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Homotransplantation Immunity of Neonatal Rabbits.* (27278)

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The development of the immune response in neonatal animals has been the subject of many investigations(1). Most of these studies have dealt with the humoral antibody responses of neonates injected with soluble protein or bacterial antigens and have revealed little or no antibody formation. At the same time in the neonatal period, homograft immunity is apparently already developed, but detailed information concerning the degree of development is not available for most species. More precise knowledge of the status of homograft immunity in the neonatal rabbit is of importance in interpreting experiments in which the immunologic behavior of adult homologous lymphoid cells, transferred to neonatal recipients, has been observed. In some of these experiments, adult cells transferred to neonates have made little or no antibody (2-5). One possible explanation for this failure has been the homograft reaction by the neonatal recipient against the transferred cells(6).

It was the purpose of this study to determine the transplantation immunity of the neonatal rabbits as measured by rejection of skin homografts. In addition, the effect of prior irradiation on survival times of homologous skin grafts was also observed.

Method. Pregnant albino rabbits were obtained from several different breeders and after delivery the nursing neonates were used as donors and recipients of skin grafts. Neonatal skin grafts were not exchanged between litter mates. A total of 172 neonates were used in this study and were divided into 2 major groups for skin grafting. The first group of animals was grafted within the first week of life and ranged in age between 2 and 6 days. The second group consisted of animals 10 to 14 days old. One-half the members of each group of neonates received adult homologous skin, the other half received neonatal homologous skin.

Thirty-three adult non-pregnant rabbits weighing between 2.5 to 3.5 kg were obtained from the same breeders and served as both skin graft donors for the neonates and as control recipients for homologous adult and neonatal skin grafts. Approximately one-half of the neonatal and adult recipients received 400 r total body irradiation 48 hours prior to skin grafting(7).

Full thickness skin grafts were taken from the dorsum of the ear of all neonates. The adult donor skin grafts were full thickness grafts from the dorsum of the ear or split thickness grafts from the abdominal skin. Split thickness grafts were used in order to arrive at a more comparable physical quantity to neonatal skin. All the skin grafts were fitted and sutured to the dorsum of the ear of the recipients and measured 0.5 × 0.5 cm in neonates and 1 × 1 cm in adults. In approximately one-half of the recipient neonates, the skin removed to create the defect

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TABLE I. Survival Times of Homologous Skin Grafts on Neonatal and Adult Rabbits with and without Sublethal Irradiation.

Donors	Recipients	No irradiation		400 r total body irrad.	
		No. of animals	MST ± S.D.*	No. of animals	MST ± S.D.*
Neonate (2-6 days)	Neonate 2-6 days	35	6.6 ± 1.3	20	9.6 ± 1.3
Adult	" 2-6 "	22	5.4 ± 1.0	22	8.1 ± 1.2
Neonate (10-14 days)	" 10-14 "	18	8.7 ± 1.4	19	12.5 ± 3.6
Adult	" 10-14 "	14	8.2 ± 1.5	15	11.9 ± 2.8
Neonate (2-6 days)	Adult	13	8.1 ± 1.1	—	—
Adult	"	16	8.5 ± .9	14	17.8 ± 4.3

* Median survival time of complete graft breakdown ± stand. dev.

for grafting was applied to the opposite ear as an autograft control. No dressing was used over the skin grafts.

The survival of the skin grafts was determined by daily inspection. The gross visual scoring of the grafts was supported in approximately one-half the cases by biopsy and microscopic examination. Survival times of the grafts were determined by the same technic described by Billingham *et al.* with inbred mice and represented the time of complete graft breakdown(8).

Results. The median survival times of the skin homografts on neonatal and adult rabbits with and without prior sublethal irradiation are presented in the Table. The median survival time (MST) of homografts of neonatal skin on neonatal rabbits less than 6 days of age was 6.6 days as compared with an MST of 8.1 days for neonatal skin homografts on adult animals. The difference between the MSTs of these 2 groups when compared statistically was found to be significant ($P = .03$). When adult skin grafts were applied to neonates, an MST of 5.4 days was obtained as compared with an MST of 8.5 for adult grafts on adult recipients and the difference between these groups was highly significant ($P = .01$). In the older neonates (10 to 14 days of age), the MSTs of homologous 2-week-old neonatal and adult skin grafts were 8.7 and 8.2, respectively, and were not significantly different from the adult recipient group.

