THE BEHAVIOR OF SKIN GRAFTS INCOMPATIBLE WITH RESPECT TO SKIN ALLOANTIGENS ON MICE RENDERED TOLERANT AT BIRTH WITH LYMPHOID CELLS*

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When the phenomenon of acquired immunological tolerance was first described it was believed that all cells possess the same array of transplantation antigens (1). Indeed, even after the demonstration that some fetal or neonatal animals injected with allogeneic lymphoid cells permanently accept these cells but do not permanently accept skin allografts of the same genetic origin, it was presumed that rejection results from the greater susceptibility of skin to transplantation immunity (2). We now know that the ability of permanent hemopoietic chimeras to reject skin grafts from the hemopoietic cell donor is due to skin-specific (Sk) antigens. Thus, Boyse and his associates (3) observed that adult C57BL/6 (hereafter B6) mice lethally irradiated and restored with (B6 x A) hemopoietic cells subsequently reject (B6 x A) or A-strain skin grafts, although cytotoxicity and hemagglutination tests fail to distinguish between these chimeric recipients and normal (B6 x A) controls. It seems, therefore, that the A-strain skin grafts are rejected by (B6 x A) lymphoid cells which have lost tolerance of A-strain Sk antigens after being transferred to recipients which do not express these antigens. (The possibility remains that rejection in this system is mediated by a residuum of radiation-resistant host cells.) Further support for this contention is provided by the finding that tolerance of putative Sk antigens is maintained when A-strain epidermal cells are administered intravenously and intraperitoneally at the time of irradiation and restoration (4).

Whereas these results are in accord with the concept of Sk antigen they do not agree with previous studies concerned with tolerance induction in neonatal mice (2). These early studies indicate that the resistance of neonatal B6 mice to the induction of tolerance of A-strain skin allografts can be overcome if they are exposed to a sublethal dose of X irradiation before they are inoculated with a suspension of (A x B6) lymphoid cells. Thus, 12 of 24 B6 mice exposed to 460 R and 20 million (A x B6) spleen cells at birth, were subsequently found to be

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Abbreviations used in this paper: MST's, median survival times; Sk, skin-specific differentiation alloantigens.
highly tolerant of A-strain skin grafts; i.e., such grafts survived for more than 50 days. However, since four of these animals rejected their grafts by the 56th day after transplantation and since no grafts were followed for more than 60 days, it is conceivable that all grafts might have been destroyed eventually had the observation period been extended. In this case rejection would be consistent with the notion of Sk antigen.

In the experiments described below the observation period was extended to determine whether neonatally X-irradiated B6 animals, inoculated with \((A \times B6)\) lymphoid cells and subsequently challenged with A skin grafts, ultimately reject these grafts while maintaining their chimeric state. Moreover, the response of these mice to adult skin grafts was compared with their response to skin grafts from neonatal donors.

**Materials and Methods**

**Mice.** Male and female mice of the isogenic B6/Ss (H-2\(^b\)) and A/Ss (H-2\( ^a\)) strains as well as \((A \times B6)\) hybrids of these two strains were used.

**Tolerance Induction.** When less than 18-h old, B6 and A mice were inoculated intravenously, via the orbital branch of the anterior facial vein, with 20 million spleen and lymph node cells from \((A \times B6)\) donors (5).

**Cell Suspensions.** All cell suspensions were prepared in Hanks' balanced salt solution according to procedures described elsewhere (5). Cells were administered in a standard vol of 0.1 ml of medium.

**Irradiation.** Mice which were irradiated before receiving the tolerance-inducing inoculum, were exposed to 400 or 500 R from a \(^{137}\)Cs source.

**Skin Grafting.** All neonatally inoculated B6 animals were challenged with strain A skin grafts and all treated A mice received B6 skin grafts after 12-14 wk (6). The skin grafts were prepared from the ventrum of adult female donors or from females less than 24 h of age. All grafts were full thickness disks of skin measuring 1.2-1.5 cm in diameter. Graft beds were prepared in the lateral thoracic wall of the hosts. First and third grafts were always transplanted on the right side of the body and second grafts on the left side. Primary inspections were carried out at 8 or 9 days after transplantation and grafts were evaluated daily for 30 days and twice weekly thereafter. Surviving neonatal skin grafts were observed for at least 200 days and adult grafts were observed for at least 100 days. Animals were tested for cellular chimerism after they had rejected their test grafts by hemagglutination and cytotoxicity tests.

