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Coupled Skin Grafting for Use in the Studies of the Rejection Mechanism

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Summary. **Mice are convenient for the elucidation of the mechanism of skin graft rejection because they are genetically well defined and economically available. Recently, the responsiveness of the T cell subset to the major histocompatibility complex (MHC) class I and II antigen has been analyzed and an immunohistochemical method has also been developed. For re-evaluation of the mechanism of skin graft rejection, we endeavored to develop more useful techniques of murine skin grafting. We implanted a couple of fullthickness skin pieces of 0.8 cm diameter to the graft beds prepared in the dorsal part of a recipient. Allogeneic coupled skin grafts were rejected at almost the same date and there was no difference in the survival days between the biopsied and non-biopsied groups. By the "coupled skin grafting" it became possible to observe one graft for skin rejection and analyze the other histologically and immunohistochemically.**

INTRODUCTION

Techniques of skin grafting were originally described by Medawar's group[1] and thereafter improved by others.[2,3] Mechanisms of skin graft rejection in turn have benn studied and it is generally understood that an H-2 different-skin graft is rejected in approximately 10 days by the mediation of T lymphocytes. Currently, functionally different subsets of T lymphocytes are clarified using monoclonal antibodies[4] and murine stains differing in the defined locus of MHC or non-MHC become available. In addition, immunohistochemical methods are remarkably improved. To keep pace with these changes, mechanisms of graft rejection seem therefore to require reevaluation.

In this short report, we describe an improved skin graft technique, named "coupled skin grafting", which uses recently developed materials. The technique might be useful for the studies on the mechanisms of graft rejection.

MATERIALS AND METHODS

Mice. Female C57BL/6(B6), (B6×B6. H-H-2^{bm1}(bm1)) F1 and B6 nude mice were used at 8-12 weeks of age. Strain bml is a mutant of B6 mice at the MHC class I (H-2K) locus. All of these mice were originally derived from the Jackson Laboratory (Bar Harbor, ME, USA) and maintained at our Facilities of Animal Experimentation.

Preoperative preparation

Anaesthesia. A 10-times-diluted solution of Nembutal was used for the grafting operation at a dose of 0.1 ml/10 g body weight. Nembutal was injected either intravenously or intraperitoneally. During the biopsy of the skin, ether was used.

Removal of hair. Mice were fixed on a wood board with pins and the hair of the dorsal region was depleted with barium sulfate. The region on the donor was more widely depleted than that of the recipient. The dorsal hair was removed two or three days before the grafting operation to avoid preparing damaged epidermis of the depilated mice. Mice are known to show cyclic changes in hair growth from telogen (resting phase) through anagen (growing phase) and catagen (regressive phase).[5] The graft during anagen was not suitable for grafting because it was easily affected by insufficient blood supply (in Fig. 1).

