Allografts in Genetically Defined Rats: Difference in Survival between Kidney and Skin

Abstract. *Although skin allografts from inbred donors of the Fisher strain to inbred male Lewis recipients regularly show acute rejection within 12 days, orthotopic kidney allografts between untreated animals, in this same combination of strains, usually remain functionally intact for longer than 100 days. Since such renal allografts persist despite previous or concomitant rejection of skin allografts, neither acquired tolerance nor nonspecific immunosuppression can explain the surprisingly prolonged kidney survival. Many factors appear to be responsible for the disparate survival times observed. Tentatively, these factors are (i) antigenic differences between kidney and skin, (ii) intervention of immunological enhancement, and (iii) physiological differences in vulnerability between kidney and skin.*

Prolonged survival of kidney allografts among normal unrelated recipients is infrequent; it appears to depend primarily on matching of multiple histocompatibility antigens between donor and recipient (*1*). Histocompatibility typing has been variably successful in several species by diverse methods. Among rodents, typing by skin grafting, tumor induction or suppression, hemagglutinating and cytotoxic antibodies, and graft-versus-host reactions (GVHR) have received the most attention (see *2*).

With respect to "strength" of differences in histocompatibility, some inconsistencies appear when results of skin allografts are compared with those of organ grafts or with GVHR (*3*). Skin is acutely rejected in certain strain combinations of rats after 1 or 2 weeks; such prompt reactivity has been assumed to be characteristic of strong histocompatibility barriers. No GVHR occurs, however, between Lewis and Fisher rats over a considerable variation in dosage of immunocompetent donor cells, even though skin grafted between these two strains is rejected, with median survival times of 9.0 to 9.8 days (*3, 4*). Lewis and Fisher rats evidently share the same allele at the major *H-1* (*Ag-B*) histocompatibility locus, but differ with respect to several other histocompatibility genes that may have cumulative effects insofar as skin allograft incompatibility and mixed leukocyte reactions are concerned (*5*).

Renal allografts, made across strong histocompatibility barriers in inbred strains of rats, also reportedly provoke acute rejection (*6*). One may then ask whether renal transplantation in connection with weaker incompatibilities leads to a substantially different sequence of physiological and morphological events. This is what we have found, for renal allografts between otherwise normal adult Fisher and Lewis rats regularly show prolonged survival, even with concomitant rejection of Fisher-skin allografts. Another remarkable feature of the persistent survival of kidney allografts in this strain combination is its resemblance to that found in humans receiving sufficient immunosuppression to curtail reactions to "weaker" donor antigens (*7*).

Male Lewis and Fisher rats, 3 to 6 months old and weighing 200 to 350 g, were purchased from Microbiological Associates, Inc., Walkersville, Maryland; eight of them were initially used for determination of responses to isografts and allografts of skin. Each Fisher rat provided one skin graft for exchange within the Fisher group, and one allograft for a Lewis recipient. Each Lewis rat similarly provided one skin graft for reciprocal exchange within the Lewis group, and each received an allograft of Fisher skin. Eight other Lewis rats received single allografts of Fisher skin; animals in this category totaled 16.

All skin grafts were 1.0 to 1.5 cm in diameter and were sutured in place orthotopically with 7-0 chromic gut over the panniculus carnosus of the dorsal aspect of the rib cage. Plaster or tape bandages were removed on the 8th day, and the grafts were then scored daily until rejection was complete, or for at least 100 days with compatible isografts. Each original Fisher donor of a skin allograft was retained for later donation of a kidney to the same Lewis recipient.

