Dissertations

Vitiligo and Piebaldism. Treatment of Leucoderma by Transplantation of Autologous Melanocytes

Mats J. Olsson
Section of Dermatology and Venereology, Department of Medical Sciences, Uppsala University, University Hospital, SE-751 85 Uppsala, Sweden. mats.olsson@medsci.uu.se.

Leucoderma is a term used for disorders, congenital or acquired, in which the skin turns white in some areas. This is often due to a lack of melanocytes in the epidermis and sometimes also in the matrix of the hair follicles. Piebaldism, different kinds of vitiligo and some cases of chemical leucoderma are examples of this. Many different medical treatments have been tried over the years, but the results have often not been satisfying and the response to medical treatments for patients with piebaldism and segmental vitiligo has been very limited.

More recently, however, surgical methods have been introduced to achieve skin repigmentation. In order to improve the surgical options for restoring the epidermal melanin unit, we have worked out new and modified methods in an attempt to cover the needs for both extensive and small areas of leucoderma.

Clinic and aetiology

Piebaldism

Piebaldism is inherited as autosomal dominant, congenital, stable leucoderma affecting 1:14,000. The typical piebald macules are located in the centre of the forehead with a characteristic white forelock, ventral part of the trunk and symmetrically bilateral on the mid parts of the legs and arms.

Vitiligo

Vitiligo is an acquired disorder with an unpredictable course, affecting about 1–4% of the population all over the world.

Based on distribution and localization, vitiligo can be clinically grouped as vitiligo vulgaris (generalised vitiligo), halo naevi and segmental vitiligo.

Vitiligo vulgaris is the most common type of leucoderma and the distribution is most often symmetrically bilateral. Köbner reactions after physical injuries to the skin, such as cuts, abrasions and burns, are frequently seen.

Vitiligo vulgaris is considered by many researchers to be an autoimmune disorder and has been shown to exist in association with other disorders such as thyroiditis, diabetes type I and alopecia areata, all of which are suspected of having an immune-mediated aetiology.

It has been postulated that the genetics of the immune system may be a predisposing factor for developing autoimmune vitiligo. Increased prevalence of certain HLA haplotypes have been seen, and genetic factors probably play a role in the pathogenesis, but the exact genetic alteration(s) re-
mains to be identified. The bilateral progression with infiltration of mononuclear cells in the lesional margins indicates an up-regulated immunological activity. T cells are abundant in these infiltrates and the existence of melanocyte-specific cytotoxic T-lymphocytes has been shown. Autoantibodies against cell-surface antigens of melanocytes have been found as well as antibodies against melanocyte-specific proteins such as tyrosinase, TRP-1, TRP-2 and Pmel-17 in sera from patients with vitiligo vulgaris.

**Halo naevi** occurs in approximately 1% of the population. The depigmentation starts in the border of the naevi and progresses centrifugally, giving rise to approximately Ø 2–4 cm maculae. In histology, a dense lymphocytic infiltration with a relatively high portion of CD8+ cells has been observed.

**Segmental vitiligo.** In about 5–10% of the total vitiligo population the achromic spots appear as a unilateral area, not extending beyond the mid-sagittal line of the body. It follows the whole or parts of a region innervated by one or neighbouring sensory nerve segments. The trigeminus region seems to be the most common location. The follicular melanocytes are most often affected and the hair in the lesion white. Spontaneous repigmentation is uncommon.

A neuron hypothesis suggests that local abnormalities in the expression of neuropeptides or other neurochemical mediators secreted by the nerve endings are capable of harming the melanocytes. Communication between the nervous system and epidermal melanocytes with direct contact between the intra-epidermal nerve endings and the melanocytes has been demonstrated with confocal and electron microscopy, and the expression of NPY (neuropeptide Y) and CGRP (calcitonin gene-related peptide) has been shown to be increased.

**Methods**

The work described in my thesis, defended in September 2001, was based on the establishment of three new models for transplanting autologous melanocytes from normally pigmented skin onto denuded leucodermic areas. Before the transplantations were begun, profound cell biology work was carried out, resulting in a defined melanocyte cell culture medium which we felt safe to use, i.e. free from serum, pituitary extracts and phorbol esters. It is now marketed as the M2-medium by PromoCell in Heidelberg, Germany. The surgical transplantation methods used were: 1) expansion of melanocytes from a shave biopsy by culture under serum-free conditions, 2) transfer of ultra-thin epidermal sheets from pigmented skin, 3) transplantation of a suspension of basal epidermal cells obtained from a shave biopsy.

