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MODIFICATION OF SKIN ALLOGRAFT IMMUNITY BY EXTRACORPOREAL IRRADIATION OF LYMPH[1]

by

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DISCLAIMER

SUMMARY

Thoracic duct lymphocytes of calves were continuously destroyed by extracorporeal irradiation of the lymph (ECIL) for up to 68 days. Skin grafts which were placed within the drainage bed of the thoracic duct (posterior grafts) were compared to grafts from a second donor which were not drained exclusively by the thoracic duct (anterior grafts). Pregraft ECIL for 10 to 22 days produced lymphopenia and prolonged survival of anterior and posterior grafts for 8 - 10 days. When ECIL was continued after grafting, anterior grafts were rejected as before, but posterior grafts remained intact until ECIL was discontinued. Postgraft ECIL delayed and markedly suppressed the cytotoxic antibody activity in the serum although small amounts could be detected while grafts were intact. Following the termination of ECIL, the cytotoxic activity increased, however, at the time of rejection it was still well below that found in untreated calves.

INTRODUCTION

It is now widely believed that rejection of solid tissue allografts is mediated by immunologically "activated" cells of the lymphocytic series rather than by a humoral antibody[2,10]. Observations on adoptive transfer of allograft sensitivity have suggested that "activated" lymphoid cells are first found in the regional lymph nodes and blood, and later in other lymphoid tissue[1,11]. It may be hypothesized that in the process of skin allograft rejection, antigenic information is transported to the regional lymph nodes via the afferent lymphatics[8]. In the lymph node immunocompetent cells, presumably lymphocytes[6,9], proliferate and give rise to "activated" cells which enter the blood via the efferent lymphatics, migrate to the graft and effect its destruction. This hypothesis was, in part, examined experimentally in calves. Thoracic ducts of calves were cannulated and the lymphocytes destroyed by continuous extracorporeal irradiation of lymph (ECIL). Skin grafts placed within the drainage of the thoracic duct (posterior grafts) were compared to grafts from a second donor placed in an area not exclusively drained by the thoracic duct (anterior grafts). The results indicate that "activated" lymphocytes enter the blood via efferent lymphatics and that destruction of these cells will prevent graft rejection.

In conjunction with these studies, the cytotoxic antibody response was measured to determine the effect of postgraft ECIL on the production of circulating antibody, and to examine the relationship between cytotoxic activity of the serum and graft rejection in instances where rejection

was prolonged by ECIL.

MATERIALS AND METHODS

Animals. Holstein calves, weighing between 80 and 125 kg, were used.

Skin grafting. Multiple, full thickness pinch grafts[3], were removed from the dorsum of the ear and transplanted either in the area near the iliac crest (posterior grafts) or on the right side of the withers (anterior grafts). Anterior and posterior grafts were from different donors.

Thoracic duct cannulation, lymph collection and irradiation. The thoracic duct was cannulated using Teflon[2] cannulas and Silastic[3] tubing as previously described[4]. Lymph was collected continuously into glass bottles or plastic bags and pumped back into the jugular vein via a Silastic coil surrounding the irradiator[7] (Co^{60} or Cs^{137} gamma ray source). The dose of radiation to the lymphocytes as they passed through the radiation field was 450 to 1100 rad. The collection system was kept sterile and continuously heparinized.

Cytotoxic antibody. Cytotoxic antibody assays were performed by mixing 0.1 ml serum with 0.1 of Medium 199 containing 1×10^6 donor blood lymphocytes and 0.1 ml of guinea pig serum. After 30 min incubation at 38.5C, 0.2 ml of trypan blue (1:750) in saline was added and the per cent of stained cells determined. Sera having high cytotoxic activity were serially diluted and retested. The results were expressed as the number of donor cells killed per ml recipient serum. When cytotoxicity could not be demonstrated, even at the time of graft rejection, the sera were

retested using rabbit serum as the source of complement. This increased the sensitivity of the assay.

