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Variable rejection patterns of cultured keratinocyte allografts in the rabbit

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SUMMARY. **There are many conflicting reports on the survival of cultured keratinocyte allografts (CKAs). Studies were performed in the rabbit model to elucidate further the fate of CKAs. Of 24 CKAs, 7 were identified as technical failures, 13 displayed classical macroscopic evidence of rejection with a prolonged mean survival time of 2.8 days, compared to non-cultured allograft controls ($p < 0.01$), and 4 failed to display typical macroscopic evidence of rejection. Furthermore, the time to eventual wound healing of the classically rejected CKAs were delayed by 6.0 days ($p < 0.0001$), compared to identical non-grafted control wounds.**

The use of cultured keratinocyte autografts to close burn wounds is well documented (O'Connor *et al.*, 1981; Gallico *et al.*, 1984; Cuono *et al.*, 1986; Teepe *et al.*, 1986). Unfortunately the time required for culture of clinically useful quantities of cultured autografts reduces their effectiveness.

Cultured keratinocyte allografts (CKAs) can be available when patients first present. Evidence that cultured keratinocyte sheets lack MHC class II antigen bearing cells (Morhenn *et al.*, 1982; Hefton *et al.*, 1984), particularly Langerhan's cells, suggests that CKAs may be accepted as permanent skin cover.

The literature is divided on the question of whether CKAs are permanently accepted. Two studies report that such allografts are rejected, but with slightly prolonged survival, in the pig (Eisinger, 1985) and human (Aubock *et al.*, 1988). Two studies report the permanent acceptance of cultured allografts in the mouse (Hammond *et al.*, 1987) and human (Faure *et al.*, 1987). Another claims that cultured allografts are permanently accepted on split-thickness wounds and lost in full thickness wounds (Madden *et al.*, 1986). A further report (Ninneman and Good, 1974) retracts the authors' previous findings that CKAs are accepted (Summerlin *et al.*, 1973).

More recent studies report that, although CKAs appeared to be clinically accepted, blood group and sex typing revealed the healed grafts to be of recipient genotype, suggesting failed "take" or rejection (Brain *et al.*, 1989; Burt *et al.*, 1989; Kaawach *et al.*, 1991). Several other reports are anecdotal only.

We have used a rabbit model to help resolve these discrepancies. The rabbit was chosen firstly to enable identical standardised full thickness wounds to be created for clearly defined controls of non-grafted wounds and cultured keratinocyte autografts synchronous with the CKAs. Secondly, of the readily available, easily housed animals, the rabbit displays the closest degree of homology with humans in the arrangement and function of its Major Histocompatibility Complex (Kulaga *et al.*, 1987).

Materials and methods

29 rabbits were derived from two sources: outbred rabbits were obtained from Commonwealth Serum Laboratories (Melbourne, Australia) and our internal colonies provided three separate closed strains of inbred rabbits.

All allografts were performed across these closed strains to ensure that a Major Histocompatibility Barrier existed. Furthermore, all allografts were performed across sex and blood group incompatibilities.

Disaggregated rabbit keratinocytes were cultured into confluent sheets of stratifying keratinocytes suitable for grafting by a modification of the method of Green *et al.* (1979), as previously descried (Breidahl *et al.*, 1990). All cultures were maintained for at least 21 days prior to removal onto vaseline gauze with Dispase, and careful washing to remove culture medium and enzyme ready for grafting.

Rabbits were divided into two groups. Group I was a control group of five rabbits which received non-cultured split skin allografts on full thickness ear wounds, 10 mm in diameter. This group established non-cultured skin allograft rejection dynamics.

Group II consisted of 24 rabbits, each of which had three full thickness skin wounds created on their ears measuring 20×10 mm, under general anaesthesia. One of these wounds was not grafted (group IIa), one received a cultured keratinocyte autograft (group IIb) and one received a CKA (group IIc).

All wounds in group II were identically dressed: vaseline gauze supporting the cultured keratinocyte sheets was secured on the wound with 8-0 silk sutures, covered with a layer of sterile paraffin to protect against desiccation and sealed with a liberal coat of Op-Site spray. Exposed radiograph film was secured above this dressing with 4-0 silk sutures, as a protective splint.

