THE RESTORATIVE EFFECT OF EARLY ESCHAR EXCISION AND GRAFTING ON DEPRESSED IMMUNE RESPONSE IN BURNED MICE.

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SUMMARY

This study was carried out to research the immunologic changes in experimental burn injuries on an animal model treated by early eschar excision and skin grafting (EEG).

We used a full-thickness burn injury model (30% TBSA) and EEG procedure at postburn 48. hours. The immunologic status was quantitated via two in vivo measurements of cell-mediated immunity (CMI) in the mouse. First by measuring the degree of sensitization to the contact antigen, 2,4-dinitrofluorobenzene and second by weighing the popliteal lymph nodes after incection of splenocytes.

A full-thickness burn covering 30 % ofbods surface area was profoundly immunosuppressive and the EEG was able to significantly restore CMI as reflected by the two in vivo assays. This study demonstrated that EEG resulted in improvement of immunosuppression caused by thermal injury although not enough to restorate to normal levels.

***Key Words** : Burn, Early excision, Skin Graft, Immunity,*

ÖZET

Bu çalışmada, deneysel olarak, yanıktan sonra uygulanan erken eskar eksizyonu ve greftlemenin immun sistem üzerindeki etkileri araştırıldı. Bu amaçla farelerde % 30 III. derece haşlanma yanığı oluşturuldu. Diğerruba ise yanıktan 48 saat sonra erken eskar eksizyonu ve greftleme işlemi uygulandı. Hücresel immun cevap iki ayrı in vivo yöntemle tayin edildi. Bunlardan birincisinde, Dinitroflorobenzen'e karşı hassaslaştırılan hayvanlarda kontakt hipersensitivite reaksiyonu ölçüldü. İkincisinde ise splenosit injeksiyonunu takiben popliteal lenf nodülleri tartıldı.

Oluşturulan % 30 civarındaki yanık travmasının organizmada ileri derecede immundepresyona sebeb olduğu görüldü. Yine immun testler yardımı ile, yapılan erken eskar eksizyonu ve greftlemenin önemli oranda immun fonksiyonları geri kazandırdığı ve korunduğu izlendi. Böylece termal yaralanmanın sebeb olduğu immundepresyonun erken eksizyon ve greftleme işlemi ile tam olarak olamasa bile büyük oranda engellenebileceği ortay kondu.

***Anahtar Kelimeler:** Yanık, Erken eksizyon, Deri Grefli, İmmünite.*

The clinical practise of aggressive early burn wound excision and skin grafting has slowly gained wide acceptance recently since the early, favorable reports by Burke and Tompkins (1-2). Investigations have shown that excision and skin grafting procedures result in temporary and partial restoration of the mixed lymphocytic responsiveness, improved survival, shorter hospitalization, diminished circulating endotoxin levels and lower burn woundn infection in burned patients (3-4-5-6). There have been numerous animal studies in the burn trauma model that immediate postburn eschar removal resulted in improvement of immunosuppression (7). Tchervenkov has also shown that early burn wound excision and skin grafting postburn trauma restored in vivo neutrophil delivery to inflammatory lesions(8). Lalonde and Demling have demonstrated in the sheep burn model that complete excision and wound closure could reverse the postburn increase in 0_2 consumption (9-10). Echinard has shown that early excision of burn eschar prevented weight loss and depression of thymic DNA synthesis of animals(11).

This study was undertaken to determine whether grafting the burn wound following early excision restored the cell-mediated immunity by in vivo monitoring.

MATERIALS AND METHODS

Female BALB/C mice from DETAM weighed 30-35 gr were used at ambient room temperature and given water and food ad libitum throughout the experiment. Animals were divided into four homogeneous groups - group A:control, group B: burned, group C: burned and early excised & grafted (EEG). Group D: excision & grafting without burn injury(Table 1).

