Immunization with Skin Isografts Taken from Tolerant Mice

Abstract. *Skin isografts from mice that were immunologically tolerant to allogeneic tissue had the ability to immunize isogeneic recipients against subsequent skin allografts. The immunizing isografts showed no gross signs of rejection themselves and appeared to be only the vehicles for transplantation antigen. It seems likely that allogeneic leukocytes derived from the spleen and bone marrow cells used to confer tolerance were contained in the skin of the tolerant mice and were transferred by the skin isografts in sufficient numbers to stimulate transplantation immunity.*

Skin isografts are not expected to stimulate transplantation immunity because their donors and recipients are genetically identical. However, my study shows that skin isografts from mice that were immunologically tolerant to allogeneic tissue had the ability to immunize isogeneic recipients against subsequent skin allografts. The immunizing isografts themselves showed no gross signs of rejection and appeared to be only the vehicles for transplantation antigen. The tolerant isograft donors were leukocyte chimeras because they had been inoculated with allogeneic bone marrow or spleen cells to make them tolerant. Allogeneic leukocytes derived from these inocula that confer tolerance probably were contained in the skin of the tolerant mice and were transferred by the skin isografts in sufficient numbers to stimulate transplantation immunity.

I obtained immunization with skin isografts from mice made tolerant by the inoculation of allogeneic spleen cells at birth, as well as from tolerant "radiation chimeras" produced by irradiating adult mice lethally and then inoculating them with allogeneic bone marrow cells. Positive results were obtained in about 75 percent of the cases in all experiments in which isografts were transplanted to tolerant mice, left in place 20 to 30 days, and then removed and transplanted again to normal secondary recipients. However, in four of 16 cases (25 percent), I was able to immunize mice with isografts that were prepared directly from the undisturbed skin of tolerant mice. Ear skin was used in two of these four cases, suggesting that the acquisition of immunizing ability was not limited to any particular area of body skin.

Good immunization has been obtained consistently in experiments similar to that outlined in Fig. 1. Strain-A mice were inoculated intravenously within 24 hours of birth with 10 million (A × C3H)F1 hybrid spleen cells. This results in the production of a high degree of tolerance to C3H skin allografts in 80 percent or more of the recipients without any of the complications of graft-against-host disease which usually follows the inoculation of parental strain cells (*1*). About 60 days later, two belly skin grafts, a C3H allograft on one side and a strain-A isograft on the other, were transplanted by the technique of Billingham and Medawar (*2*) to the chests of the now mature, presumably tolerant recipients.

As a control, strain-A skin isografts were also transplanted to a group of nontolerant A mice of comparable age. Thirty days later, the immunologically tolerant mice were identified by their full acceptance of the C3H allografts. The "tolerated" C3H allografts and the strain-A isografts were then removed and separately transplanted again to

