The Longevity of a Bilayered Skin Substitute After Application to Venous Ulcers

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Background: A bilayered skin substitute composed of allogeneic keratinocytes and fibroblasts in a collagen gel has been approved by the US Food and Drug Administration for the treatment of venous and diabetic ulcers. Its mechanism of action has not been fully determined.

Objective: To determine the longevity of allogeneic fibroblasts and keratinocytes in a bilayered skin substitute in patients with venous leg ulcers.

Methods: Ten patients with venous leg ulcers were treated with a bilayered skin substitute on day 0, days 3 to 5, and weeks 1 through 3. Biopsy specimens of the grafted wound were taken. We used polymerase chain reaction analysis to determine whether allogeneic DNA was present in the biopsy specimens.

Results: We detected allogeneic DNA in 2 of 8 specimens at 1 month after initial grafting. Neither of the 2 patients showed persistence of allogeneic DNA at 2 months after initial grafting.

Conclusions: Allogeneic cells from a bilayered skin substitute do not appear to survive permanently after grafting for treatment of venous leg ulcers. Other mechanisms of action might include cytokine release, structural support, or provision of a moist wound environment.

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Bilayered skin substitute (BSS) (Apligraf; Novartis, Inc, Canton, Mass) is a viable skin replacement that has been approved by the US Food and Drug Administration for the treatment of venous and diabetic ulcers. The epidermal layer is formed by serial passage of human keratinocytes that are seeded onto a contracted dermal matrix in culture medium. A well-differentiated stratum corneum develops after exposure of the keratinocytes to an air-liquid interface. The dermal layer is composed of human fibroblasts in a bovine type I collagen lattice. These fibroblasts divide, multiply, and produce new collagen. Although matrix proteins and cytokines found in human skin are present in the BSS, it does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels, or hair follicles.

At present, the BSS has not been shown to be immunogenic in treated patients. In vitro studies demonstrated that the BSS does not contain cells that constitutively express class II major histocompatibility complex antigens such as macrophages, lymphocytes, and Langerhans cells. These immunogenic cells are lost during serial culture and expansion of human keratinocytes and fibroblasts. When tested in a mixed lymphocyte reaction assay, cultured human keratinocytes and fibroblasts did not trigger a proliferative immune response, even when class II antigen expression was induced by interferon-γ. Therefore, it is possible that the BSS might promote healing by surviving permanently in the wound bed. However, experience in other models suggests that the BSS may be eventually replaced by host tissues.

The purpose of the present study was to determine whether allogeneic DNA from the BSS could be detected at different times after BSS grafting of venous ulcers.

RESULTS

Fourteen patients were enrolled into the study. Ten patients demonstrated results of tissue typing in which HLA antigens were not common with those of the BSS. These patients underwent evaluation for persistence of BSS cells. Eight of these 10 patients underwent testing for persistence at 4 weeks. Two ulcers healed completely during the study. The DNA from the BSS could
PATIENTS AND METHODS

Patients with venous ulcers of greater than 1 month’s duration (confirmed by means of duplex ultrasonographic findings) and an ankle-brachial pressure index of greater than 0.65 were enrolled into the study. Patients enrolled in other investigational studies within the past 3 months, patients previously treated with the BSS, and patients with HLA class I antigens common to the patient and the BSS were excluded from participating.

All patients received up to 5 applications of the BSS during the 3-week treatment. At the screening visit (days -7 to -5), blood was collected as a DNA control sample. The BSS was applied to a clean, debrided wound after thorough irrigation with isotonic sodium chloride solution. Oozing or bleeding caused by debridement was stopped by means of gentle pressure. The graft was covered with a non-adherent dressing, preevacuated gauze, and a self-adherent elastic wrap from the metatarsals to the tibial plateau. At 4, 8, and 12 weeks after the first BSS application, 3-mm punch biopsy specimens were taken from the area judged by the investigator (T.J.P. or V.F.) to be most likely to contain BSS. If the BSS was not visible on the ulcer, specimens were taken from the center of the wound. Biopsy sites were rotated so that the specimen was not taken from the same location at each visit. When DNA of the BSS could not be detected in the specimen, no further samples were taken.

To test for the persistence of the BSS on patients, we used the expression of specific HLA genes by BSS cells. The HLA phenotypes of the BSS have been determined previously (data not shown). We used this information to create sequence-specific primer sets directed toward the DNA of the BSS. In the present study, HLA-DQB1*0201–specific primers were used (Figure 1). Keratinocytes and fibroblasts from the BSS carry this gene. Primers were amplified using polymerase chain reaction (PCR) analysis.6

not be detected in biopsy specimens from 6 patients (Figure 2). Results of PCR testing showed that 2 of these wounds demonstrated the BSS DNA at 4 weeks (Figure 3). No correlation between the clinical appearance of the wound and the BSS persistence was found. Neither wound showed persistence of DNA from the BSS at 8 weeks. One of these ulcers healed; the other did not.

COMMENT

The BSS has been approved by the US Food and Drug Administration for the treatment of venous and diabetic ulcers. The healing process has not been entirely elucidated.

Some authors postulated that the presence of a dermal-like substitute in the BSS may create conditions sufficiently different from cultured epidermal allografts to allow long-term engraftment.7 In patients with acute wounds due to epidermolysis bullosa, extensive erosions were treated with the BSS, and the treated areas remained blister free, with clinical evidence of graft take and no signs or symptoms of acute graft rejection.8-9 Molecular genetic testing using a specific marker for the tissue-engineered cells confirmed persistence of the skin substitute in 4 (33%) of 12 patients with these acute wounds at 4 weeks.10 Delayed rejection of allograft may occur in these patients because of immune tolerance to allogeneic tissue in early life8,11 or because of abnormalities in immune function in patients with epidermolysis bullosa.12-15 Alternately, the mechanism of action of cultured allogeneic tissue may be different in acute wounds compared with chronic wounds. In other acute wounds (eg, split-thickness skin-graft donor sites), persistence of allogeneic DNA from the BSS occurred in 3 (27%) of 11 patients at 4 weeks.15 Additional data are needed to elucidate whether the BSS persists for longer than 4 weeks in acute wounds.

In chronic wounds, clinical investigators have observed clinical graft take and temporary persistence of the
These include interleukins 1, 3, 6, and 8; transforming growth factor-I is not found in cultures of pure human keratinocytes or of pure human dermal fibroblasts, but is present in the BSS (J.Y., unpublished data, September 1997).

The matrix components of the graft may act as a substrate to allow recruitment of cells into the wound. The dermal fibroblasts in the construct may contribute to the formation of new dermal tissue through matrix biosynthesis.19,20 The mechanisms of action of this skin substitute are of great interest and should be investigated further. However, whatever the mechanism, the BSS offers a new treatment modality for patients with venous and diabetic ulcers, and potentially for patients with other types of wounds.

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REFERENCES