Group B was divided into six subgroups and each one was studied at intervals of 1, 4, 7, 10, 14 and 21 days after induction of the burn. BALB/c mice were anesthetized with pentobarbital (generoulsy supplied by Abbott Lab. İstanbul, Turkiye), 25 mg/kg, and their backs and abdomens were shaved. They were placed in

Table 1. Experimental design and group characteristiccs:

Group	No. Animals	No. sub-groups	PBD*/POD** Day of test	Burn	Treatment
A	12	—	—	No	No
B	60	6	(1,4,7,10,14,21)*	Yes	No
C	50	5	(4,7,10,14,21)*	Yes	EEG
D	10	—	12**	No	EG

EEG : Early excision and grafting on postburn day 2.
EG : Excision and grafting without burn
PBD* : Postburn day
(DNFB sensitization and Spleen cells injection in each subgroup weer initiated on these days)
POD** : Postoperative day
(DNFB sensitization and Spleen cells injection in each subgroup were initiated on this day)

a mold that left approximately 30 % of their body surface area exposed. This exposed surface was immersed into 70 C water for 6 seconds. The animals were resusciated with an intraperitoneal injection of 1 ml of Lactat Ringer(12). In group C, burn wounds were excised and skin from C57 BL/KS/DB OLA/HSD mice was grafted with a full thickness skin allograft 48 hours after burn trauma. This group was studied at the same intervals as in group B, postburn 4, 7, 10, 14 and 21 days.

Full-thickness grafts were performed according to the standard methods. After removing the pelt from donors, subcutaneous fat and tissues were leaned and the graft applied to the defect.

Contact Hypersensitivity : This technique used was similar to described methods by Hansbrough (13-14).

Fifty microliters of (DNFB) (Lot. 119F3792, SIGMA Chemical Co.) 0.5 % 2.4-dinitrofluorobenzene in 4:1 acetone : olive oil was applied to the shaved abdominal wall skin for two consecutive days. DNFB sensitization was performed on various days 1, 4, 7, 10, 14 and 21 following burn injury. Five days later the ear thickness was measured with a spring-loaded engineers' micrometer (10-3 cm, Baty, England) and the ear was immediately painted with 25 microliters of 0.2 % DNFB. Exactly 24 hours later, the thickness of the challenged and unchallenged ears were again measured and the difference calculated. Results were reported as a percent of their respectve control (Group A). The control value was therefore always presented as 100 %.

Popliteal Lymph Node Assay (PLNA) : Measurement of CMI was performed according to published methods by Shelby (15). Splenocytes from parental DBA / 2 / OLA / HSD mice were given to BALB / c recipients for lenfoid hyperplasia. Spleen cell suspensions were prepared in balanced salt solution (BSS). The cell population was adjusted to 20 X 107 cells per ml. In 50 microliters 10 x 106 viable cells were injected into the right hind footpad of the BALB/c recipients. Five days later, the recipients were anesthetized and the popliteal lymph nodes from both injected and contralateral uninjected hind legs removed and weighed on a Mettler balance. The ratio of injected/uninjected PLN weights for individual animals were used to evaluate PLN enlargement in response to antigenic challenge. Data were expressed as mean values ± standard error (SEM). The mean enlargement ratio (MER) was determined for each group.

Statistical Methods: For statistical significance the student's t test for independent means was used.