The dressings were inspected daily, and removed at 5 days. Thereafter the grafts and control wounds were inspected daily and photographed three times per week, with the times taken to initial complete healing,

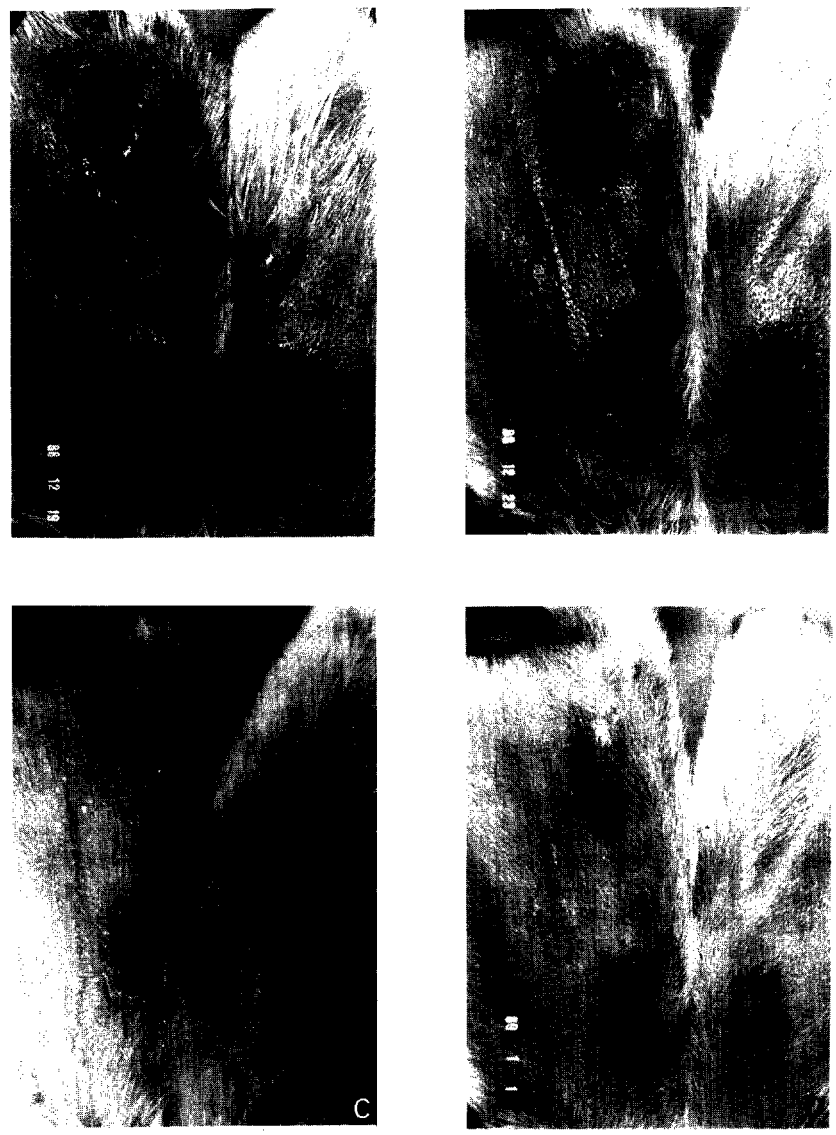

Fig. 1

Figure 1—Classical macroscopic rejection of CKAs. Right base—CKA; Left base—cultured autograft control; Left top—non-grafted control wound. (A) Day 6: Complete take of both cultured grafts and scab on the non-grafted control wound. (B) Day 10: The CKA is developing patchy necrosis whilst the cultured autograft develops a thickened cornified layer. The non-grafted control wound is considerably smaller. (C) Day 14: The CKA is completely necrosed while the cultured autograft remains healed. The non-grafted control wound has healed. (D) Day 19: The CKA has achieved final complete healing, 5 days after the non-grafted control wound. The cultured autograft control remains stable.

50% graft necrosis, and final complete wound healing recorded.

The t-test for independent samples was used for the statistical analysis of data.

Results

Group I

The times to 50% graft necrosis for five split-thickness allograft controls were 8, 7, 8, 8 and 7 days (mean survival time 7.6 days).

Groups IIa and IIb

18 non-grafted control wounds (group IIa) healed in a mean time of 14.6 days. This was statistically significantly slower than the mean time to healing of 13 cultured autograft controls (group IIb, 9.6 days, $p < 0.0001$; Fig. 4B).

Group IIc

Figure 3 documents the results of CKAs in 24 rabbits. The seven technical failures consisted of 3 CKAs being desiccated with no graft take, and the other four being infected when the dressings were removed.

Thirteen of the remaining 17 CKAs displayed classical macroscopic evidence of rejection with initial healing followed by necrosis, ulceration and finally delayed healing (Fig. 1).

