Autologous Epidermal Cultures and Narrow-Band Ultraviolet B in the Surgical Treatment of Vitiligo

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BACKGROUND. **Vitiligo is an acquired skin disorder with a great social impact. It can be successfully treated with autologous epidermal grafting.**

OBJECTIVE. **To evaluate the possibility of treating vitiligo by autologous grafting of epidermal cells and narrow-band ultraviolet B (UVB).**

METHODS. **Autologous epidermal cultures were prepared starting from small biopsies of normally pigmented skin. Cells were cultured on hyaluronic acid membranes using medium supplemented with patient's serum. Cell cultures were grafted onto laser-abraded depigmented areas. Patients underwent narrow-band UVB therapy 3 weeks after grafting.**

RESULTS. **Repigmentation of the grafted areas started 1 month after transplant and continued until 4 months after grafting. All patients were evaluated 3, 6, 12, and 18 months after grafting. At the 18-month follow-up, repigmentation was observed in 75% of patients with focal and segmental vitiligo and in 30% of patients with generalized vitiligo.**

CONCLUSIONS. **This therapy can be considered for the treatment of stable vitiligo (especially focal and segmental) resistant to standard therapies. Their results are encouraging from the clinical and esthetic point of view, although the treatment is costly and highly specialized.**

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VITILIGO IS an acquired idiopathic disorder of epidermal pigmentation involving 1 to 2% of the world population and affecting males and females and the various races equally.[1,2] It is characterized histologically by the absence of or marked reduction in melanocytes in the basal epidermal layer and clinically by the presence of depigmented lesions of variable shape and size.[3]

The course is generally unpredictable: in "stable" vitiligo, the lesions remain static for a long period (more than 2 years) and Koebner's phenomenon is absent, whereas in "active" vitiligo, old lesions progress, new lesions develop, and Koebner's phenomenon is present.[4]

The activity of vitiligo, together with parameters such as type, location, and spread, must be carefully considered in deciding the best therapy. For active vitiligo, therapeutic options include medical therapies, such as topical and systemic corticosteroids,[5] immunomodulators, vitamin D derivatives, systemic or topical photochemotherapy, narrow-band ultraviolet B (UVB),[6,7]and 308 nm excimer laser.[8] Stable forms of vitiligo are largely resistant to standard medical therapies.

Surgical techniques such as minigrafting,[9] autologous epidermal grafts,[10] epidermal cultures,[11] cultured autologous melanocytes,[12]and autologous epidermal cell suspension[4] are new possibilities. Vitiligo must be stable to avoid Koebner's phenomenon in donor areas.[2]

Several studies have demonstrated that combined therapy with cell suspension grafting and subsequent narrow-band UVB induces expansion and spread of grafted melanocytes.[13,14] Narrow-band UVB therapy has advantages over psoralen plus ultraviolet A because no drugs are required for the treatment and the effects of photocarcinogenesis and photoaging are probably reduced.[2]

Here we illustrate the results of our experience in the treatment of 93 cases of stable vitiligo, refractory to nonsurgical therapies, using autologous grafting of epidermal cells associated with subsequent narrow-band UVB.

Materials and Methods

Patients

Ninety-three patients (55 males, 38 females), ranging in age from 14 to 62 years (median 38), were treated.

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They had focal (49), segmental (26), or generalized vitiligo (18). The duration of the vitiligo varied from 6 to 24 years, and 36 patients had a family history of vitiligo. No autoimmune disorders were diagnosed, except autoimmune thyroiditis in two patients. The selection criteria for autologous grafting of epidermal cells included high patient motivation, age over 14 years, stable lesions and Koebner's phenomenon negative for at least 2 years, a lack of response to conventional topical and systemic therapies, no personal history of hypertrophic scarring or keloids, and no severe autoimmune disorders. The technique was also used to treat a 43-year-old female with piebaldism.[15] All patients were observed for up to 18 months after grafting. At each follow-up, patients were evaluated clinically by the surgical team and two dermatologists.

The results were assessed as the extent of repigmentation of the depigmented areas. The surface of repigmentation was calculated by image analysis using a special algorithm (DB-Mips, Dell'Eva, Burroni, Siena, Italy).