RESULTS

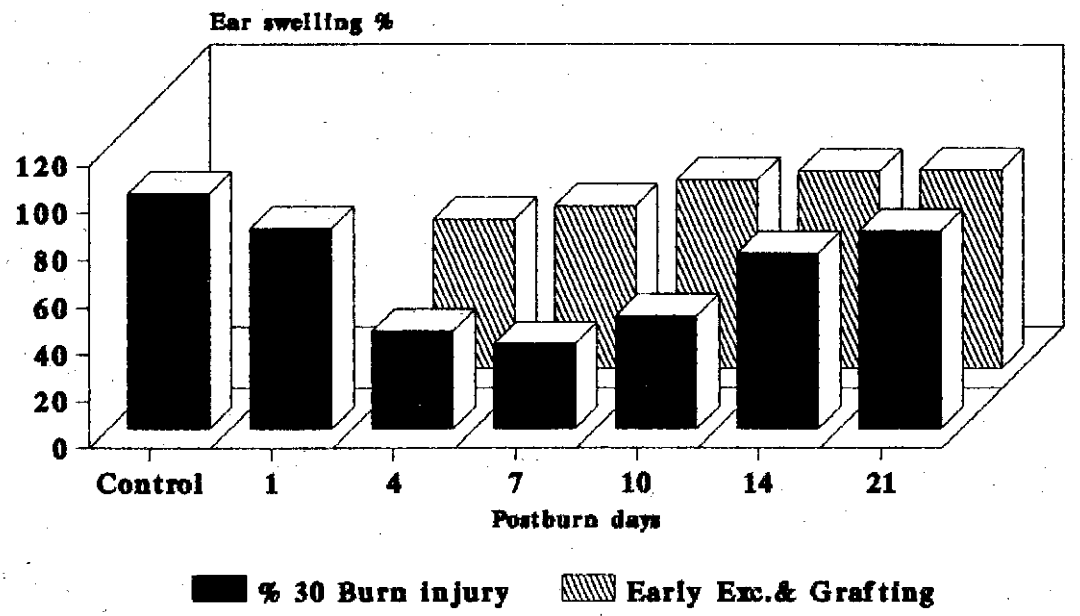
All animals receiving only 25-30 % TBSA burns survived with the mortality of 30-35 % . The majority of the injury was estimated to be full-thickness, as evidenced by a thick layer of burn eschar consisting of coagulated and necrotic skin.

Contact Sensitivity Responses:

The contact hypersensitivity response of burned mice following application of DNFB was found to be

highly sensitive to inhibition by burn injury. When DNFB sensitization was initiated 4, 7 and 10. days after the burn, ear swelling was significantly reduced (41.6±4.0 %, 36.3±7.0 % and 48.3±6.5%) ($p<0.001$). The presence of burned tissue results in a progressive degree of immunosuppression. By 21 days after burn, the wound was largely healed in most animals and cellular immunity had returned to nearly normal levels (84.2±14.3 %) (Fig.1). Excision of the eschar and skin grafting at 48 hours following burn injury resulted in no significant immunosuppression. In all operated groups showed no significant differences at postoperative day 12 (Fig.1) All of the groups which had eschar removal and grafting showed similar results but not comlete restoration of CMI. In group C the mean survival time of skin allografts was 11.3 days. The group with excision and grafting without burn injury had minimal suppressive effect on the cell-mediated immunity (group D). These results correlate well temporally with changes in popliteal lymph node assay as in Fig. 2.

Fig.1: Contact Hypersensitivity Reaction in Group B (Burn) and Group C (Burn+EEG)



DNFB sensitization was initiated these days after burn injury.Burn eschar was excised and grafted 2 days after injury.

