient combinations that involve various kinds of immunogenetic disparity, as indicated in Table 2. The intensity and frequency of the inflammatory responses that we observed in the grafts varied among the different groups of "syngeneic" recipients, but it is clear from the overall results of the experiment that the phenomenon of selective but nonspecific damage of graft elements is a common event in the case of skin. The numbers of animals in individual groups are too small to reach any firm conclusion with respect to the relationships between the intensity of the responses and the nature of the immunogenetic diversity between the allogeneic dendritic cells and their recipients. However, it is interesting to note that the most intense and destructive responses occurred in the B10.BR/C3H combination which involves multiple minor histoincompatibilities only, an observation that may be related to reports that DTH reactions to minor histocompatibility antigens are particularly strong (11). Also, in the case where there was only an H-2D region difference ( $D^k \rightarrow D^d$ ) that induces rejection responses of modest intensity, inflammation occurred in only one of seven grafts. This and an observation made in a different context that chimeric grafts involving I-A differences only were not rejected (12), suggest that the destruction of bystander cells occurs when the immune response to the dendritic cell antigens is

**Table 2.** Fate of Skin Taken from Chimeras of Various Genetic Constitutions and Placed on Marrow Donor and Recipient Strain Mice

Chimeric donor	Recipient	Disparity	Inflammation	Rejection	
				Partial	Complete
B10.BR → C3H	B10.BR	Minor HA	5/5	–	5/5
	C3H	Minor HA*	4/4	3/4	1/4
	(B10.BR × C3H)F <sub>1</sub>	None	0/5	–	–
B6AF <sub>1</sub> → B10.BR	B6AF <sub>1</sub>	---D	30/30	–	30/30
	B10.BR	K IA-D*	19/27	8/27	5/27
(C3H × C3H.SW)F <sub>1</sub> → C3H	C3H	K IA-D*	7/7	2/7	2/7
	(C3H × B10.A[5R])F <sub>1</sub>	---D*	1/7	1/7	–
	(C3H × C3H.SW)F <sub>1</sub>	None	0/7	–	–

\* Disparity with respect to donor LC only.  
HA, histocompatibility antigen.

acute and intense, circumstances that permit the destructive process to override repair and regeneration. In this connection it is important to consider that cells containing the irritant represent a limiting factor that is consumed during the response. Thus, in the case of a slowly evolving response, the intensity of inflammation may produce little damage to bystander cells and the effects on the vascular bed may be completely reversible.

*Fate of Chimeric Grafts Placed on Previously Sensitized Recipients.* The mechanisms of nonspecific but selective destruction of allografts that we propose here suggest that pre-sensitization of intended recipients with tissue from the mouse strain that served as donor of the bone marrow would lead to more vigorous and extensive damage to chimeric grafts than is observed in nonprimed mice. Accordingly, we transplanted B6AF<sub>1</sub> skin to B10.D2 mice and 2–3 wk after the grafts had been rejected, we placed B6AF<sub>1</sub> → B10.D2 chimeric skin on these rejectors which had become sensitized to B6AF<sub>1</sub> alloantigens. The results of this experiment are presented in Table 3. As expected, the chimeric skin was rejected acutely by all of the B6AF<sub>1</sub> recipients. All four chi-

meric grafts placed on nonsensitized B10.D2 mice became moderately to intensely inflamed 2–7 d after removal of the graft dressings. One of these grafts was completely rejected by day 16, two of them were partially destroyed, and the remaining graft recovered completely. In the case of the sensitized recipients, all four grafts were completely rejected, two by day 10, and two by days 15 and 16. Thus, these results affirm in a dramatic way the occurrence of selective and complete destruction of skin grafts through the interaction of host lymphocytes with a relatively small number of nonvascular targets.

### Discussion

The prime role of APCs in the induction of states of transplantation immunity, initially enunciated in terms of the passenger leukocyte hypothesis (13), has been recognized for several decades, but the possibility that these cells may serve as important targets in the rejection of allografts seems not to have been seriously considered. This oversight is surprising in view of the well-known involvement of dendritic cells in

**Table 3.** Fate of Skin Taken from B6AF<sub>1</sub> → B10.D2 Bone Marrow Chimeras and Transplanted to Mice of the Recipient Strain That Had Previously Rejected B6AF<sub>1</sub> Skin

Chimeric donor	Recipient	Disparity	Inflammation	Rejection	
				Partial	Complete
B6AF <sub>1</sub> → B10.D2	B6AF <sub>1</sub>	K IA IE <sup>-</sup>	4/4	–	4/4
	B10.D2	K IA IE D*	4/4	1/4	1/4
	B10.D2 <sup>‡</sup>	K IA IE D*	4/4	–	4/4

\* Disparity with respect to donor LC only.