**Hemagglutination Test.** Hemagglutination was accomplished according to the procedure of Gorer and Mikulska (7) with the following modifications: 0.3 ml whole blood (drawn from the retroorbital sinus) was added to 2.0 ml sodium citrate solution (3.8 g sodium citrate-100 ml of 0.85% saline/100 ml distilled water). Erythrocytes (RBC) were centrifuged at 150 g for 10 min and washed twice in 2-5 ml saline. 0.01 ml packed RBC was then suspended in 0.15 ml heat-inactivated, gamma globulin-free fetal bovine serum and one drop of this suspension was reacted in plastic hemagglutination trays with one drop of typing sera (B6 anti-ASL1, a strain A leukemia; or A anti-ERLD, a strain B6 leukemia) diluted 1/32 and 1/128 with dextran-dextrose saline (1.8% dextran, average mol wt 115,000-1.08% dextrose). Controls included the dextran-dextrose saline but omitted the antiserum. Hemagglutination trays were covered to prevent evaporation, and incubated at 37°C for 90 min. Tests were read on glass slides by tilting the mixture and comparing controls with the two serum dilutions.

**Cytotoxicity Test.** The cytotoxicity test was performed essentially according to the method described by Boyse et al. (8), using lymphocytes obtained from biopsied axillary and brachial lymph nodes. Briefly, equal volumes (0.05 ml) of H-2 antiserum (serially diluted, e.g., 1/40 to 1/320), lymphocyte suspensions (5 × 10\(^6\) cells/ml), and absorbed guinea pig serum diluted 1/4 (complement source), were incubated at 37°C for 45 min in a rocking water bath. After incubation the tests were placed on ice. A freshly prepared solution of trypan blue dye was added to each tube, and the cells were counted in a hemacytometer. Dead cells stained with the dye.

**Median Survival Times (MST's).** MST's were computed by the method of Litchfield (9).
Survival of Adult Strain A Skin Grafts on B6 Mice Inoculated at Birth with 20 Million (A x B6)F1 Lymphoid Cells

| Sex of recipients | No. of animals | Graft survival times | MST  | SD  |
|-------------------|----------------|----------------------|------|-----|
|                   |                | days                |      |     |
| ff                | 15             | 2 x 9, 10, 3 x 12, 7 x 13, 15, 16 | 12.2 | 1.21|
| mm                | 11             | 9, 2 x 10, 2 x 12, 6 x 13 | 12.0 | 1.25|
| ff and mm         | 26             | 3 x 9, 3 x 10, 5 x 12, 13 x 13, 15, 16 | 12.1 | 1.18|

Results

Survival of Adult Strain A Skin Grafts on B6 Mice Inoculated at Birth with (A x B6) Cells. The first experiment was performed to confirm the report (2) that it is not possible to render B6 mice more than slightly unresponsive of strain A skin grafts by exposing them at birth to (A x B6) spleen and lymph node cells. Accordingly, 26 neonatal B6 mice, 15 females and 11 males, were inoculated with 20 x 10⁶ of these cells and were subsequently challenged with strain A skin grafts. As noted in Table I none of these grafts survived for more than 16 days (MST, 12.1 days). The MST of strain A skin on 22 untreated B6 mice was about 8 days with none of the grafts surviving more than 9 days.

Survival of Adult A Skin Grafts on X-Irradiated B6 Mice Inoculated at Birth with (A x B6) Cells. To determine whether neonatal B6 mice could be made tolerant of A skin grafts if the recipients were exposed to X irradiation before receiving (A x B6) lymphoid cells, as previously reported (2), B6 newborns received either 400 or 500 R before they were inoculated with (A x B6) lymphoid cells. Although the mortality after this treatment was not recorded, we estimate that at least 50% of the subjects died before they could be challenged with skin grafts. Nevertheless, 13 recipients (6 females and 7 males) of 400 R and 29 (13 females and 16 males) which received 500 R survived in apparently healthy condition. The response of these animals to strain A skin grafts is summarized in Table II. Although these grafts survived for significantly longer periods than similar grafts on unirradiated animals, and although they appear to have survived better on males than on females, no graft survived for more than 69 days. These rejections were not accompanied by a loss of cellular chimerism. Thus, when 21 animals in this experiment were tested serologically for the persistence of (A x B6) cells all proved to be highly chimeric, even though 10 of these animals had rejected their A-strain skin grafts within 14 days (see Table II).