Epidermal Cultures

A superficial skin biopsy (400 μm) of about 2 to 4 cm^2 was obtained from pigmented areas of the body (buttock) using an electric dermotome (Colibrì Intramatic 4000, 100 watt, AM Medica, SAS, GE, Italy). The biopsies were sent to our laboratory, where they were used to grow primary epidermal cultures by the classic method.[16] Briefly, the tissue was washed in phosphate-buffered saline (Sigma, Sigma Aldrich, St. Louis, MO, USA) supplemented with penicillin and streptomycin (Sigma) and cut into thin strips, which were placed in Petri dishes (Kostar, Corning Costar, Rochester, NY, USA) containing a solution consisting of trypsin (0.5 g/L) and ethylenediaminetetraacetic acid (EDTA) (0.2 g/L) (Sigma). The dishes were incubated for at least 3 hours at 37°C. A cell suspension was obtained by scraping the tissue on the dermal side. The cells were washed in Dulbecco's Modified Eagle's Medium (DMEM; Sigma) supplemented with antibiotics, 20% patient's serum, and L-glutamine (Sigma) and then resuspended in 15 mL culture epithelial cell (CEC) medium consisting of DMEM and Ham's F12 (3:1) (Sigma) with 20% patient's serum, 0.584 mg/mL L-glutamine (Sigma), 100 IU/mL penicillin (Sigma), 100 mg/mL streptomycin (Sigma), 0.4 μg/mL hydrocortisone succinate (Sigma), 5 μg/mL insulin (Sigma), 5 μg/mL transferrin (Sigma), 2×10^{-9} M triiodothyronine (Sigma), 10^{-10} M cholera toxin (Sigma), and 1.8×10^{4} M adenine (Sigma), without epidermal growth factor (EGF). The cell suspension was seeded at a density of 30,000 cells/cm^2 in 75 cm^2 culture flasks (Falcon, BD Falcon, BD Bioscience, Bedford, MA, USA) prepared with lethally irradiated 3T3 fibroblasts (density 20,000 cells/cm^2). Subconfluent primary cultures were detached from the flasks with trypsin and EDTA. Secondary cultures were obtained from the resulting cell suspension, seeded at a density of 30,000 to 50,000 cells/cm^2 on carrier sheets of hyaluronic acid ester polymer (Laserskin, FAB, Abano Terme [PD], Italy) in square Petri dishes prepared the day before with lethally irradiated 3T3 fibroblasts. The medium (CEC with EGF) was changed every 2 days until subconfluence. The sheets were placed in CEC medium without cholera toxin for 48 hours before grafting. After 2 weeks of culture, the autologous keratinocytes on the carrier sheets were grafted.

Grafting

All persons underwent grafting as outpatients. The achromatic graft area was cleaned with nonalcoholic disinfectant and anesthetized with 0.5% bupivacaine. The epithelium was removed with a pulsed erbium:yttrium-aluminum-garnet (Er:YAG) laser (Derma 20, ESC Medical System Ltd, Yognean, Israel) (2 mm spot-sized handpiece; pulse energy 5 J/cm^2) using four pulse series.

Epidermal residues after laser treatment were removed with saline-moistened gauze. The area was then covered with the keratinocyte sheets, held in place with petroleum jelly gauze, which was changed every 5 days. The carrier was left in place until it detached spontaneously (7–10 days).

The patients were allowed to go home after grafting and were cautioned against physical injury that could displace the dressing with negative effects on the graft taking. During this period, the patients were on systemic antibiotics. The dressing was removed after 7 to 10 days.

Narrow-Band UVB

Three weeks after grafting, patients started narrow-band UVB therapy (twice a week for 4 months, with an interruption of 1 month in the middle) (Spectra 305/350, Daavlin, Bryan, OH, USA). To stimulate melanocyte's activity and spread, the starting dose of 200 mJ/cm^2 was slowly increased to 1800 mJ/cm^2.