Renal grafting was performed by a reported technique (*8*), with certain modifications, the most significant of which involved double nephrectomy of each recipient—removal of both host kidneys at the time of transplantation. Ischemia time of most kidneys was about 1 hour; after perfusion with cold heparinized isotonic saline and removal from the donor, each kidney was kept cold until the recipient was ready to receive it. Lewis recipients were divided into three groups of nine animals each. Each rat in group 1 received a Lewis-kidney isograft, and at varying intervals thereafter eight received Fisher-skin allografts. Each animal in group 2 received only a Fisher-kidney allograft. Each rat in group 3 received a Fisher-skin allograft and a Fisher-kidney allograft in two combinations: (i) two received kidney and skin concurrently; and (ii) seven received kidney and, at intervals thereafter, first-set skin grafts. After rejection of the skin allografts, all received second-set grafts of Fisher skin.

In both Fisher-to-Fisher and Lewis-to-Lewis combinations of skin isografts, the grafts healed well and remained fully viable indefinitely (>100 days). Sixteen Fisher-skin allografts were rejected by Lewis recipients with a median survival time of 10.2 (± 1.2) days (a range of 8 to 12 days, with 95 percent confidence limits indicated in parentheses). Visible onset of rejection, manifested by edema and inflammation or partial scabbing (or both), occured after a median time of 8.2 days. Lewis-to-Lewis renal isografts in group 1 have survived, with no apparent loss of function, longer than 100 days so far. However, Fisher-skin allografts in this group were rejected within 8 to 15 days, with a mean survival time of 10.1 (± 1.1) days. In sharp contrast, Fisher-to-Lewis renal allografts in group 2 uniformly show persistent survival now ranging from >80 to >100 days.

The group 3 results, involving Fisher-to-Lewis skin and renal allografts, are summarized in Table 1. The skin allografts were rejected with a mean survival time of 14.0 (± 2.2) days. After brief convalescence, animals in all groups (apart from one death in group 3) have remained healthy and alert. Very prolonged survival of kidney allografts then is the rule, even in the face of immunity sufficient to cause acute rejection of skin allografts.

Multiple criteria for sequential assessment of the functional integrity of the transplanted kidneys are still under evaluation. In addition to the overriding consideration of survival times of the recipients, the criteria include changes in body weight, urine volume and specific gravity, blood and protein contents

Table 1. Histories of kidney and skin allografts in rats; Fisher donors to Lewis recipients (group 3). Total time of survival after renal grafts, for all animals except (*), longer than 100 days. When the skin graft followed the kidney, the time of neither onset nor completion of rejection of the skin graft depended on the interval between the two grafts over the range 16 to 43 days.

Rats (No.)	Order of grafts	Rejection of skin (days later)		Days of survival after first-set skin graft
		Onset	Completion	
2	Skin and kidney concurrently	9, 12	15, 15	> 100, > 100
7	Skin 16 to 43 days after kidney	10, 10, 16, 16, 18, 10, 15*	11, 11, 23, 22, 25, 12, 19*	> 84, > 78, > 72, > 70, > 65, > 57, 30*

* Died 30 days after receiving the skin graft.

of urine, and blood urea nitrogen. Generally within 1 to 2 weeks after kidney grafting, these parameters all returned to normal or near-normal values. The allografted animals, groups 2 and 3, appeared to be somewhat slower in returning to normal.

Punch-type renal biopsies were taken from many animals for histological evaluation, and whole kidneys from animals with similar isografts and allografts were removed for study of histopathological changes. In renal specimens taken 3 to 50 days after transplantation, a very mild lymphocytic infiltration, diffuse or local (or both), appeared in some kidneys. The frequency and intensity of this cellular infiltration were greater in Fisher-to-Lewis renal allografts, group 2, than in Lewis-to-Lewis isografts, group 1.

Primary rejection of Fisher skin by Lewis rats occurred with a median survival time of 10.2 (± 1.2) days; an earlier published result was 9.0 (± 0.8) days (*3*). This slight difference may reflect weaker male-to-male reactions as well as differences in scoring criteria. The *H-1* (*Ag-B*) locus compatibility of the Fisher and Lewis strains appears sufficient to allow prolonged survival of kidney but not skin allografts. Elkins and Palm (*3*) could detect no humoral antibodies resulting from grafting of skin or injection of cells reciprocally in either strain. Lewis and Fisher erythrocytes also reacted identically with antiserums prepared in other strains of rats; moreover, red cells from either strain reciprocally absorbed all detectable hemagglutinating antibodies from various antiserums.