Survival of Neonatal Strain A Skin Grafts on B6 Mice Inoculated at Birth with (A x B6) Cells. When only weak histoincompatibilities prevail, neonatal skin grafts often survive in situations where adult skin grafts of the same genotype are rejected (10–12). Therefore, we wished to determine how irradiated B6 mice inoculated at birth with (A x B6) cells would behave towards neonatal A-strain grafts. Accordingly 35 such mice (13 females and 22 males which had received 500 R) were challenged with neonatal A-strain skin grafts. In contrast to the eventual destruction of all adult A-strain skin grafts on identically treated hosts (noted above) more than half of these grafts were tolerated.
BEHAVIOR OF Sk-INCOMPATIBLE SKIN GRAFTS ON TOLERANT MICE

TABLE II

Survival of Adult Strain A Skin Grafts on B6 Mice Inoculated at Birth with 20 Million (A × B6)F1, Lymphoid Cells after Receiving X Irradiation

| X-ray dose | No. of animals | Sex | Graft survival times | MST | SD |
|------------|----------------|-----|---------------------|-----|-----|
| 400 R      | 6              | ff  | 2 × 12, 13, 15, 18, 20 | 13.5| 1.33|
|            | 7              | mm  | 14, 2 × 15, 19, 22, 23, 51 | 19.0| 1.22|
|            | 13*            | mm and ff | 2 × 12, 13, 14, 3 × 15, 18, 19, 20, 22, 23, 51 | 16.3| 1.34|
| 500 R      | 13             | ff  | 5 × 13, 3 × 14, 15, 16, 17, 23, 45 | 13.2| 1.20|
|            | 16             | mm  | 4 × 13, 18, 20, 22, 24, 25, 27, 30, 36, 41, 44, 65, 69 | 24.0| 1.88|
|            | 29‡            | mm and ff | 9 × 13, 3 × 14, 15, 16, 18, 20, 22, 23, 24, 25, 2 × 27, 30, 36, 41, 44, 45, 65, 69 | 17.2| 1.98|

* Two females with grafts surviving 12 and 13 days and six males with grafts surviving 14, 2 × 15, 22, 23, and 51 days tested for chimerism.
‡ Eight females with grafts surviving 3 × 13, 3 × 14, 15, and 27 days and five males with grafts surviving 13, 18, 36, 44, and 65 days tested for chimerism.

throughout the observation period of 200 days (Table III). Indeed, 26 of the 35 grafts survived longer than the longest surviving adult skin grafts. Moreover, whereas 9/13 (69%) females rejected such grafts, only 6/20 (30%) males did so.

Because of the prolonged and in most instances indefinite survival of these neonatal grafts on irradiated recipients, we wished to learn whether such grafts would survive as well on similarly inoculated but unirradiated hosts. The persistence of neonatal A skin grafts on inoculated but unirradiated B6 hosts would indicate that failure of adult A skin grafts to survive on similarly treated hosts is due not to H-2 antigens but to weaker (putative Sk) antigens. In this event, the resistance of unirradiated neonatal B6 mice to tolerance induction (with respect to adult A skin) could be attributed to their ability to respond to weak (Sk) but not strong (H-2) antigens (13). We found that although unirradiated B6 animals which had received (A × B6) lymphoid cells at birth occasionally accepted neonatal A strain grafts slightly longer than adult A grafts, the MST's of both adult and neonatal grafts were similar (Table IV). Moreover, when 17 inoculated but unirradiated B6 animals were assayed for H-2k antigens after they had rejected their test grafts, none proved to be chimeric.