RESULTS

Continuous ECIL resulted in a marked reduction in both the blood lymphocyte count and the thoracic duct cell output. These changes are illustrated in Fig. 1(a) for Calf 206 which received 25 days of ECIL. Similar changes were observed in the other 8 calves given extended ECIL.

Untreated animals. The survival time of skin allografts in untreated calves was between 8 and 11 days[13] (Table I). Calf 203 received anterior and posterior grafts from different donors. Both sets were rejected within 10 days (Table I).

Continuous pregraft ECIL. Calf 186 received 22 days of ECIL followed by anterior grafting, and Calf 239 received 10 days of ECIL followed by anterior and posterior grafting (Table I). There was no postgraft ECIL. In both calves graft survival was prolonged and rejection less violent as compared to untreated calves. Rejection occurred on days 17 and 18 with anterior and posterior grafts (Calf 239) being rejected simultaneously.

Continuous pre- and postgraft ECIL. Calves 200 and 202 (Table I) received 7 days pregraft ECIL. Both calves were grafted anteriorly and posteriorly from different donors. Calf 200 received 27 days of postgraft ECIL. Anterior grafts were rejected in 22 days, however posterior grafts survived for 39 days, i.e. 12 days after ECIL was discontinued. Calf 202 received 61 days postgraft ECIL. Anterior grafts were rejected by day 27. On the 61st day following grafting the calf developed acute septicemia

and died. The posterior grafts were intact at the time of death.

Continuous postgraft ECIL. To determine if lymphopenia prior to grafting was necessary for the maintenance of posterior grafts, 4 calves (206, 284, 257 and 296) received only postgraft ECIL (Table I). In Calf 206 the anterior grafts were rejected by day 13, but the posterior grafts were not rejected until 3 days after ECIL had been terminated (day 28). Calf 284 received posterior grafts from different donors on days 5,4,3,2,1 and 0, prior to commencement of ECIL. Also on day 0, grafts from a 7th donor were placed both anteriorly and posteriorly. The anterior and posterior grafts from the 7th donor were simultaneously rejected on day 15. All other posterior grafts remained intact until day 20 (3 days after ECIL was discontinued). These results support those of Billingham et al.[1] who found that cells capable of transferring homograft sensitivity were first demonstrable in the blood and regional nodes of mice 6 days after grafting.

In calves bearing both anterior and posterior grafts, edema of posterior grafts was observed during active rejection of anterior grafts. In the event this was the result of a sharing of histocompatibility antigens, Calves 257 and 296 received only posterior grafts (Table I). No edema was observed during the period of postgraft ECIL (21 and 28 days respectively). Grafts were rejected 5 and 9 days after ECIL had been terminated.

Calf 295 (Table I) received only anterior grafts and 11 days of postgraft ECIL. Graft survival was 16 days.

Effect of postgraft ECIL on the cytotoxic antibody response. The serum antibody response of 3 untreated calves (273, 304, 305) is illustrated in Fig. 1(b). Cytotoxic activity of the serum rose sharply

1 - 2 days prior to gross rejection and reached a peak about the time of rejection or shortly thereafter. The antibody response in Calves 257 and 295, which received posterior grafts and postgraft ECIL, are illustrated in Fig. 1(c). When the sera were assayed with guinea pig C', little or no cytotoxicity was detected even at the time of graft rejection. When rabbit serum was used in place of guinea pig serum as the source of complement, cytotoxic antibody was detected 10 to 15 days following grafting. After the termination of ECIL, the activity increased reaching a peak about the time of rejection. Cytotoxic antibody was also detected in thoracic duct lymph at the same time as it appeared in the serum, however it did not increase after termination of ECIL.

The antibody response in Calf 295, which received only anterior grafts and 11 days postgraft ECIL, is illustrated in Fig. 1(c). Although the lymph draining the graft was not being irradiated directly, the antibody response was delayed and suppressed as compared to untreated calves.