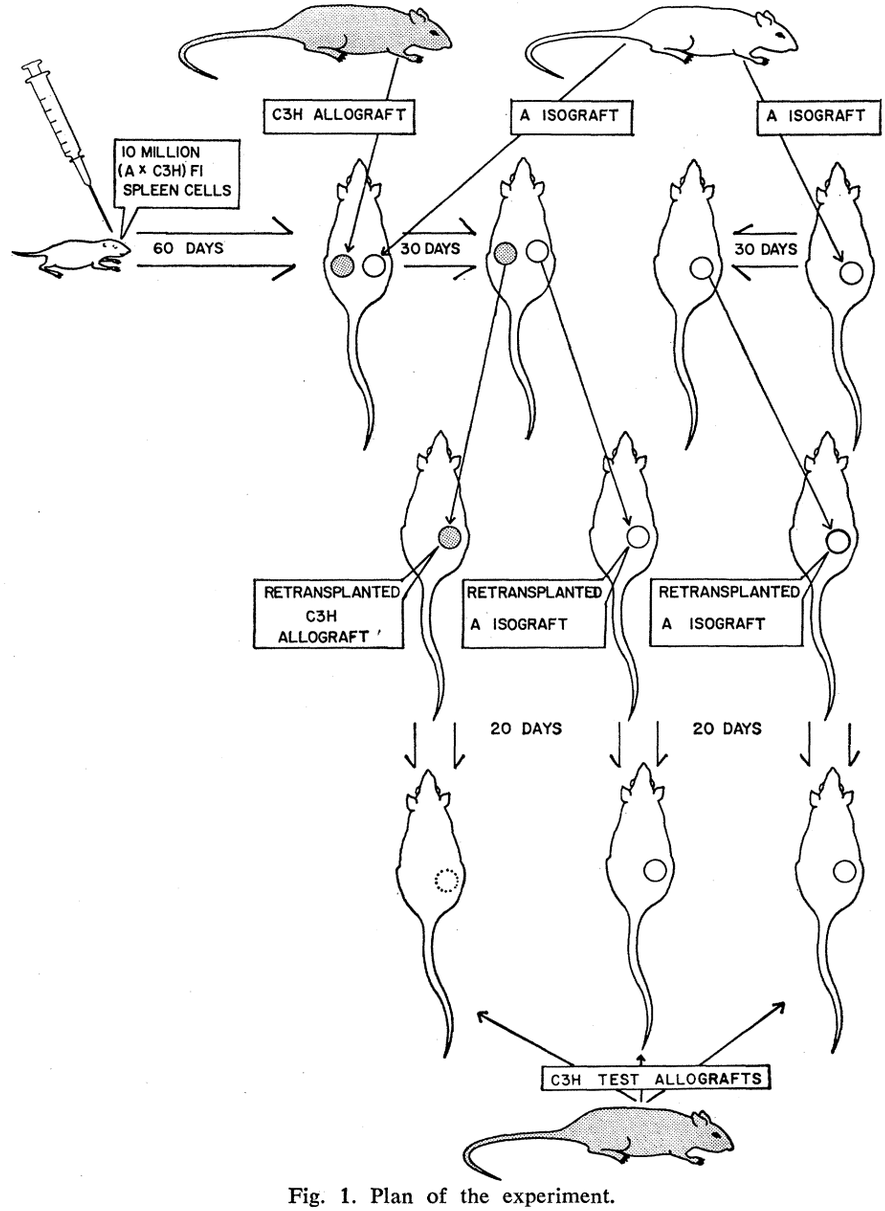

Fig. 1. Plan of the experiment.

Table 1. Survival of C3H test skin allografts on A mice immunized by skin grafts from A mice tolerant to C3H, MST, median survival time ± 95-percent confidence limits; SD, standard deviation.

Immunizing graft	Strain A recipients (No.)	C3H test allografts	
		MST (days)	SD
C3H allograft (tolerant donor)	21	6.3 ± 0.7	1.3
A isograft (tolerant donor)	22	6.8 ± 0.8	1.3
A isograft (nontolerant donor)	18	11.6 ± 0.7	1.1

two groups of nontolerant secondary recipients, represented by the third row in the figure. The isografts were also removed from the control group and similarly transplanted again to a third group of nontolerant A recipients. In the first group, the retransplanted allografts were destroyed within 15 days. In contrast, the retransplanted isografts in both the other groups were accepted, and they flourished. At 20 days after transplantation, all three groups of secondary recipients were challenged with C3H skin allografts. Dressings were removed 6 days later, and the survival times of the test allografts were estimated by gross observation daily.

The results of such an experiment are given in Table 1. The three rows refer to the three groups of secondary recipients in Fig. 1. The nature of the retransplanted graft is shown on the left; the median survival time and standard deviation of the C3H test grafts, as computed by Litchfield's nomographic method (*3*), are given on the right. It is no surprise that mice that have recently rejected a skin allograft reject a second one of the same specificity in the accelerated manner indicative of a preexisting state of transplantation immunity (*4*). However, one would not expect mice bearing isografts to react in this manner. Nevertheless, the recipients of the isografts from the tolerant donors were also obviously immune. In contrast, the mice bearing skin isografts from the control group of nontolerant donors were not immune. Clearly, the tolerant state of the isograft donors was responsible for the accelerated rejection of the test grafts in the second group.