Capacity of Neonatal Skin Grafts to Induce Unresponsiveness of Adult Skin Grafts. With respect to weak antigenic differences, neonatal skin grafts not only survive longer than adult grafts, but they also may induce unresponsiveness of the latter (10–12). This phenomenon is most frequently observed when the neonatal graft has been in residence for some time before the host is challenged with the adult graft. Therefore, we wished to determine the fate of adult A-strain grafts on B6 hosts which had been challenged with newborn grafts after their neonatal treatment, especially on those hosts which had permanently accepted these grafts. 13 B6 mice (8 females and 5 males) irradiated with 500 R and inoculated with (A × B6) cells at birth, which had rejected neonatal A-strain skin grafts, and 10 similarly treated mice (3 females and 7
TABLE III
Survival of Neonatal Strain A Skin Grafts on B6 Mice Inoculated at Birth with 20 Million (A × B6)F1 Lymphoid Cells after Receiving 500 R

| No. of animals | Sex of recipients | Graft survival times | Surviving >200 days |
|----------------|-------------------|----------------------|-------------------|
| 13             | ff                | 15, 16, 20, 26, 54, 71, 85, 105, 106, 4 × >200 | 31                |
| 22             | mm                | 15, 39, >53, 54, 80, 87, 93, >134, 14 × >200 | 64                |
| 35             | ff and mm         | 2 × 15, 16, 20, 26, 39, >53, 2 × 54, 71, 80, 85, 87, 93, 105, 106, >134, 18 × >200 | 51                |

* Animal died with graft intact.

TABLE IV
Survival of Neonatal Strain A Skin Grafts on B6 Mice Inoculated at Birth with 20 Million (A × B6)F1 Lymphoid Cells

| Sex of recipients | No. of animals | Graft survival times | MST | SD |
|-------------------|----------------|----------------------|-----|----|
| ff                | 11             | 2 × 8, 9, 3 × 10, 2 × 11, 2 × 12, 19 | 9.5 | 1.16 |
| mm                | 12             | 2 × 8, 9, 10, 3 × 12, 15, 20, 2 × 21 | 12.0 | 1.60 |
| ff and mm*        | 23             | 4 × 8, 2 × 9, 4 × 16, 2 × 11, 5 × 12, 15, 18, 19, 20, 2 × 21 | 10.8 | 1.58 |

* Eight females with grafts surviving 3 × 10, 2 × 11, 2 × 12, and 19 days and nine males with grafts surviving 10, 3 × 12, 15, 18, 20, and 2 × 21 days tested for chimerism.

males) which had accepted these grafts for 200 days were rechallenged with a second A skin graft, this time from an adult donor. The fate of these grafts is summarized in Table V. Whereas rejection of the initial neonatal A strain skin graft was always followed by the destruction (usually acute) of the subsequent adult graft, even in situations in which the neonatal graft had survived for more than 70 days, this was not the case when the neonatal graft had been accepted. Thus 8 of 10 animals bearing neonatal A skin, retained both grafts in excellent condition (Table V).

Survival of Neonatal Strain A Skin Grafts on B6 Mice which had Rejected Adult Strain A Grafts. Not only are neonatal skin grafts frequently able to induce unresponsiveness of adult grafts on normal hosts but they are sometimes able to render sensitized animals unresponsive of such grafts (10, 11). To determine if this phenomenon might occur in the present situation, 15 animals which had rejected adult skin grafts after being exposed to 400 or 500 R and (A × B6) cells at birth were rechallenged with neonatal skin grafts 30–68 days after receiving the adult grafts. Although these neonatal grafts were almost always rejected and frequently in accelerated fashion (compare their survivals in Table VI with the survival of neonatal grafts shown in Table III), they persisted on four recipients. One animal died after the neonatal graft had been on for 40 days, but the other three recipients maintained the neonatal graft for 100 days at which time they received a second adult A-strain skin graft. After this procedure, two of the hosts promptly rejected the neonatal graft and subse-
BEHAVIOR OF sk-INCOMPATIBLE SKIN GRAFTS ON TOLERANT MICE

TABLE V

Survival of Adult Strain A Skin Grafts on B6 Mice which had been Exposed 200 Days Previously to Neonatal Strain A Skin Grafts after Receiving 500 R and 20 Million (A × B6)F1, Lymphoid Cells at Birth

| No. of animals | Sex | Graft survival times adult graft / graft survival times neonatal graft |
|----------------|-----|---------------------------------------------------------------------------------------------------|
| 11             | ff  | 10/15, 9/16, 10/26, 15/64, 9/71, 9/85, 10/105, 10/106, 96/262, 2 × >100/>300                      |
| 12             | mm  | 18/15, 13/39, 15/54, 13/80, 12/93, 20/221, 6 x >100/>300                                      |
| 23             | ff and mm | 10/15, 18/15, 9/16, 10/26, 11/39, 2 × 15/54, 9/71, 13/80, 9/85, 12/93, 10/105, 10/106, 20/221, 96/262, 8 x >100/>300 |