Three CKAs were not observed to undergo necrosis or ulceration, but became erythematous and contracted, compared with their autograft controls,

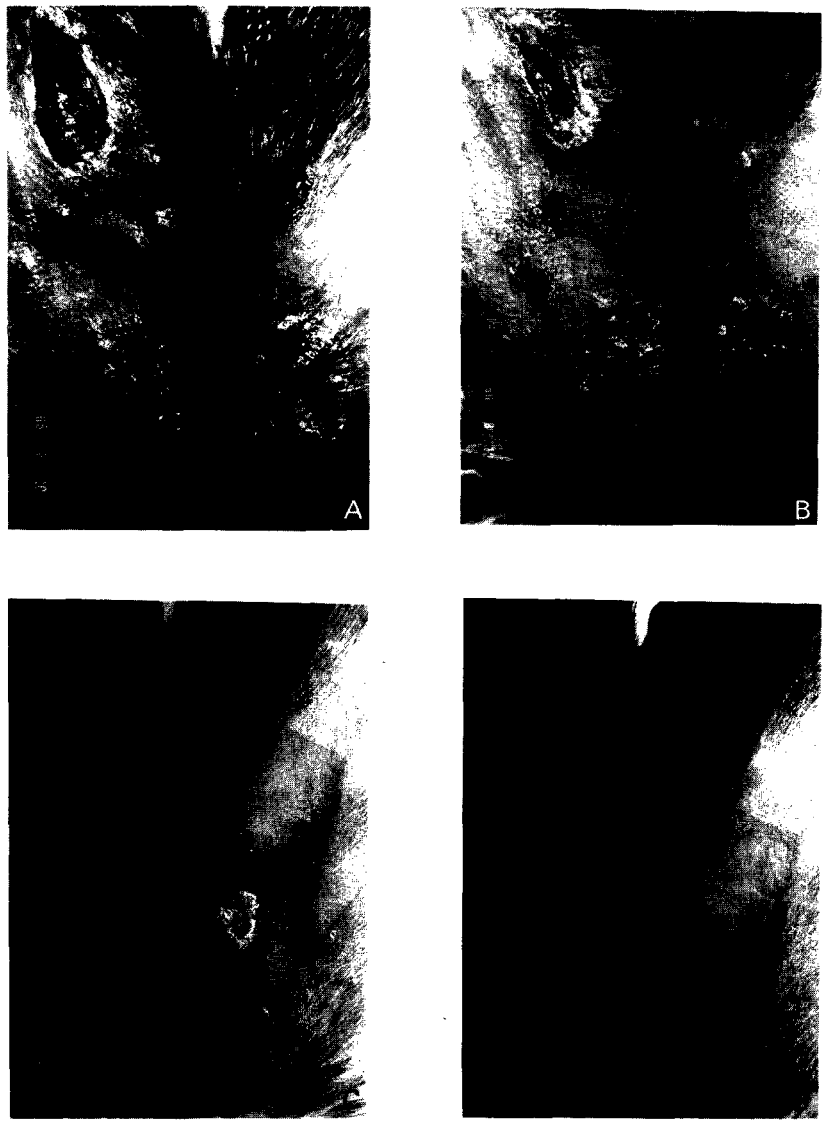

Fig. 2

Figure 2—Lack of macroscopic evidence of rejection of CKAs, displayed by three rabbit recipients. Right base—CKA; Left base—Cultured keratinocyte autograft control; Left top—non-grafted control wound. (A) Day 8: Incomplete "take" of both cultured autograft and CKA, scab on non-grafted control. (B) Day 10: Cultured autograft completely healed and settling to normal skin colour. CKA almost healed but erythematous. Non-grafted control approaching complete healing. (C) Day 17: Cultured autograft healed with normal skin hue and minimal contraction. Non-grafted control healed and contracted into fine scar (covered by surrounding hair). CKA still erythematous and contracting. (D) Day 28: All wounds healed and quiescent with no macroscopic evidence of necrosis in cultured allograft.

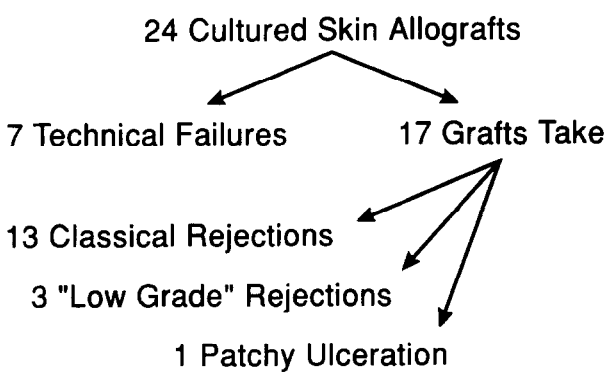

Fig. 3

Figure 3—Fates of CKAs.

eventually resembling their control wound scars (Fig. 2).

The final CKA was well healed at 6 days and, although spotty ulceration was observed in the graft from time to time, it was not observed to redden or contract.

The mean survival time (mst) of CKAs (group IIc, 10.4 days) was slightly prolonged compared with the split-thickness allograft controls (group I, 7.6 days, $p < 0.01$; Fig. 4A).

The mean time to eventual complete healing of CKAs (group IIc, 20.6 days) was significantly longer than for the non-grafted control wounds (group IIa, 14.6 days, $p < 0.001$; Fig. 4B).