Another experiment eliminated the tolerated skin allografts as a possible source of antigen for the immunizing isografts. Strain-A mice were injected at birth with numbers of (A × C3H) F1 spleen cells known to produce a high degree of tolerance. As adults, they were given only skin isografts from strain A, and these were removed a month later and again transplanted to normal secondary A recipients which were subsequently tested for immunity to C3H allografts. In 10 of 14 cases, immunization was obtained just as with isografts retransplanted from mice whose tolerance had been confirmed by their acceptance of C3H skin allografts. In this experiment, the inoculum that confers tolerance was the only possible source of allogeneic antigen in the isograft donors.

Mice made tolerant by inoculations of living allogeneic bone marrow or spleen cells are usually leukocyte chimeras (*5*). High percentages of donor-type cells are usually detected in the blood and lymphoid tissues of tolerant mice in parent-to-parent combinations. In contrast, very few donor-type cells are usually found in F1 hybrid-to-parent combinations (*5*). As all my mice were made tolerant with F1 hybrid cells to avoid graft-against-host disease, there was some question as to their chimerism. Accordingly, the spleens and lymph nodes from four of the tolerant A mice were tested for their ability to immunize normal A recipients against C3H skin allografts. No quantitative assays were made, but enough (A × C3H)F1 cells were apparently present to immunize the recipients on a one-to-one donor-recipient basis in all four cases.

If it is known that the leukocytes of the tolerant mice were indeed a mixture of autochthonous and allogeneic cells, the simplest explanation for the immunizing ability of the skin isografts is that they contained allogeneic leukocytes which could be released and could stimulate immunity without prejudicing the survival of the isogeneic skin grafts which carried them into their new hosts. The 75-percent incidence of immunization obtained with the retransplanted isografts, as opposed to the 25 percent obtained with the first-passage isografts, can be explained by the relatively greater number of leukocytes in the recently traumatized site of a skin isograft. I have compared histologic sections of isografts and normal body skin of the same mice at several intervals after grafting. Because of problems of cell identification and sampling, it is difficult to make precise quantitative comparisons. However, there clearly are more identifiable leukocytes in the isografts even 30 days after grafting than in areas of normal skin of the same size. Moreover, enough leukocytes are apparently present in both cases to account for the immunization. A few hundred thousand allogeneic leukocytes can stimulate transplantation immunity by the intraperitoneal route (*6, 7*), and even fewer might do so if released from an orthotopic skin graft. Billingham *et al.* (*7*) showed that an allogeneic "micro skin graft" containing fewer, perhaps, than 50,000 viable cells stimulates an immunity which is still maximum after 30 days.

Although the hypothesis of allogeneic leukocyte containment is the simplest explanation for the immunizing ability of skin isografts from tolerant mice, other explanations are possible. Subcellular transplantation antigen might be released from circulating allogeneic cells into the blood or tissue fluid and absorbed by the skin, particularly in an area of chronic inflammation. Mannick *et al.* (*8*) detected transplantation antigen in tissue-culture media in which rabbit spleen cells had been cultivated. Moreover, Hellmann and Duke (*9*) claim to have immunized mice with skin isografts which had previously been incubated in vitro with skin allografts or with media that had contained skin allografts. Guttmann *et al.* and Burrows *et al.* (*10*) have described the rejection of mouse skin isografts and autografts which had been soaked in allogeneic RNA. They suggested that the mechanism of this rejection is an antigenic transformation induced by RNA. In their experiments, as well as in those by Hellmann and Duke, isografts behaved like allografts and were themselves rejected. In my experiments, however, the immunizing isografts were accepted and were only the vehicles for transplantation antigen.