Importance of the Persistence of the Neonatal Skin Graft in Maintaining Unresponsiveness of the Adult Graft. The persistence of tolerance apparently depends upon the persistence of antigen (14). It follows that if neonatal skin grafts can induce tolerance to Sk, then the removal of these neonatal grafts before challenge with adult skin should terminate the unresponsive state (see reference 4). Indeed, unlike animals with persisting neonatal skin grafts, animals which rejected these grafts, even after long periods of time, always rejected subsequent adult A-strain grafts (in many instances acutely, see Table V). To ascertain the importance of the persistence of the neonatal grafts in rendering adult animals unresponsive of adult grafts, six neonatal grafts were removed after they had been in place on neonatally treated B6 males for 200 days. 50 days after this procedure the hosts were challenged with adult A-strain grafts. All of these adults grafts were rejected. Moreover, their survival times (20, 25, 35, 40, 43, and 47 days) compared favorably with the survival of similar grafts on male recipients which had not been exposed to neonatal skin (see Table II).

Survival of Adult B6 Skin Grafts on X-Irradiated A-Strain Mice Inoculated at Birth with (A × B6) Cells. The experiments reported above are all concerned with the behavior of A-strain skin grafts on B6 mice. However, it follows that if A-strain skin grafts possess Sk antigen absent in B6 mice, then B6 skin cells should possess an alternative allele at the relevant locus (assuming that the alternative is a productive and not a null allele) and express Sk antigen absent in A mice.

To determine the capacity of A-strain animals to react against this putative B6 antigen, the survivors of four litters of strain A mice which had been irradiated at birth with 500 R, and inoculated with (A × B6) lymphoid cells, were challenged with adult B6 skin grafts. In contrast to the response of similarly treated B6 animals to A-strain grafts, 8 of 10 survivors permanently accepted their B6 test grafts. Indeed, at no time during the 200 day observation period did the grafts display any signs of rejection. The two animals which rejected their B6 skin grafts did so in 29 and 71 days, respectively (Table VII). When these two rejectors were assayed for chimerism, B6 (H-2b) antigens could not be detected.
Table VI
Survival of Neonatal Strain A Skin Grafts on B6 Mice which had Rejected Adult Strain A Skin Grafts after being Irradiated and Inoculated at Birth with 20 Million (A × B6)F1 Lymphoid Cells

| X-ray dose | No. of animals | Sex | Interval between adult and neonatal grafts | Survival times adult graft/survival times neonatal graft |
|------------|----------------|-----|-------------------------------------------|-------------------------------------------------------|
|            |                |     | days                                      | days                                                  |
| 400 R      | 2              | ff  | 42                                       | 12/9, 13/9                                            |
| 500 R      | 1              | ff  | 30                                       | 13/11                                                 |
|            | 3              | mm  | 30                                       | 13/40,* 13/9, 13/12                                   |
|            | 1              | mm  | 40                                       | 16/12                                                 |
|            | 1              | ff  | 40                                       | 25/15                                                 |
|            | 2              | mm  | 42                                       | 23/100‡                                               |
|            | 1              | mm  | 56                                       | 41/11                                                 |
|            | 2              | mm  | 68                                       | 27/78, 30/100‡                                        |

* Animal died with graft intact.
† These animals were regrafted with a second adult strain A skin graft after the neonatal graft had survived for 100 days. The female rejected its neonatal graft 9 days after challenge with the adult graft and the second adult graft survived for 43 days. The male accepted the second adult A graft for more than 100 days and its neonatal graft also remained in excellent condition, surviving for >200 days.
§ This animal was regrafted with a second adult strain A skin graft after the neonatal graft had survived for 100 days but was undergoing rejection. The neonatal graft was destroyed at 112 days and the second adult A graft survived for 30 days.

Table VII
Survival of Adult B6 Skin Grafts on Strain A Mice Inoculated at Birth with 20 Million (A × B6)F1 Lymphoid Cells after Receiving 500 R

| No. of animals | Sex | Graft survival times |
|----------------|-----|----------------------|
|                |     | days                 |
| 3              | ff  | 29, 71, >200        |
| 7              | mm  | 7 × >200            |
| 10             | ff and mm | 29,* 71,* 8 × >200 |

* Tested for chimerism.