DISCUSSION

Although sensitization to kidney transplants is known to occur as the result of direct vascular anastomosis[12], the results of these experiments are in accord with the hypothesis that sensitization to skin allografts is principally via the afferent lymph[8]. Undoubtedly posterior grafts were vascularized within 2 or 3 days, but rejection did not occur until after ECIL was discontinued. The experiments also indicate that, for first set skin graft rejection, "activated" cells from the sensitized regional lymph nodes must enter the blood in a viable state and that the

principal route of entry is via the efferent lymphatics. Homograft immunity does not apparently spread to distant lymphoid tissue provided that cells in the efferent lymph of regionally sensitized lymph nodes are destroyed.

Postgraft ECIL effectively delayed and suppressed the cytotoxic antibody response. The peak antibody activity correlated closely with graft rejection; however, when compared to untreated calves, the level of antibody in calves which received postgraft ECIL was very low even at the time of rejection. One explanation for this suppression is that ECIL destroyed sensitized cells emerging from the regional nodes thus preventing the expansion of the immune response to other lymphoid tissue[5]; therefore, the low level of antibody in the serum resulted from antibody produced only in the regional lymph nodes. A reduction in immunologically competent cells as a result of extensive postgraft ECIL is another factor to be considered.

FOOTNOTES

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2. E. I. DuPont DeNemours and Co., Inc., Wilmington, Del.

3. Dow Corning Corp., Midland, Mich.

TABLE LEGEND

TABLE I. Effect of extracorporeal irradiation of thoracic duct lymph (ECIL) on the survival of skin allografts in calves.

TABLE I

Calf	Duration (Days) of ECIL		Day of Graft Rejection	
	Pregraft	Postgraft	Anterior	Posterior
12 control[a]	0	0	8-11	---
203	0	0	10	10
186	22	0	17	---
239	10	0	18	18
200	7	27	22	39
202	7	61	27	61[b]
206	0	25	13	28
284	0	17	15	20-25[c]
257	0	21	---	26
296	0	28	---	37
295	0	11	16	---

[a] 12 recipient calves used to establish normal graft survival[(3)].

[b] Died of acute septicemia on day 61. Grafts were intact at time of death.

[c] Received posterior grafts from 7 different donors, the first set grafted 5 days before ECIL commenced (see text).

FIGURE LEGENDS

Figure 1(a) The effect of extracorporeal irradiation of thoracic duct lymph (ECIL) on the blood lymphocyte count and thoracic duct cell output.

R-A = rejection of anterior grafts.

R-P = rejection of posterior grafts.

Figure 1(b) The cytotoxic antibody activity of sera from 3 untreated calves which received skin allografts on day 0.

R = graft rejection. Sera were assayed with guinea pig C'.

Figure 1(c) The effect of postgraft extracorporeal irradiation of thoracic duct lymph (ECIL) on the cytotoxic antibody activity of sera from 3 calves. ECIL was started on the day of grafting (day 0). Calves 257 and 296 received only posterior grafts and Calf 295 only anterior grafts. ▲-▲-Calf 257 assayed with guinea pig C'.

●--●-Calf 296 assayed with guinea pig C'.

Δ-Δ- Calf 257 assayed with rabbit C'.

○-○-Calf 296 assayed with rabbit C'.

□-□-Calf 295 assayed with guinea pig C'.

R = graft rejection.

