• Development of Blood Irradiators

Extracorporeal irradiation of blood, using repeated brief exposures, has been shown to suppress rejection of tissue transplants and to inhibit progression of chronic lymphocytic leukemia. This project is designed to study the basic processes by which blood irradiation produces such effects, to establish the conditions of dose administration which optimize therapeutic effect, to improve the techniques of blood irradiation through the development of improved and portable blood irradiators, and to move this technique toward clinical applications.

A FULLY PORTABLE BLOOD IRRADIATOR

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A fully portable blood irradiator has been developed using the beta emitter thulium-170 as the radiation source and vitreous carbon as the body of the irradiator, matrix for isotope encapsulation and blood interface material. These units have been placed in exteriorized arteriovenous shunts in goats, sheep and dogs and the effects on circulating lymphocytes and on skin allograft retention times measured. The present work extends these studies by establishing baseline data for skin graft rejection times in untreated animals.

Dogs selected for reciprocal grafting were paired on the basis of having no common ancestor in the prior two generations. Following removal from the anesthetized dog, skin patches, uniformly 2 cm x 2 cm, were prepared by slicing with a sharp blade through the skin patch at the level of the hair follicle. In this way, there was minimal damage to the dermal and epidermal tissues transplanted. Skin patches were briefly held in normal saline prior to application to the lateral rib cage area of the host. Bleeding of small vessels in the transplant area was suppressed by applying a small amount of 1:50,000 epinephrine in saline and by clamping large vessels. Typically, two identical allografts were placed on each animal. In some dogs, an autograft was also applied, making three patches on each animal. Patches were sutured into place with 3-0 silk and covered with a gauze pad held in place by a bandage wrap around the animal. The gauze pad, which provided initial pressure to assist in initiating vascular growth, was removed a day or two after surgery; sutures were removed about 1 wk following graft placement. Animals received systemic antibiotics but no local treatment of the graft areas.

Table 72 gives the results of the skin graft tests. Dogs 1098 and 1075 were regrafted 1 mo following placement of the initial grafts to confirm that an immune reaction had been initiated, and to observe the course of a second rejection. It was clear that the second set of skin patches provoked immediate vascular congestion, so that rejection depended only on the time necessary for the skin to become distinctly necrotic. Animal numbers grouped by two in the table indicate that skin grafts were reciprocal between the pairs. While aseptic techniques were used, some of the patches (Dogs 1142, 1138, 1295) appeared to be infected, resulting in early loss. This was not confirmed by direct culture of the organisms.

The biopsy samples taken from dogs on day 7 (1137, 1156, 1159, 1169), on day 5 (1142, 1122, 1100, 1073), or on day 4 (1138,

TABLE 72. Full-Thickness Skin Grafting on Beagle Dogs

Dog Number	Allograft Rejection Time, Days	Autograft Retention Time, Days
1098	15	NA
1075	10	NA
1098 regraft	7	Take
1075 regraft	7	Take
1076	13	Take
1099	14	Take
1137	10, 11[a]	NA
1156	11, 11	NA
1159	10[a], 10	NA
1169	11, 11	NA
1073	15[a], 8	NA
1100	6[a], 14	Take
1142	6[a], 6	9
1122	9[a], 12	NA
1138	9[a], 9	4
1285	14[a], 14	Take
1259	14, 14	Take
1281	9, 9	9
1295	7, 7	7
1163	7, 11	Take
1665 (1161)	29, 28	NA

[a] Biopsies removed for histologic examination
NA - not applicable, no autograft was placed

1285, 1259, 1281) were not helpful in interpreting the rejection reaction. Overall, the extent of necrosis was moderately severe; mild neutrophilic cell infiltration and hemorrhage was apparent; and there was little evidence of fibrocytic proliferation. There was no clear distinction between the histology of the allografts as compared to the autografts.

Dog 1665 received a pair of skin patches from dog 1161 following 12 days of treatment with a vitreous carbon-thulium 170 (VCTm) irradiator that delivered a transit dose of 30 rad. The irradiator remained in place, continuing to give a daily dose to blood of about 4500 rad during the entire period. It is clear that rejection of the skin patches was delayed well beyond any of the times observed for previously treated animals. Lymphocyte levels in Dog 1665 were unusually low ($1.7 \times 10^3/mm^3$) prior to any treatment, and fell to only $0.5\text{-}0.7 \times 10^3/mm^3$ following continued treatment with the VCTm unit. Therefore, it would be difficult to conclude from the findings for this dog alone that prolonged retention resulted from the blood irradiation. However, this prolonged retention is consistent with earlier data reported for goats, a baboon and dogs.

The unit designed last year, which was used to hold the irradiator on dog 1665, is difficult to use because of limited accessibility to the irradiator and its connections to the shunt tubing. An alternative system, using a nylon jacket designed for beagle dogs (Alice Chatham, 5043 Oaknoll Ave., Los Angeles, CA) was used to stabilize the irradiator, which was fastened to the jacket dorsally, at the base of the neck. A thermoplastic cover surrounded the irradiator and the exposed shunt tubing. The shunt tubing was tunneled unde the skin from the cannulas leading from the carotid artery and the jugular vein to points on the midlateral surface of each side of the neck. While this approach offers many potential advantages, more tests will be needed to establish our ability to maintain prolonged flow through shunts routed in this way.

We anticipate augmenting our information about the immune reaction and the skin graft response by pretesting the animals for their mixed-lymphocyte response in order to assure that donor and recipient animals are immunologically dissimilar. The mixed-lymphocyte response can also be used to evaluate the extent of recovery in the cellular immune system associated with fractionated as compared to acute exposures.