Discussion
Our results with neonatally treated mice are in accord with those of Boyse and his associates (4) with lethally irradiated and restored adult mice. Thus B6 mice X irradiated at birth and inoculated immediately thereafter with (A × B6) lymphoid cells are apparently rendered tolerant of the foreign antigens present on these cells, but not on A-strain skin, since the hosts reject A skin grafts even though they are persistent leukocyte chimeras. We conclude, therefore, that the prompt rejection of the A-skin grafts occurs not only because the hosts are naive with respect to A-strain Sk antigens, but also because the cells of the (A × B6) inoculum lose tolerance of these antigens as a result of their transfer to an
behavior of Sk-incompatible skin grafts on tolerant mice

environment in which the antigens are not expressed. Consequently the A skin grafts are most probably rejected by the combined action of radiation resistant host cells and of the descendants of the F, hybrid cells inoculated neonatally.

Other evidence indicating the existence of Sk-differentiation alloantigens is provided by the observation that the serum of (B6 × A) → B6 chimeras which have rejected strain A skin grafts, is cytotoxic for dispersed strain A epidermal cells but not strain A lymphoid cells (15). Moreover, leukocytes from these tolerant mice are stimulated in mixed cultures by strain A epidermal cells but not by strain A leukocytes (16). The antigens have been designated Sk.1 and Sk.2 in strains A and B6, respectively (4). More recently evidence has been obtained that in B6 and A mice two independently segregating loci determine expression of Sk antigens (Wachtel and Boyse, unpublished data). Preliminary data indicate that incompatibility with regard to the Sk antigens determined by both these loci in A-strain mice results in more rapid rejection of A skin grafts by (B6 × A) → (B6 × A) × B6 backcross chimeras than incompatibility with regard to either of these antigens alone.

Since, in contrast to the destruction of all adult strain A skin grafts by B6 animals rendered chimeric at birth, only 2 of 10 adult B6 skin grafts were rejected by similarly treated A newborn mice, we assume that the Sk.2 antigens are not as strong in A animals as Sk.1 antigens are in B6 mice. In fact, the two strain A animals which eventually rejected their B6 skin grafts evidently did so not because they were able to react specifically against the Sk antigens present in their B6 skin grafts, but rather because of a loss of chimerism which permitted them to regain competence with respect to all foreign antigens present in the graft (14). This weaker response on the part of neonatally treated A-strain animals against B6 skin has also been reported in lethally irradiated adult A-strain mice restored with (B6 × A) marrow and spleen cells. In this situation, however, the immunologic response of approximately 50% of the A animals which eventually rejected their B6 skin grafts appears to have been directed solely against Sk antigens of the graft (3).

The uniformity with which B6 mice irradiated at birth and inoculated with (A × B6) lymphoid cells reject adult A-strain skin grafts, contrasts with the frequent acceptance of neonatal A skin grafts by similarly treated hosts. Moreover, the persistence of these grafts often results in acceptance of subsequent adult A skin grafts. This ability of neonatal A-strain skin to induce unresponsiveness of adult A-strain skin in chimeric B6 mice, provides further evidence that the rejection of the adult grafts by similar hosts not previously exposed to newborn skin, is due to their capacity to respond to Sk antigens. It also indicates that Sk antigens behave like other weak transplantation antigens since these too may induce unresponsiveness when presented in newborn skin (10, 11). This similarity includes the infrequent capacity of neonatal skin grafts to transform sensitized hosts into unresponsive animals (10, 11).

The fact that neonatal skin grafts may induce unresponsiveness of Sk antigens is advantageous in studies concerned with the relationship between persistence of antigen and the form in which it persists, and the maintenance of the unresponsive state. In this case, the tolerance maintaining antigens are confined to the graft itself and so removal of the graft allows one to eliminate the
source of persisting antigen. Thus, whereas animals exposed to neonatal grafts for 200 days are usually unresponsive to adult skin grafts, removal of the neonatal grafts for 50 days before challenge with adult skin is sufficient to break tolerance. These results are similar to those obtained in studies with H-Y antigen (10) and provide a useful basis for determining the factors responsible for the maintenance of unresponsiveness to Sk antigens.