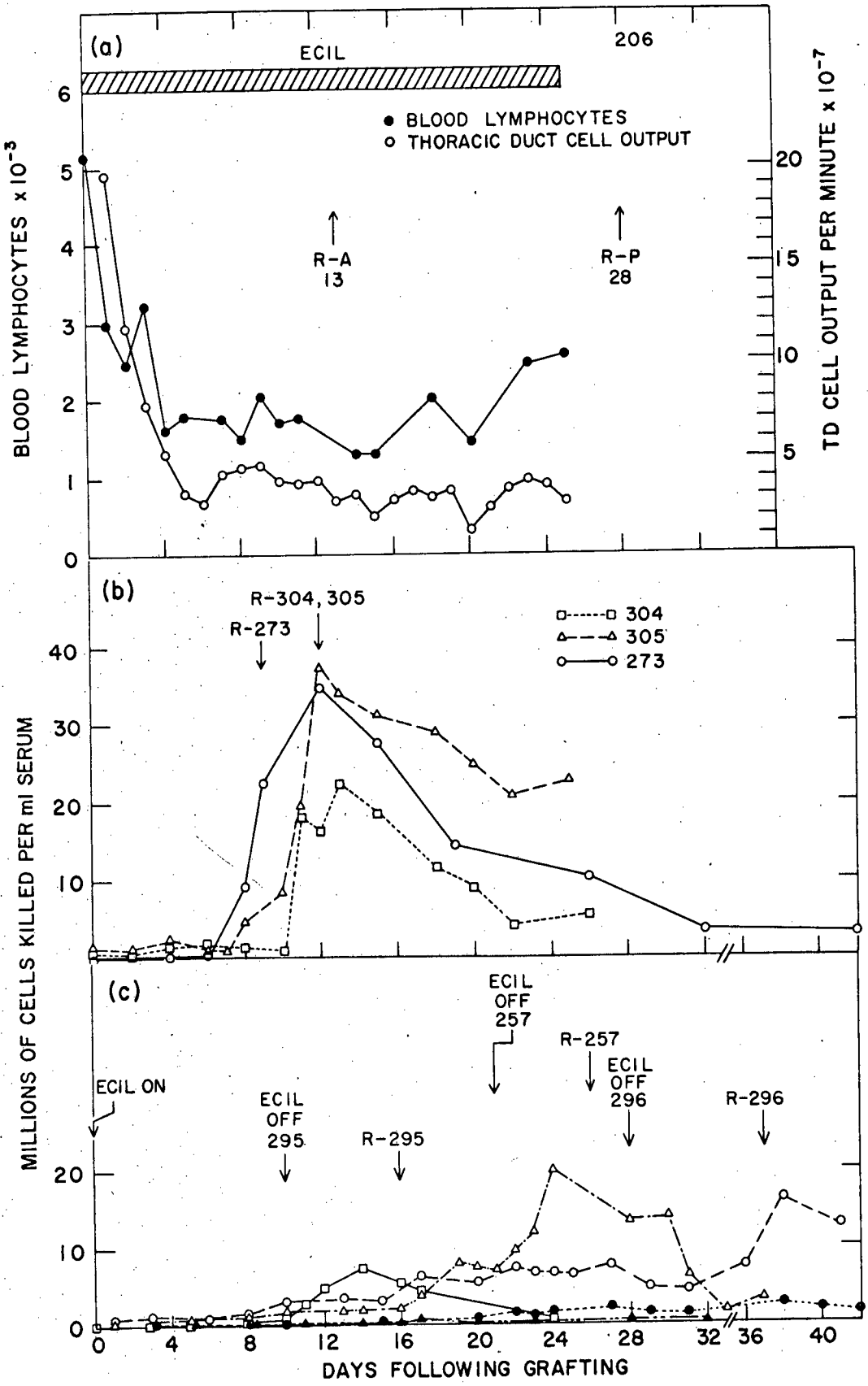
(a)
ECIL
206
BLOOD LYMPHOCYTES
THORACIC DUCT CELL OUTPUT
R-A
13
R-P
28
BLOOD LYMPHOCYTES x 10⁻³
TD CELL OUTPUT PER MINUTE x 10⁻⁷
(b)
R-304, 305
R-273
304
305
273
(c)
ECIL ON
ECIL OFF 295
R-295
ECIL OFF 257
R-257
ECIL OFF 296
R-296
MILLIONS OF CELLS KILLED PER ml SERUM
DAYS FOLLOWING GRAFTING

FIGURE 1

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