Results

Compliance was excellent in all cases. Apart from hypertrophic scars in two cases, no side effects were observed in our patients. The first repigmented areas were observed 1 month after grafting. They resembled pinkish brown pigmented islands that spread to form patches, which were sometimes hyperchromic. In

several cases, these patches joined up, completely banishing preexisting vitiligo lesions. Pigmentation was similar to that of the surrounding, normally pigmented skin. In some cases, spontaneous repigmentation of untreated areas was observed, revealing a stimulating effect of autologous grafting beyond the grafted areas. In most cases, pigmentation continued to increase up to 4 months after grafting. The best results were observed in focal and segmental vitiligo (repigmentation in 75% of patients); observation after 3, 6, 12, and 18 months showed complete repigmentation in 60% of patients, partial repigmentation ($\geq$ 50%) in 30% of patients, and negligible repigmentation ($<$50%) in 10% of patients (Figures 1–4). The results in generalized vitiligo were not excellent (repigmentation in 30% of patients). No Koebner's phenomenon was observed in the site of skin biopsy, and no cases of relapse were observed at follow-up after 18 months.

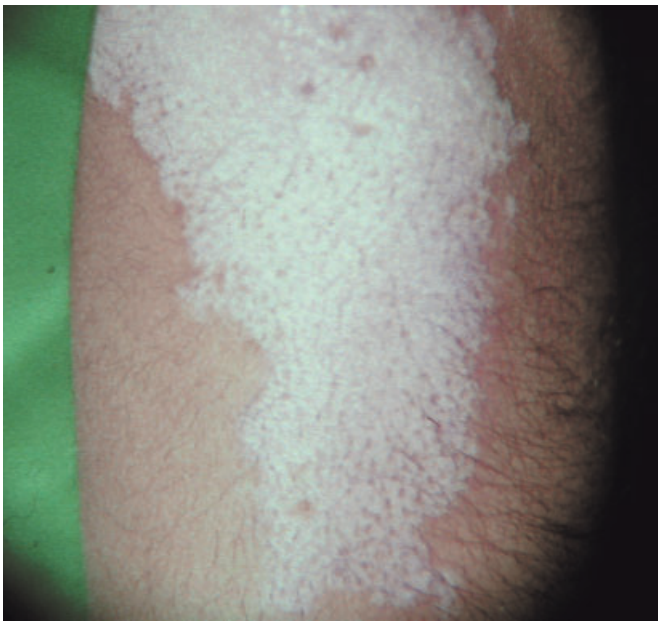

Figure 1. Achromic areas of the right arm of a 39-year-old patient, before treatment.

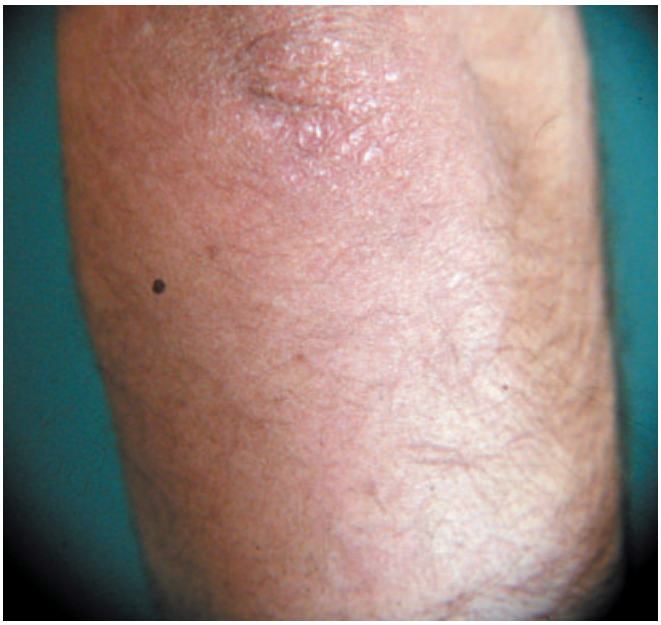

Figure 2. The same patient at the 12-month follow-up, showing complete repigmentation.