The treatment outcome in a short perspective for each respective method was individually reported and finally supplemented with a comparative long-term follow-up study in 132 patients treated with one or several of these methods on a total of 176 transplantation occasions at over 1,000 individual sites, between the years of 1993 and 1999. The long-term follow-up statistical analysis was of importance for pinpointing the type of patient that would benefit the most from this type of treatments and which kind of patient would be less suitable. The results of the transplantations were related to the method used, anatomical distribution of the lesions, disease duration, age of the patient, gender, progression of the lesions, extent of vitiligo, associated diseases and some other variables.

**Results**

Patients with stable forms of leucoderma, i.e. segmental vitiligo, focal vitiligo and piebaldism, almost always respond with a complete, 100% repigmentation regardless of the method used or the anatomical location treated and with no loss of pigmentation seen at follow-up several years post-surgery. Patients with extensive and unstable vitiligo vulgaris were less often fully repigmented. Several vitiligo vulgaris patients had experienced some loss of pigmentation in the treated areas. Vitiligo vulgaris patients treated using the three different methods displayed at follow-up an average percentual pigmentation of the treated areas as follows: cultured melanocytes 42%, 6.6 years post-surgery (y ps), ultra-thin epidermal sheets 59%, 4.2 y ps, and basal-layer suspension 49%, 3.5 y ps, respectively. When comparing different anatomical locations it was clear that the smooth areas of the lower leg and trunk responded best and that hands, feet and elbows were the most diffi-
cult areas for achieving repigmentation. Significant strong correlation was found between total body surface of vitiligo-affected skin and transplant outcome, showing that patients with extensive generalised vitiligo had a much lower odds ratio in achieving good repigmentation compared to similar patients with less extensive areas. It was also evident that in the group of patients with vitiligo in a progressive state, it was more likely that some or all of the repigmentation obtained was lost during the follow-up period. Young patients and patients with short disease duration responded better than older patients and patients with long disease duration. Initial postinflammatory hyperpigmentation of the transplanted lesion was common, especially after the treatment with ultra-thin epithelial sheets. Ultra-thin epithelial sheets are not suited for areas with lots of movement, like the elbows or eyelids. In these areas the methods with cells that were free in suspension gave much better results. The group of vitiligo vulgaris patients with associated autoimmune hypothyroidism responded somewhat less well to the treatment compared to those without hypothyroidism.

Discussion and Conclusion

Autologous melanocyte transplantation is an effective option for obtaining repigmentation in patients with stable leucoderma, such as in piebaldism, segmental vitiligo, focal vitiligo and in patients with limited and stable vitiligo vulgaris. Corticosteroids or UV-therapy is not to recommend in piebaldism or segmental vitiligo since there is no autoimmunity to reduce or any melanocytes to recruit from the hair follicles. Here transplantation is the treatment of choice.

The findings that patients with extensive and active vitiligo vulgaris responded less well to the transplantation and that they also had a reduced chance of regaining their repigmentation, might indicate that they had an up-regulated autoimmunity against the melanocytes and should therefore not be considered for transplantation. Here it is important to stop the progression of the disease and if possible reverse it by suppressing the immune attack and to stimulate the recruitment of new pigment cells from the hair follicles or the stem-cell containing bulge area by light-treatment. The discovery that patients with associated autoimmune hypothyroidism also responded less well also supports the importance of immunology in the aetiology, progress of the disease and probability of achieving a good and longstanding treatment outcome.

Spontaneous repigmentation, no appearance of new spots over the past years, a non-extensive total vitiligo area, young patients and a short disease duration are good indications when decisions are to be made about whether to treat by transplantation or not.

Future

In the field of generalised vitiligo the research must be continued to forward a better understanding of the underlying mechanisms of melanocyte destruction. New work in characterising the HLA-system and the lymphocyte CD-expression of the cells from this group of patients is about to be started in Uppsala.

The Nordic Vitiligo Association was founded recently and its Web site can be visited at www.vitiligo.nu. Researchers and clinicians interested in the field of pigmentation are more than welcome to contact me for more information. Our University Vitiligo Information page can be visited at www.medsci.uu.se/dermatology/vitiligo.

List of original publications