The effect of prior sublethal total body irradiation with 400 r was most marked in the adult rabbits, which showed approximately a 2-fold prolongation of the survival of homologous skin grafts with an MST of 17.8 days. In the neonatal rabbits, the effect of prior irradiation was less marked with MSTs of 9.6 and 8.1 for homologous neonate and adult skin grafts in the two- to six-day-old rabbits and MSTs of 12.5 and 11.9 for neonatal and adult skin grafts on 10- to 14-day-old rabbits.

Sixty-three autografts were applied as controls of the technic of neonatal skin grafting. All but 2 of these autografts survived and remained viable during the experimental period of observation (35 days).

Discussion. The results of these experiments indicate that homotransplantation immunity as measured by skin graft survival is at least as well developed in neonatal rabbits as in adults. It is interesting to note that the rejection time of neonatal skin homografts on neonates was significantly more prompt than that seen with neonatal homografts on adult recipients. The difference between the rejection time of adult skin homografts on neonates and adult homologous skin on adults was even more striking than neonatal skin grafts on neonate and adult rabbits.

The 2-week-old neonates were indistinguishable from adult animals, and thus accelerated rejection during the first week of life appeared to be a short lived phenomenon. Accelerated rejection in the neonatal rabbits was difficult to explain and differed from the situation in neonatal mice and rats which actually had slightly prolonged MSTs for homologous skin grafts(9). Immunologic maturity has been noted in rabbits during embryonic life(10) and tolerance to homologous tissues could rarely be accomplished after the 22nd day of gestation(11). On the other hand, tolerance has been induced in

mice(12) and rats(13) during the post natal period.

One possible explanation for the accelerated rejection seen in the neonate may be related to the increased cellular proliferation at this time which might result in a speed-up of the homograft reaction. This rapid cellular response of the neonate was also manifested by the rapidity of reepithelization of the graft bed during the slough. This process, in many instances, required less than 2 days and frequently seemed almost to eject the dying grafts.

The period of accelerated rejection (2 to 6 days of age) did not seem to be the immunologic "null period" described by Billingham(14), since 14 neonates which were rechallenged after 6 weeks with skin grafts from the original donor showed a heightened sensitivity as evidenced by accelerated skin graft rejection.

The temporary abrogation of homotransplantation immunity with sublethal irradiation as measured by skin graft survival has been reported(15). In the present study, there was a definite prolongation of the MST of skin homografts in adult recipients pretreated with 400 r total body irradiation. However, in the neonates, the effect of prior irradiation was not as marked as in adult recipients. The reason for this lesser sensitivity of neonates to irradiation might be explained by the ability of the neonate's cells to proliferate rapidly and afford a prompt recovery from such injury.

Finally, these results might shed some light on the apparent unfavorable environment provided by neonatal recipients for transferred adult lymphoid cells. The fact that the neonate was capable of rapid homograft rejection of solid grafts suggested that rapid rejection of passively transferred lymphoid cells might in some situations interfere with the immune response of the latter. This was particularly pertinent since the rejection time of adult skin grafts (5.4 days) closely parallels the time necessary for antibody production by the transferred cells. The validity of this premise has also been demonstrated in mice by Nossal(4) and by Mark(16) who showed adequate antibody formation following transfer of sensitized adult spleen cells to isologous neonates and no antibody production following transfer of sensitized cells to homologous neonates.

Summary. Neonatal rabbits in the first week of life were found to reject neonatal or adult grafts significantly more rapidly than adults grafted with homologous adult or neonatal skin. Transplantation immunity comparable to adult rabbits was observed in 2-week-old rabbits. Sublethal irradiation of recipient animals prior to skin grafting resulted in a 2-fold prolongation of graft survival in adults, but only a 50% prolongation in one-week- and two-week-old neonatal recipients.

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