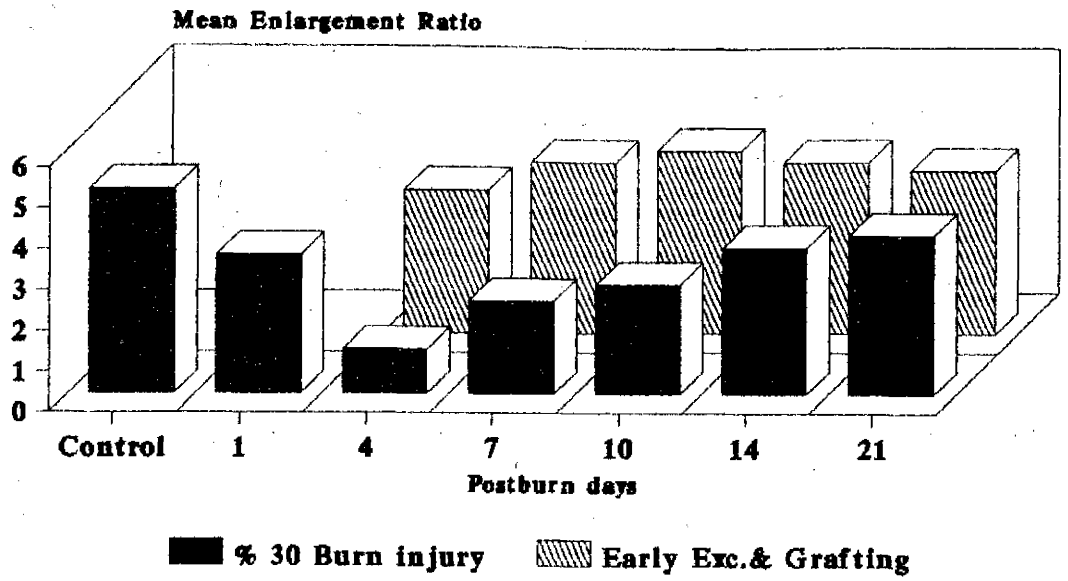
Popliteal Lymph Node Assay results:

After the injection of spleen cells, the popliteal lymph nodes of unburned mice consistently grew and resulted in an MER of 5.0±0.6. Burned mice showed a severe depression in their ability to induce a HVG reaction in the popliteal node with MER as follows: The depression was maximal at 4 postburn day and statistically significant when compared to control (MER. 1.1±0.2, $p<0.001$). No significant deppression was observed at excised and allografted groups (4,7,10,14 and 21 days after thermal injury, (Group C). Immediate postburn eschar excision and grafting resulted in return of the both parameters toward normal levels, although the values remained statistically reduced from that of control (unburned) animals (Fig 2). By contrast no significant depression was noted in grafted group without burn.

DISCUSSION

Generalized immunodepression has now been recognized to occur in the very early period after burns. The etiology of immunodeppression after thermal injury is undoubtedly multifactorial, involving circulating toxins or inhibitors hormonal changes, specific cellular defects, augmented immune regulation, and/or an injury triggered host deficiency state(14). The presence of immunosuppressive substances such as burn eschar in burn sera appears to precede tho immunologic dysfunction of animal lenfoid cells. There is convincing clinical and experimental evidence that removal of the immunosuppressive substances restore many aspects of immunity(7-16-17).

Fig. 2: Popliteal Lymph Node Assay in Group B (Burn) and Group C (Burn + EEG)



DNFB sensitization was initiated these days after burn injury.Burn eschar was excised and grafted 2 days after injury.

Early skin grafting has also advantages; ie, replaces damaged skin, and stops microbial invasion from burned tissue reconstructing a first line of defense providing mechanical barriers. In addition, the dead burn eschar offers on excellent culture medium for bacterial growth and proliferation. Sepsis, itself, has been shown to be immunosuppressive(13-18).

Numerous studies have documented the reliability of the CHR and PLNA as an in vivo monitor of immune status of animals. Because cellular immunity can be most easily studied in animal experiments, we used a well characterized model of cell-mediated immunity involving skin sensitization of the mice with DNFB. We also used popliteal lymph node assay which is considered to be an in vivo one-way mixed lymphocyte reaction, for evaluation of host versus graft responses. Thes technique is appropriate for measurement of CMI reactions and permits measurement of lenfoid response in the burn milieu (13-14-15-18). This model also allows for the precise quantitative, and reproducible measurement of CMI following burn injury.

These results indicate that treatment of burned mice with EEG can maintain normal or near normal cell-mediated immun responce in period of otherwise maximal immunosuppression. The treatment with EEG of burned mice thus appears to accurately reflect the potential of this operation to preserve normal cell-mediated immunity after severe burn injury.

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