Discussion

Modern therapies for vitiligo aim to arrest the progression of the disease and promote pigmentation of achromic skin. Medical therapies for vitiligo include topical and systemic corticosteroids,[5] immunomodulators,

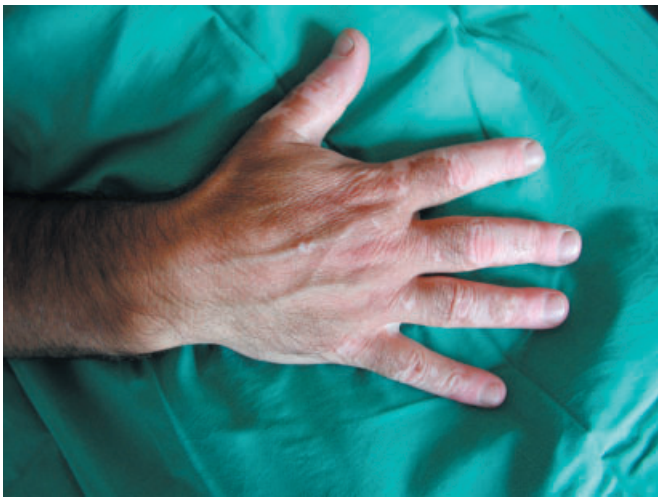

Figure 3. Dorsal area of the right hand of a 45-year-old patient, before grafting.

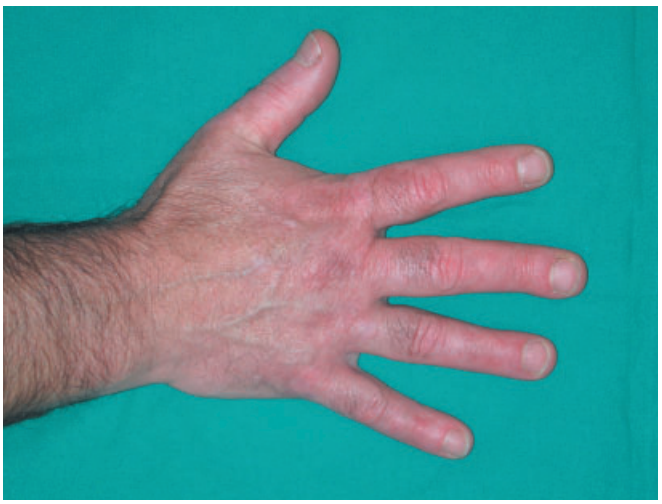

Figure 4. Complete repigmentation of the same area, 18 months after treatment.

vitamin D derivatives, systemic or topical photochemotherapy, narrow-band UVB,[6,7] and 308 nm excimer laser.[8] Only stable vitiligo can be treated using surgical therapies such as tissue grafts (full- and split-thickness grafts, suction blister grafts) and cell grafts (noncultured keratinocytes and melanocytes, cultured melanocytes, autologous cultured epidermal cells), which aim to transfer autologous melanocytes from pigmented donor skin to achromic sites.[2,17,18]

Autologous cultured epidermal grafting is a surgical technique based on composite cultures of autologous melanocytes and keratinocytes grown on perforated membranes of hyaluronic acid, which are grafted directly onto dermabraded recipient achromic areas.[19]

The great advantage of this technique is that large achromic areas can be treated in one session using cultured epidermal cells grown from a small biopsy of normally pigmented skin. The Er:YAG laser enables precise and homogeneous superficial de-epithelialization of achromic areas without scarring.[20]

Combination with narrow-band UVB therapy accelerates repigmentation of treated areas by stimulating melanocyte activity and spreading from the graft.[4,21,22] Unlike other surgical treatments, such as minigrafts, this procedure does not cause a "cobblestone" appearance.[4]

Our experience shows that excellent results can be obtained in focal and segmental vitiligo (repigmentation in 80 to 100% of cases). Partial repigmentation was achieved in generalized vitiligo (30% of patients).

Successful therapy of focal and segmental vitiligo depends on the body areas to be grafted. Good results are obtained on extensory limb surfaces (80–100%), whereas only 30% of patients with vitiligo lesions on the face, genitals, and extremities were successfully treated, probably owing to the difficulty of immobilizing the membranes.

Another important aspect is the possibility of repigmentation of untreated vitiligo areas after grafting. We noted the appearance of enlarging and progressively confluent islands of repigmentation inside untreated achromic lesions, which sometimes led to total repigmentation.

In our experience,[19] although epidermal culture alone gives good results,[20] it does not guarantee the confluence of repigmentation islets. The idea of treating vitiligo by combining innovative surgery and narrow-band UVB phototherapy sprang from the need to provide a new cellular component (in areas in which the follicular pool is often depleted) and to promote migration to obtain confluence of the islets of pigmentation.