‡ Rejected normal B6AF<sub>1</sub> skin 1 mo previously.

the expression of DTH reactions. It seems reasonable to expect that the dendritic cells in skin, for example, would present their resident alloantigens to effector cells just as they do with environmental antigens. Indeed, in view of our understanding of the function of MHC molecules it seems inevitable that DTH reactions would be found to be involved in the rejection of allografts. Thus our observations, which can be explained only on the basis of the interaction of sensitized cells of the host with dendritic cells in the grafts, might well have been anticipated, and what is surprising is that they have not been reported much earlier. The reasons that this potentially important pathway of responsiveness has gone almost unnoticed have both conceptual and experimental origins. As mentioned above, the striking selectivity of graft rejection seemed intuitively incompatible with DTH reactions as an explanation for graft rejection, and the discovery of CTL with their specificity for target cells provided a more satisfying albeit unproven explanation for the phenomenon. The data that we report here, however, make it abundantly clear that the selectivity of tissue destruction that is seen in grafts undergoing rejection need not entail the killing of cells individually by effector substances, and need not remove indirect effector mechanisms from consideration of the pathways of rejection.

Nevertheless, there are experimental data that have been offered as strong evidence against the involvement of indirect mechanisms. These data come from studies in which skin from tetraparental mice was grafted onto recipients that shared the MHC antigens of one set of parents but were incompatible with those of the other parents. Thus, these grafts consisted of a complex mosaic of histocompatible and histoincompatible cells, and induced responses that resulted in the destruction of variable proportions of the transplanted tissue. Because the surviving tissue contained only histocompatible cells, it was concluded in the report of the initial study (14) that there had been selective destruction of the incompatible cells. However, that conclusion cannot be sustained because it is virtually impossible to determine the genotypes of all of the individual cells that comprise the grafts. Whereas it can be determined that all of the incompatible cells have been destroyed and that only compatible ones remain, it cannot be known whether or not significant numbers of compatible cells were rejected. Indeed, in a subsequent study, Rosenberg and Singer (15) found that there was substantial loss of compatible as well as incompatible cells. However, they concluded that the inflammation that led to nonspecific destruction of epidermal cells did not cause rejection of the grafts, and that "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."

We come to a very different conclusion on the basis of our results but we find no difficulty in resolving the difference. The skin that we used for grafting came from mice that had received lethal doses of ionizing radiation and rescuing doses of allogeneic bone marrow cells 4 mo before serving as donors. There was time, then, for all or nearly

all of the LC that were present in skin at the time of irradiation to have been replaced by cells from the donor marrow, thereby insuring an ample supply to serve as allogeneic stimulators and targets of effector cells when grafted to mice otherwise syngeneic with the donor. The skin of tetraparental mice, on the other hand, consists of cells of two distinct genotypes, the relative numbers of which vary from time to time and place to place in individual mice. Accordingly, the density of LC that are foreign to recipients of either parental type is variable and very likely to be lower than is the case in our experimental system. Furthermore, in the experiments of Rosenberg and Singer (15) the grafts were in place on B6 nude mice for 31 d before the animals received spleen cells from euthymic B6 donors. That period of time would have resulted in replacement of some allogeneic LC by syngeneic ones, resulting in further reductions of immunogenicity and responsiveness to effector cells. Like Rosenberg and Singer, we have found that portions of grafts that become intensely inflamed frequently recover, and occasionally there is recovery of all or nearly all of the grafted tissue. However, when the responses to the grafts were sufficiently intense, as in the case where the recipients had been sensitized to the alloantigens of the LC in the chimeric skin, all of the grafts were completely rejected, and whenever there was strong immunogenetic disparity between the LC and the recipient, a significant proportion of first set grafts was completely rejected. We conclude that the interaction between LC and T cells specifically sensitized to the alloantigens of the LC can lead to a highly selective and complete rejection of skin grafts. Thus, we do not see our results as conflicting with those found in studies using tetraparental mice as donors. Rather, we feel that our experimental system involves inflammatory responses that are more intense and more pervasive than is the case for tetraparental grafts, and that such intense responses can cause complete and selective destruction of skin grafts in the absence of specific attacks on cells other than LC. The selectivity is due, in our view, to the anatomic arrangement of the microvasculature of the graft. These small vessels, which are especially responsive to vasoactive and thrombogenic factors released by the interaction of T cells and LC, are so placed as to have ready access to such substances, and their pattern of distribution is such that they nourish only cells of the graft. Thus, we see selectivity of destruction in these experimental systems as a reflection of irreversible damage to the microvascular bed.