All available evidence indicates that (i) the serum antibody response to these weak antigens either is deficient or escapes detection by conventional serology, (ii) the weak antigens fail to react effectively because they are present in such low concentrations in (or even absent from) the indicator red cells, or (iii) these factors are combined.

The fact that group 1 Lewis recipients, bearing kidney isografts, have survived well beyond 100 days is consistent with the supposed isogenic constitution of this strain. Yet the persistent survival of rats sustained by Fisher-kidney allografts (group 2) beyond 100 days was surprising—this despite a rejection time of only 8 to 12 days for skin allografts in the same combination. The animals grafted at differing intervals with both skin and kidney (group 3) served to clarify this disparity in the reactions to allografts of either skin or kidney alone. In general, the rejection times of skin allografts in group 3 were delayed by several days relative to the controls which received skin allografts alone. In fact the skin-allograft survival times in groups 1 and 3 were sufficiently similar to indicate that the stress of kidney grafting as such is only slightly immunosuppressive. The uniformly acute or subacute rejection of skin allografts in all these experiments essentially rules out the possibility that the prolonged survivals of kidney allografts could be attributed to specific induction of tolerance or to nonspecific immunosuppression.

Alternatively one must consider that skin and kidney from the same donor source nevertheless differ in effective antigenicity and in vulnerability to the alloimmune responses of the host. The continuing survival of kidney allografts, long after skin from the same donors had failed completely (Table 1), indicates a marked difference in vulnerability. However, the failure of prior kidney transplantation to sensitize recipients for accelerated destruction of skin allografts in these experiments complicates interpretation. Indeed, the delayed rejection of skin grafts in about two-thirds of these recipients strongly suggests the intervention of immunological enhancement wherein graft survival is really promoted by specific IgG antibodies (*9*).

In other experiments still in progress, Lewis recipients preimmunized by rejection of Fisher-skin allografts also show surprisingly prolonged survival (> 50 days) after receiving later Fisher-kidney transplants. Unfortunately, no serological method is currently available for testing for antibodies to antigens other than H-1; however, this enhancement question may well prove to be testable in other combinations of strains.

The finding of prolonged survival of renal or ovarian allografts relative to skin grafts has been reported (*10*), although ours is the first evidence of substantial disparity in kidney and skin allografts in genetically defined animals under diverse conditions. Many investigators now accept the contention that skin is especially susceptible to immune rejection. The reasons for this susceptibility are not clear. Difference in the alloantigenic constitution of skin has yet to be discerned in relation to other tissues, but few studies of weaker histocompatibility antigens have been undertaken in this connection. Also, the known cumulative effects of multiple, weak histocompatibility differences in curtailing survival of skin allografts (*5*) may not apply in the light of our results to kidneys.

Multiple physiological factors, involving blood supply, lymphatic drainage, and reparative or regenerative capacity, may partially account for the marked differences in survival of kidney and skin allografts. Kidneys orthotopically transplanted, as in our study, certainly do not occupy an immunologically privileged environment. Acute rejection of such allografts is the rule in dogs and humans in the absence of immunosuppressive therapy. Thus the consistently prolonged survival of Fisher kidney, in contrast to skin allografts in otherwise untreated Lewis recipients must be assumed to reflect fundamental differences in the constitution of these two tissues as well as in their vulnerability to alloimmune reactions.