Steinmüller and Lofgreen (17) also present evidence that A and B6 mice differ with respect to at least one Sk antigen and that this can function as a histocompatibility antigen. They report that (B6 × A) → B6 radiation chimeras accept neonatal A-strain heart tissue but not neonatal A-strain skin. This difference in the survival of neonatal skin grafts (compared with our findings) is probably due to the fact that they used skin grafts only 2 mm in diameter. Finally, our observation that adult and neonatal A-strain skin grafts persist longer on treated males than on treated females, agrees with several other reports in which, under a variety of conditions, females display greater immunological reactivity than males (11).

Summary

It has been reported that lethally irradiated adult B6 mice restored with (A × B6) hemopoietic cells subsequently reject adult A-strain skin grafts because they are not tolerant of A-strain skin-specific (Sk) antigens. This thesis has been confirmed by a series of experiments conducted on neonatally treated animals. Thus sublethally irradiated neonatal B6 mice, inoculated with (A × B6) lymphoid cells, permanently accept these cells while subsequently rejecting adult strain A skin grafts. Further evidence that the destruction of these grafts results from the reaction of the host to Sk antigens, is provided by the fact that similarly treated recipients often permanently accept neonatal A-strain skin. Such grafts usually induce tolerance of adult A-strain skin grafts.

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References

1. Billingham, R. E., L. Brent, and P. B. Medawar. 1956. Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 239:357.
2. Billingham, R. E., and W. K. Silvers. 1962. Some factors that determine the ability of cellular inocula to induce tolerance of tissue homografts. J. Cell. Comp. Physiol. 60(Suppl. 1):185.
3. Boyse, E. A., E. M. Lance, E. A. Carswell, S. Cooper, and L. J. Old. 1970. Rejection of skin allografts by radiation chimeras: selective gene action in the specification of cell surface structure. Nature (Lond.). 277:901.
4. Boyse, E. A., E. A. Carswell, M. P. Scheid, and L. J. Old. 1973. Tolerance of Sk-incompatible skin grafts. Nature (Lond.). 244:441.
5. Billingham, R. E. 1961. The induction of tolerance of homologous tissue grafts. In Transplantation of Tissues and Cells. R. E. Billingham and W. K. Silvers, editors. The Wistar Institute Press, Philadelphia, Pa. 87.
6. Billingham, R. E. 1961. Free skin grafting in mammals. In Transplantation of Tissues and Cells. R. E. Billingham and W. K. Silvers, editors. The Wistar Institute Press, Philadelphia, Pa. 1.
7. Gorer, P. A., and Z. B. Mikulska. 1954. The antibody response to tumor inoculation. Improved methods of antibody detection. *Cancer Res.* 14:651.
8. Boyse, E. A., L. J. Old, and I. Chouroulinkov. 1964. Cytotoxic test for demonstration of mouse antibody. *Methods Med. Res.* 10:39.
9. Litchfield, J. T. 1949. A method for rapid graphic solution of time-per cent effect curves. *J. Pharmacol. Exp. Ther.* 97:399.
10. Silvers, W. K. 1968. Studies on the induction of tolerance of the H-Y antigen in mice with neonatal skin grafts. *J. Exp. Med.* 128:69.
11. Wachtel, S. S., and W. K. Silvers. 1971. Skin homografts: tolerogenic versus immunogenic influences in mice. *J. Exp. Med.* 133:921.
12. Wachtel, S. S., and W. K. Silvers. 1972. The role of passenger leukocytes in the anomolous survival of neonatal skin grafts in mice. *J. Exp. Med.* 135:388.
13. Billingham, R. E., and W. K. Silvers. 1960. Studies on tolerance of the Y chromosome antigen in mice. *J. Immunol.* 85:14.
14. Lubaroff, D. L., and W. K. Silvers. 1973. The importance of chimerism in maintaining tolerance of skin allografts in mice. *J. Immunol.* 111:65.
15. Scheid, M., E. A. Boyse, E. A. Carswell, and L. J. Old. 1972. Serologically demonstrable alloantigens of mouse epidermal cells. *J. Exp. Med.* 135:938.
16. Gillette, R. W., S. Cooper, and E. M. Lance. 1972. The reactivity of murine lymphocytes to epidermal cells. *Immunology.* 23:769.
17. Steinmuller, D., and J. S. Lofgreen. 1974. Differential survival of skin and heart allografts in radiation chimeras provides further evidence for Sk histocompatibility antigen. *Nature (Lond.)*. 248:796.