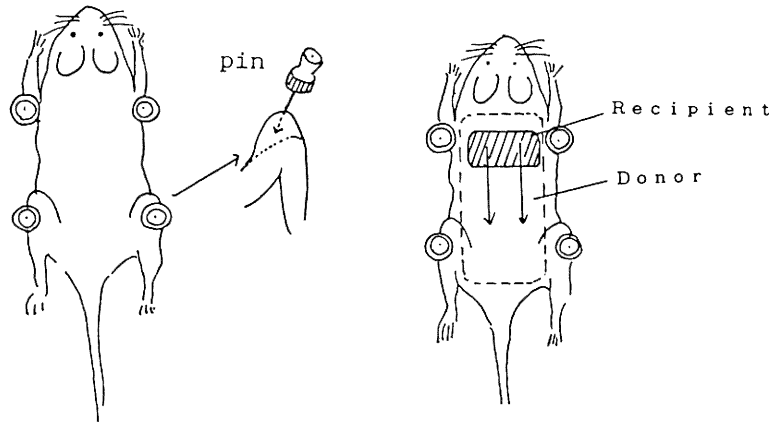

Fig. 1 Removal of hair

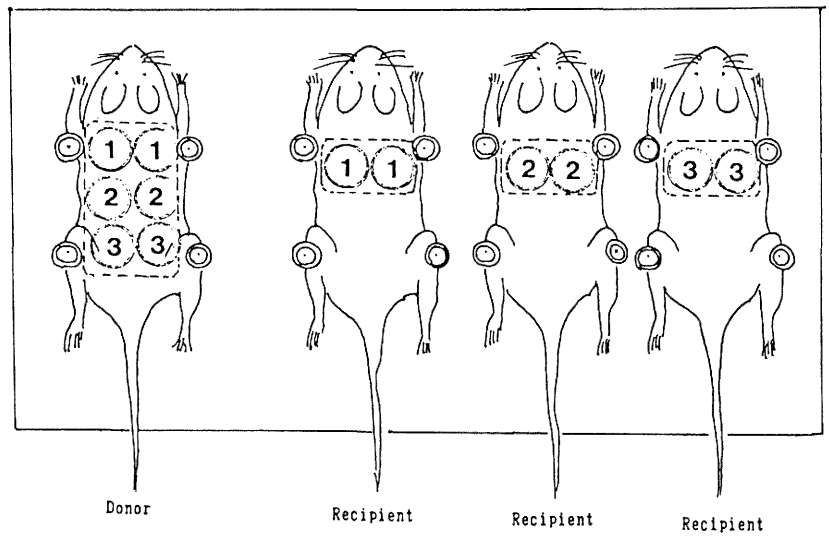

Fig. 2a Positions of grafts and marking

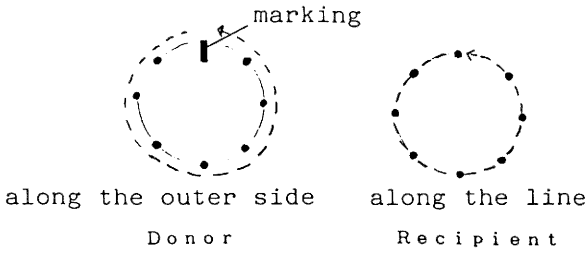

Fig. 2b Cutting of graft and bed

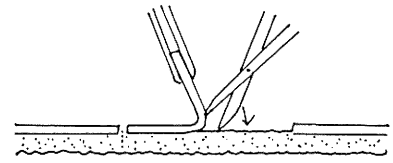

Fig. 2c Removal of skin

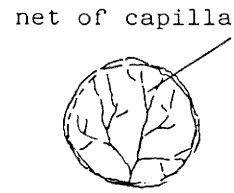

Fig. 2d After trimming

Skin grafting

Removal of skin and preparation of graft bed. After general anaesthesia (strong for donors and rather weak for recipients) the mice were fixed on a wood plate with pins. Circles were marked on the dorsal skin of the donor and 3 or 4 recipients using a cap (0.8 cm in diameter) the skin was carefully cut along the outer side of the marked circle on the donor and along the marked line on the recipient. After careful trimming, net of the capillary vessels could be seen in the recipient's bed, which was kept moist by covering with sterilized gauze slightly dipped in minimum essential medium. Careful trimming, without bleeding from capillary vessels, was required because the mice's subcutaneous tissue had steady panniculus carrosus and much areolar tissue (Fig. 2a. b. c. d).

Fixation. The graft was carefully placed on the recipient in reverse and fixed at 8 points with bond (Aron Alpha A®, Sankyo, Tokyo) (in Fig. 3).

Postoperative care

The grafts were covered with Gentacin ointment® (Schering Plough co., USA) and tied over with a transparent plaster (Op Site®, Smith & Mephew, Med. Ltd, Hull, UK) which was removed on the 7th day after grafting. Silky Tex® (Tokyo Eizai Lab. co. Ltd, Tokyo), a non-transparent plaster, was used for the nude mice because it held them more tightly.

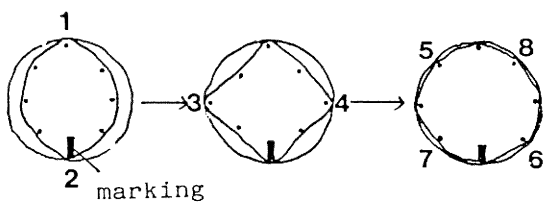

Fig. 3 Fixation

Judgment of graft rejection

When only one side graft was removed on the 7th day after grafting we judged it our technical error. The implanted grafts were observed every day and the conditions of the grafts were recorded by using a scale of rejection referring to the color, contraction and hair of the grafts.

RESULTS AND DISCUSSION

When the graft was successfully implanted, it showed delicate pink flush on the 7th day and gradual pigmentation and hair growth after the third week. By using our skin graft techniques, the surviving graft was easily recognized because the graft's hairs were growing in the opposite direction as the recipient's. The rejected graft turned dark-colored and its epidermis become dry and rough. Finally, it contracted and was replaced by the surrounding epithelium of the recipient.

From our experiences, the transparent bandage (Op Site®, Smith & Nephew, Med. Ltd, Hull, UK) was more convenient than the non-trasparent one (Silky Tex®, Tokyo Eizai Lab. co. Ltd, Tokyo) for the observation of the graft. However, the transparent plaster could not fix the graft onto nude mice tightly and was loosened by the mice, so the latter is recommended. The first removal of the plaster should not, if possible, be performed before the fifth day after grafting because it sometimes causes a graft to come away with it.

Comparing the left and right sides of the coupled grafts, we could easily judge the graft survival days and recognize our technical errors in grafting. In 35 cases, we had 2 in which only one side became necrotic by the 7th day after grafting. These errors, however, can be decreased through more careful grafting.

There were almost no differences in the graft survival days between competently transplanted coupled grafts, and any diffecence was always within one day, as shown in the following.

Table 1. Effect of biopsy on MHC class I-disparate skin allograft survival

Donor	Treatment	Biopsy	Survival times (days)	MST±SEM (days)
$F_1(B_6 \times bml)$	conorol	−	9 ,12,14,	11.7±2.5
		+	12,12,12,12,14,14,15,15,	13.3±1.4
	anti-Lyt-2	−	9,12,12,14,15,16,	13.0±2.5
		+	10,11,12,12,13,14,14,15,	12.6±1.7

$$\frac{\sum_{k=1}^{n} | \text{The left side graft survilal days} - \text{the rigth side's} |}{n}$$

$$= \frac{4}{10} = 0.4$$

There were no differences in the survival days between biopsied group and non-biopsied one (shown in Table 1). These data show that "coupled skin grafting" enabled us to observe the skin rejection of one graft and analyze the adequate process of the rejection histologically and immunohistochemically by sampling the other one.

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