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Mice Unilaterally Sensitized for Audiogenic Seizures

Abstract. *Strain SJL/J mice exposed to loud bell-ringing (primed) with one ear blocked do not convulse, but are susceptible to audiogenic seizures 48 hours later when stimulated only through the ear open at priming. Mice stimulated through the ear blocked at priming do not convulse, but are convulsible when retested on the opposite ear. The site of sensitization appears to be either in the ear or in those portions of the auditory system receiving input only from one side.*

Henry (*1*) demonstrated that "seizure-resistant" C57BL/6J mice can be made highly susceptible to audiogenic seizures by exposing them to the sound of a bell during a sensitive period, which includes portions of the 2nd and 3rd weeks after birth. Similar dependence of the induction of audiogenic seizure upon prior sensitization has been found in SJL/J mice by Fuller and Collins (*2*). In this strain, convulsibility develops 30 to 36 hours after a priming exposure to bell-ringing at 3 weeks of age. Repeated exposures to bell-ringing at 6-hour or 12-hour intervals following priming, but not at 18-hour intervals, interfere with sensitization. Once sensitized, an SJL/J mouse remains convulsible for more than 20 weeks. Sensitization becomes progressively less predictable with age, and by 8 weeks it is demonstrable in 10 percent, or less, of subjects. Attempts in our laboratory to prevent sensitization following priming exposure to the bell or to induce sensitization by other procedures have been unsuccessful, although a wide variety of agents, including general anesthetics, anticonvulsant drugs, electroconvulsive shock, and food deprivation, have been used. The stability of the process in the presence of these diverse treatments led us to suspect that the site of sensitization might be relatively localized, possibly in the ear itself.

To test this possibility, mice were exposed to bell-ringing for the first time (priming exposure) with one ear blocked, and were tested for susceptibility to audiogenic seizure by a second exposure with either the ipsilateral or contralateral ear blocked. Failure to elicit convulsions when the contralateral ear was blocked would be evidence for a peripheral locus of sensitization. Forty-two SJL/J mice, 3 weeks old, from the production colony of the Jackson Laboratory (*3*) were exposed to the sound of an electric bell (sound level, approximately 95 db above 0.0002 dyne/cm^2) for 30 seconds, half with the right ear and half with the left ear blocked by flooding the external auditory canal with glycerine. This procedure, performed bilaterally, had been shown to protect against seizures in known convulsible mice and to prevent sensitization in 3-week-old mice exposed to a normally adequate sound stimulation. Immediately following priming the contralateral ear was also blocked to insure that both ears would have similar prior treatment at the time of testing.

Forty-eight hours after priming each mouse was exposed to the same bell-ringing for 60 seconds or until it convulsed. In half the mice, the same ear was blocked as at priming (group I, ipsilateral) and in the other half, the opposite ear (group C, contralateral). Twenty out of 22 mice in group I convulsed; one out of 20 in group C (chi square, 27.5; $P < .0001$). Motor patterns in all seizures were bilaterally symmetrical, and no correlation was observed between the direction of the running phase of the seizures and the ear that was blocked either at priming or at test.

A further demonstration of the unilateral nature of sensitization in these mice, and the dependence of convulsibility upon conditions at priming and not upon a history of previous convulsibility, was obtained by retesting all subjects 24 hours after the first test. At this time group I was subdivided into groups II and IC, with the second letter designating the blocked ear in relation to conditions at priming. Similarly group C was subdivided into groups CI and CC. The ratios of convulsions to numbers tested were: group II, 7:12; group CI, 9:10; and combined ipsilaterals, in second test, 16:22. In the combined contralateral groups IC and CC, during the second test, there was one convulsion in 20 mice tested (chi square, 17.2; $P < .0001$). Groups II and CC responded similarly on tests 1 and 2, except for a slight reduction of seizures in group II which we attribute to a postictal refractory state observed in other experiments. Groups IC and CI showed reversed susceptibility between tests, but in opposite directions.

The site of sensitization, therefore, resides either in the ear itself or in parts of the auditory system which receive input solely or chiefly from one side. Delimitation of the location of the process will permit better direction of research on the nature of sensitization. The possibility must be considered also that genetic differences among mouse strains in audiogenic seizure susceptibility are based upon variations in the areas within which sensitization has been demonstrated to occur.

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3. Standards of laboratory animal care as prescribed by the National Society for Medical Research are followed in this laboratory.

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