There are also two reports (12, 16) in which skin from chimeric mice was transplanted to mice syngeneic with the bone marrow recipients using experimental systems much like those that we have employed in our study. In neither report was the rejection of such grafts recorded. However, in one case the skin was taken from the chimeras shortly after they had been reconstituted with allogeneic bone marrow cells, and as the author has pointed out, it is unlikely that this skin contained more than a small number of allogeneic LC (16). In the other case, the marrow donors and recipients differed only with respect to an I-A antigen that induced only a moderate rejection response (12). It is not surprising or in-

consistent with our observations that the authors of the report observed transient crises in some grafts but no overt destruction of the grafted tissue. The results of both studies are in keeping with our suggestion that slowly evolving responses to chimeric grafts may remove the source of antigen without causing irreversible damage to the grafts.

Our results do not, of course, exclude mechanisms of rejection involving specific killing of targets in the absence of bystander effects. Indeed, the studies carried out with the tetraparental animals provide strong evidence for such a mechanism. Moreover, rejection in some circumstances may be traceable largely or exclusively to specific mechanisms. For example, when mixtures containing small numbers of syngeneic tumor cells and very much larger numbers of allogeneic tumor cells are implanted into mice, the latter grow into easily measurable tumors which regress, and the former develop at the site of rejection into progressively growing tumors with no evidence that the times of appearance or rates of growth of these syngeneic cells have been affected by the rejection of the allogeneic cells (17). These observations point to a highly specific attack on individual target cells. The additional observation that the rejection of allogeneic tumors can be completely abrogated by depleting host CD8 cells alone (18), supports the view that the tumor cells in these cases are destroyed individually by CTL. It may be significant that there are no donor dendritic cells to serve as stimulators or targets in these tumor grafts. The importance of selective, but nonspecific forms of damage in the rejection of other types of grafts remains to be determined.

Finally, it is important to consider the relationship between

the process of rejection that we describe here and a related process that has been considered from time to time over the past four decades viz destruction of grafted tissues by DTH reactions. In this process it is suggested that graft alloantigens are processed, and peptides derived thereby are presented by recipient APC so as to activate CD4<sup>+</sup> T cells which enter the graft and incite DTH reactions by interacting with host APC presenting donor antigens. Implicit in this formulation is the suggestion that rejection involves damage to bystander cells. This hypothesis has always had some merit and the presentation of donor-derived peptides by host APC, implicit in the widespread observation of humoral antibody responses to MHC antigens, has recently received direct support from studies carried out in several laboratories (19, 20). However, it has not been possible to obtain direct evidence for nonspecific mechanisms of rejection, and the high selectivity of tissue destruction observed during the rejection process has been widely viewed as being inconsistent with damage caused to bystander cells. The data presented here do not deal directly with the DTH hypothesis insofar as our experimental system seems to involve direct recognition of alloantigens on donor cells during both the inductor and effector stages of the immune response. Nevertheless, they do make it clear that rejection of grafts by mechanisms that destroy bystander cells in an antigen nonspecific manner does occur and is entirely consistent with the selective destruction of allografts. Accordingly, they not only provide direct and convincing evidence for the path of tissue destruction described here, but they also remove a major objection to other postulated mechanisms of rejection that involve indirect destruction of grafts.

---

We thank Janet Quinn and Susan Shea for their technical assistance, and Frances Stewart for secretarial assistance.

This study was supported by U.S. Public Health Service grants AI-31050 and AI-23413 from the National Institutes of Health.

Address correspondence to Dr. Henry J. Winn, General Surgical Services, White 535, Massachusetts General Hospital, Boston, MA 02114.