The possible side effects of this therapy include temporary hyperpigmentation (which often disappears spontaneously after several months), long-lasting erythema, and transformation of cells into a malignant clone.[2–4,20] Using the Er-YAG laser, complications such as infections are rare because laser light sterilizes the wound. Bleeding is also rare because ablation of the epidermal layer is very precise. These complications were not infrequent in other surgical techniques used for vitiligo therapy.[20] We observed hypertrophic scars only in two cases.

Finally, the disadvantages of this therapy are the need for special laboratory equipment and the high cost of cell expansion and quality controls.[4,19,20]

Conclusion

Autologous epidermal grafting associated with narrow-band UVB irradiation proved successful for the treatment of stable vitiligo (especially focal and segmental), and clinical trials would now be appropriate. We observed repigmentation of ungrafted vitiligo lesions in some cases. The side effects are minor if the patients are selected accurately.

References

1. Mulekar SV. Melanocyte-keratinocyte cell transplantation for stable vitiligo. Dermatol Surg 2003;42:132–6.
2. Taneja A. Treatment of vitiligo. J Dermatol Treat 2002;13:19–25.
3. Yaar M, Gilchrest BA. Vitiligo: the evolution of cultured epidermal autografts and other surgical treatment modalities. Arch Dermatol 2001;137:348–9.
4. van Geel N, Ongenae K, Naeyaert JM. Surgical techniques for vitiligo: a review. Dermatology 2001;202:162–6.
5. Kanwar AJ, Dhar S, Dawn G. Oral minipulse therapy in vitiligo. Dermatology 1995;190:251–2.
6. Ongenae K, Geel N, Naeyaert JM. Surgical treatment of vitiligo. Pigment Cell Res 2003;16:590–1.
7. Njoo MD, Westerhof W. Vitiligo. Pathogenesis and treatment. Am J Clin Dermatol 2001;2:167–81.
8. Hadi SM, Spencer JM, Lebwohl M. The use of the 308-nm excimer laser for the treatment of vitiligo. Dermatol Surg 2004;30:983–6.
9. Falabella R, Barona M, Esobar C, et al. Surgical combination therapy for vitiligo and piebaldism. Dermatol Surg 1995;21:853–7.
10. Na GY. Autologous suction blister grafting for the treatment of vitiligo. Ann Dermatol 1996;8:9–24.
11. Falabella R, Escobar C, Borrero I. Treatment of refractory and stable vitiligo by transplantation of in vitro cultured epidermal autografts bearing melanocytes. J Am Acad Dermatol 1992;26: 230–6.
12. Olsson MJ, Julhin L. Repigmentation of vitiligo by transplantation of cultured autologous melanocytes. Acta Derm Venereol 1993;73: 49–51.
13. Olsson MJ, Juhlin L. Transplantation of melanocytes in vitiligo. Br J Dermatol 1995;132:587–91.
14. Kumagai N, Uchikoshi T. Treatment of extensive hypomelanosis with autologous cultured epithelium. Ann Plast Surg 1997;39:68–73.
15. Olsson MJ, Juhlin L. Long term follow-up of leucoderma patients treated with transplants of autologous cultured melanocytes, ultrathin epidermal sheets and basal cell layer suspension. Br J Dermatol 2002;147:893–904.
16. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 1975;6:331–43.
17. Halder RM, Young CM. New and emerging therapies for vitiligo. Dermatol Clin 2000;18:79–89.

18. Westerhof W, Löntz W, Braathen L, et al. Vitiligo: news in surgical treatment. J Am Acad Dermatol Venereol 2001;15:510–1.
19. Andreassi L, Pianigiani E, Andreassi A, et al. A new model of epidermal culture for the surgical treatment of vitiligo. Int J Dermatol 1998;37:595–8.
20. Guerra L, Primavera G, De Luca, et al. Erbium:YAG laser and cultured epidermis in the surgical therapy of stable vitiligo. Arch Dermatol 2003;139:1303–10.
21. Scherschun L, Kim JJ, Lim HW. Narrow-band ultraviolet B is a useful and well-tolerated treatment for vitiligo. J Am Acad Dermatol 2001;44:999–1003.
22. Ongenae K, Van Geel N, Naeyaert JM. Autologous cellular suspensions and sheets in the treatment of achromic disorders: the need for future controlled studies. Dermatology 2001;202: 158–61.