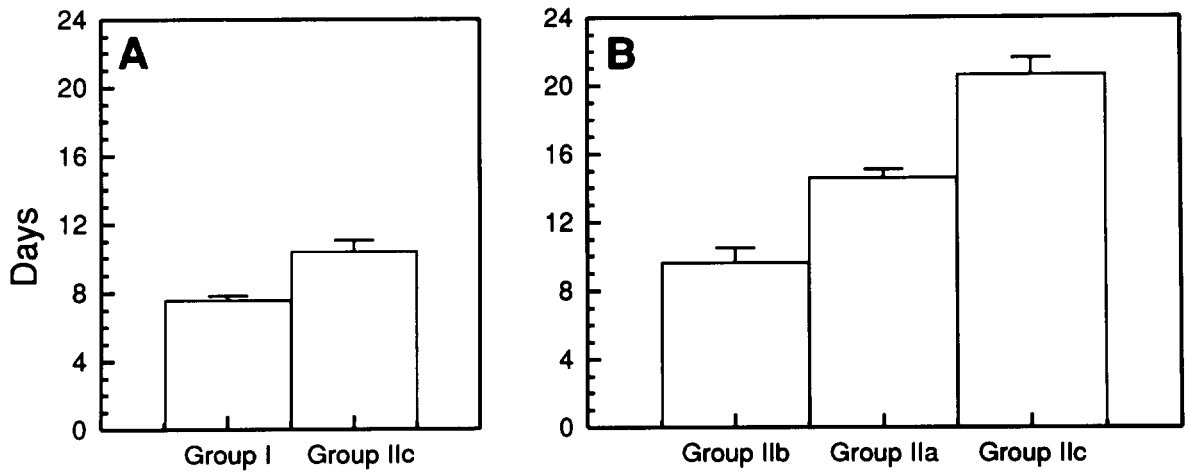

Fig. 4

Figure 4—(A) Mean survival times, +standard error of mean, of non-cultured split skin allografts (Group I, 7.6 days) and CKAs (Group IIc, 10.4 days). Statistically significant difference at $p < 0.01$ level (t-test). (B) Mean time to final wound healing, +standard error of mean, for cultured autografts (Group IIb, 9.6 days), non-grafted wounds (Group IIa, 14.6 days) and CKAs (Group IIc, 20.6 days). All statistically significant differences at $p < 0.0001$ level (t-test).

Discussion

Although our experiments were performed in the rabbit, which may be less relevant than human studies, such precisely controlled experiments are difficult to perform in humans. Our results, although they cannot be directly extrapolated to humans, can help explain some of the discrepancies observed in the literature.

Firstly, the use of full thickness wounds diminishes the chance of rapid healing from adnexal remnants in the wound base, which may be hastened by occlusive dressings or a "scab" of a necrotic graft (Grillo and Gross, 1964).

Secondly, the non-grafted control wounds (group IIa) clearly defined wound healing dynamics in the experimental model.

Thirdly, the cultured autograft controls (group IIb) gave a picture of the dynamics and clinical appearance of initial "take". Thus technical failures were easily identified and excluded.

Fourthly, that CKAs were rejected with a longer mst than non-cultured split skin allografts (group IIc versus group I) confirms that allograft rejection is modified by culture. It has been postulated that in humans this modification may be manifested as progressive replacement of donor cells with cells of host origin, with no clinical evidence of rejection.

This has been demonstrated in four patients with clinically accepted CKAs showing progressive replacement of donor HLA antigens by recipient antigens, beginning at day 14 (Gielen *et al.*, 1987). Controls for HLA markers during normal wound healing were not examined, and these membrane markers may undergo modulation during the normal healing process (Brain *et al.*, 1989).

It has been argued that DNA markers are more specific. Three papers using a Y probe all conclude that there was no evidence of CKAs "taking" to the wound, despite accelerated healing with no clinical or histological signs of rejection (Burt *et al.*, 1989; Brain *et al.*, 1989; Kaawach *et al.*, 1991).

Fifthly, our findings that CKAs delayed eventual wound healing (group IIc versus group IIa) correlates with human findings that both traditional allografts and CKAs delay wound healing (Miller, 1974; Aubock *et al.*, 1988). If CKAs are first accepted and then rejected (so no cells with donor markers remain) one would expect wound healing to be slower than normal, not faster.

Finally, our finding that four of seventeen cultured allografts did not show classical macroscopic evidence of rejection is evidence of a variable response to CKAs. This may result from a modification by culture which is great enough to affect CKA rejection in some individuals, presumably those with weaker host resistance. Matching for sex and blood group may further enhance CKA survival.

With current techniques of culture, such a diminution of rejection only occurs in isolated individuals and one would expect the majority of individuals to demonstrate a delay in wound healing if treated with CKAs that "take" successfully.

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