*Received for publication 3 November 1993 and in revised form 20 January 1994.*

## References

1. Rosenberg, A.S., and A. Singer. 1992. Cellular basis of skin allograft rejection: an in vivo model of immune-mediated tissue destruction. *Annu. Rev. Immunol.* 10:338.
2. Mayer, T.G., A.K. Bhan, and H.J. Winn. 1988. Immunohistochemical analysis of skin graft rejection in mice. *Transplantation (Baltimore)* 46:890.
3. Waksman, B.H. 1963. The pattern of rejection in rat skin homografts and its relation to the vascular network. *Lab. Invest.* 12:46.
4. Dvorak, H.F., M.C. Mihm, B.A. Barnes, E.J. Manseau, and S.J. Galli. 1979. Rejection of first-set skin allografts in man: the microvasculature is the critical target of the immune response. *J. Exp. Med.* 150:322.
5. Jooste, S.V., R.B. Colvin, W.D. Soper, and H.J. Winn. 1981. The vascular bed as the primary target in the destruction of skin grafts by antiserum. I. Resistance of freshly placed xenografts to antiserum. *J. Exp. Med.* 154:1319.
6. Jooste, S.V., R.B. Colvin, and H.J. Winn. 1981. The vascular bed as the primary target in the destruction of skin grafts by antiserum. II. Loss of sensitivity to antiserum in long-term xenografts of skin. *J. Exp. Med.* 154:1332.
7. Dvorak, H.F., M.C. Mihm, Jr., A.M. Dvorak, R.A. Johnson,

- E.J. Manseau, E. Morgan, and R.B. Colvin. 1974. Morphology of delayed type hyper-sensitivity reaction in man. I. Quantitative description of the inflammatory response. *Lab Invest.* 31:111.
8. Billingham, R.E., and P.B. Medawar. 1951. The technique of free skin grafting in mammals. *J. Exp. Biol.* 28:385.
  9. Baldamus, C.A., I.F.C. McKenzie, H.J. Winn, and P.S. Russell. 1973. Acute destruction by humoral antibody of rat skin grafted to mice. *J. Immunol.* 110:1532.
  10. Katz, S.I., K. Tamaki, and D.H. Sachs. 1979. Epidermal Langerhans cells are derived from cells originating in the bone marrow. *Nature (Lond.)*. 282:321.
  11. Burdick, J.F. 1986. Strong cellular immune responses induced in vivo against minor antigens in the mouse. *Immunology*. 58:615.
  12. Woodward, J.G., B.L. Shigekawa, and J.A. Frelinger. 1982. Bone marrow derived cells are responsible for stimulating I region incompatible skin graft rejection. *Transplantation (Baltimore)*. 33:254.
  13. Snell, G.D. 1957. The homograft reaction. *Annu. Rev. Microbiol.* 11:439.
  14. Mintz, B., and W.K. Silvers. 1970. Histocompatibility antigens on melanoblasts and hair follicle cells. *Transplantation (Baltimore)*. 9:497.
  15. Rosenberg, A.S., and A. Singer. 1988. Evidence that the effector mechanism of skin allograft rejection is antigen-specific. *Proc. Natl. Acad. Sci. USA.* 85:7739.
  16. Steinmuller, D. 1980. Passenger leukocytes and the immunogenicity of skin allografts. *J. Invest. Dermatol.* 75:107.
  17. Klein, G., and E. Klein. 1956. Genetic studies of the relationship of tumor-host cells. *Nature (Lond.)*. 178:1389.
  18. Smith, D.M., F.P. Stuart, G.A. Weinhoff, J. Quintans, and F.W. Fitch. 1988. Cellular pathways for rejection of class-I-MHC-disparate skin and tumor allografts. *Transplantation (Baltimore)*. 45:168.
  19. Benichou, G., P.A. Takizawa, C.A. Olson, M. McMillan, and E.E. Sercarz. 1992. Donor major histocompatibility complex (MHC) peptides are presented by recipient MHC molecules during graft rejection. *J. Exp. Med.* 175:305.
  20. Kievits, F., and P. Ivanyi. 1991. A subpopulation of mouse cytotoxic T lymphocytes recognizes allogeneic H-2 class I antigens in the context of other H-2 class I molecules. *J. Exp. Med.* 174:15.