In addition to uptake of subcellular antigen and antigenic transformation, a third explanation might be based on the presence of stem cells in the inocu-

la that confer tolerance; these cells could give rise to components of the skin itself, in addition to blood cells. In the older literature, the simultaneous origin of vascular endothelium and blood from the same mesenchymal elements in the embryo is emphasized, suggesting that, even in the adult, blood-forming tissues may on occasion give rise to epithelial or connective tissue cells (*11*). Dunn observed the development of a stratified, squamous epithelium on the surface of skin wounds is rats when the wounds were covered by thymus tissue, and suggested that this epithelium was derived from reticulum cells of the thymus (*12*). Furthermore, Andrew (*13*) believes that lymphocytes can transform into epithelial cells in the intestine and the skin. According to the stem-cell hypothesis, the greater immunizing ability of retransplanted skin isografts as opposed to first-passage isografts would follow from their greater proportion of allogeneic components due to the extensive revascularization and regenerative hyperplasia that invariably accompanies the union of a skin graft with its host. In both cases, the proportion of allogeneic components would have to be large enough to immunize, yet small enough so that its destruction would not result in substantial damage to the graft as a whole.

As yet, there is no decisive evidence for any of these alternatives. If the leukocyte containment hypothesis is correct, it raises the question of the extent to which the immunizing ability of skin grafts in general is dependent on contained leukocytes.

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Actinomycin D Effect on Amino Acid Absorption from Rat Jejunal Loops

Abstract. *The absorption of amino acids from jejunal loops was suppressed in anesthetized rats treated previously with 1.0 or 1.5 micrograms of actinomycin D per gram of body weight. The absorption of the acidic, neutral, and basic amino acids showed selective differences in response that were relative to the degree of inhibition and to the time interval required to demonstrate actinomycin sensitivity.*

Inhibitors of RNA formation and protein synthesis affect the membrane transport of biological materials (*1*). Piperno and Oxender (*2*) reported results suggesting the necessity of a specific binding protein in the uptake of branched-chain amino acids by *Escherichia coli*. We have studied the effect of actinomycin D on the absorption of amino acids from the intestine.

Jejunal loops were prepared in 200-g male rats (Sprague-Dawley) according to the procedure of Delhumeau *et al.* (*3*). The rats were randomly allotted to the various groups for treatment and were injected intraperitoneally with actinomycin D (1.0 or 1.5 μg per gram of body weight) 2, 4, or 8 hours before preparation of the jejunal loop. Some animals were not treated with actinomycin and served as controls. Experiments were also performed on animals injected with saline. All animals were fasted for 30 hours and then anesthetized with sodium pentobarbital.

Two loops were made in the upper part of the jejunum of each animal; 1 ml of a solution of amino acids (total of 90 μmole) simulating the composition of casein with 0.05 percent glucose added was placed in one loop, and 1 ml of 0.05 percent glucose solution was placed in the other as a blank. After 15 minutes, the loops were removed and washed several times with citrate buffer (*p*H 2.2). The combined washings for each loop were analyzed for amino acid content by ion exchange chromatography (*4*). The blank loops all contained very minute quantities of the amino acids (from 0.01 to 0.29 μmole), and these amounts were disregarded in the calculations.

In some experiments a dose of 1.5 μg per gram of body weight was required for actinomycin to reduce amino acid absorption; in others, a dose of 1.0 μg/g was sufficient (Table 1). In the experiment reported in Table 1, inhibition of absorption was obtained with 1.0 μg of actinomycin per gram of body weight. Compared to controls, rats that received actinomycin 2 hours before the loop operation had no significant decreases in the absorption of amino acids, except proline. In animals 4 hours after injection of actinomycin, the percentages of absorption for all acidic and neutral amino acids were significantly less than those of the controls. For the same time interval, the absorption of the basic amino acids appeared to be slightly decreased, but not significantly. In animals injected with actinomycin 8 hours before the loop operation, the absorption of the basic amino acids further decreased, and the absorption of some, notably lysine, arginine, and tryptophan, was significantly reduced relative to that in control rats. In experiments carried out on animals injected with saline instead of actinomycin, the amounts of amino acids that were absorbed were similar to those of control rats in all instances, and the data were not included in the table.

In addition to differences in the length of time necessary for actinomycin to influence the absorption of the various amino acid groups, treatment with the antibiotic appeared to elicit differences in the degree of inhibition. Absorption of the acidic amino acids was inhibited to the greatest extent by actinomycin administration. Among the neutral amino acids, the antibiotic treatment inhibited the absorption of some more than it did of others. The amino acids in these two subgroups paralleled the amino acids that Oxender and Christensen (*5*) postulated were absorbed at the