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Transplantation of Human Malignant and Premalignant Skin Lesions of Epidermis to Nude Mice

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Abstract—*Lesions of solar keratoses and squamous cell carcinoma maintained their histological appearance and an increased tritiated thymidine autoradiographic labelling index after being grafted on to nude mice. However, the values for their mean epidermal thickness and individual cell size appeared to decrease slightly during the 24-week period of study. As judged by the immunolocalization of involucrin antibodies, the grafts maintained a human epidermal antigenic profile. However, immunolocalization studies with HLA antibodies showed only a patchy positivity in the original premalignant lesions and were negative after grafting. These results indicate the potential value of the nude mouse as a model for studying the progress of premalignant and malignant skin lesions in an immunologically privileged non-human site and further indicate that solar keratoses can be maintained independently of systemic donor influences.*

INTRODUCTION

SOLAR keratoses (SK) are premalignant skin lesions usually present on the sun exposed areas of a lightly pigmented, elderly Caucasian population. The incidence of these premalignant lesions is high in an elderly population. One report by Marks *et al.* [1] stated that the incidence was 56.9% of the population over 40 yr of age in Maryborough, Australia. Solar keratoses possess many of the cytological features associated with a premalignant state, although very few of these lesions appear to transform to frankly malignant squamous cell carcinomas (SCC) [2]. The low rate of progression to malignancy may be due to spontaneous resolution or to being held in check by some unknown mechanism. The so-called lichenoid keratoses [3] may represent lesions in which immunological rejection has begun.

A number of studies have shown that the athymic nude mouse is a useful model for the *in vivo* study of human tissues [4, 5]; in particular, human skin lesions can be maintained for the whole lifespan of the mouse [6]. This study was undertaken to determine the fate of SKs and SCCs in a non-human immunologically privileged environment and to assess the utility of the system as a model for the study of epithelial premalignancy.

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MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM) and foetal calf serum (FCS) were obtained from Gibco Biocult (Paisley, U.K.). Involucrin antiserum was a gift from Dr Fiona Watts (Kennedy Institute).

Monoclonal antibody against human HLA, A, B and C and fluorescein isothiocyanate (FITC) anti-mouse IgG were purchased from Sera Laboratories (Crawley Down, U.K.). [^{3}H]Thymidine (sp. act. 25 Ci/mmol) was bought from Amersham International PLC (Amersham, U.K.).

Mice

Congenitally athymic BALB/c nu/nu female mice aged 5–7 weeks were used as receipients for the human skin lesions. Mice were kept under pathogen-limited conditions in covered filtered cages containing autoclaved sawdust and received

a diet of irradiated pellet and acidified water (pH 2.8) *ad libitum*. No antibiotics were used.

Grafting technique

Tissue was collected at the time of biopsy and divided into two. Half was placed in formalin for routine histological studies, the remainder placed in DMEM containing 10% FCS and transported to the animal house. The grafting technique used was a modification of that of Briggaman and Wheeler [7]. The mice were anaesthetized with ether and a graft bed approximating to the size of the human lesion was prepared on the dorsal flank region of the nude mouse (full thickness skin was removed). The lesion was placed on the bed, covered with Vaseline, gauze and adhesive plaster, and left in place for 14 days.

Histopathological techniques

The original and grafted lesions were fixed in 10% formalin, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin.

A detailed study was made of each lesion before and after grafting. Particular attention was paid to cellular atypia, epidermal thickness and epidermal cell size, para- and hyperkeratosis and the presence and extent of dermal inflammatory cell filtrate. Epidermal thickness was measured both in absolute terms (μm) and as the mean number of cells constituting the thickness of the viable epidermis using a 'Quantimet 720' image analysis device (Cambridge Instruments Ltd.) Epidermal cell size was calculated as mean keratinocyte height (MKH) (μm) [8].

Immunological techniques

Indirect immunofluorescence. Paraffin embedded grafts were sectioned at 5 μm. Sections were dewaxed and incubated with antiserum to involucrin (obtained from Dr Fiona Watt) for 30 min at 37°C. Sections were washed three times in phosphate-buffered saline (PBS) and air-dried; fluorescein isothiocyanate (FITC) sheep anti-rabbit IgG was added and the slides were further incubated for 30 min at 37°C. The sections were subsequently washed three times with PBS with a final wash with distilled water, air-dried and mounted using 'Hydromount' (Raymond Lamb). The above method was used for HLA localization in grafted tissue using frozen sections (7 μm) and incubating the HLA antiserum for 1 hr at 37°C.

Results were read immediately under an epifluorescence Nikon u.v. microscope.

Controls were run in parallel, using immunonegative sera for negative controls and normal human skin and normal mouse skin as positive controls.

Labelling index determination

[^{3}H]Thymidine (sp. act. 25 Ci/mmol) was injected intraperitoneally into the nude mice at a concentration of 1 μCi/g 1 hr prior to termination. Tissue was placed in 10% neutral buffered formalin, processed and embedded in paraffin wax. Five-micron paraffin sections were prepared for autoradiography using a modification of the dipping film technique of Joftes and Warren [9]. The number of labelled basal and suprabasal epidermal cells were counted and expressed as a percentage of the number of basal cells (labelling index; LI).

RESULTS

To date a total of 34 grafting experiments have been performed, of which 23/30 SK and 3/4 squamous cell carcinomas (SCC) have been maintained in a viable state on nude mice for periods of up to 6 months. Twenty lesions have been removed from the mice, all of which maintained a histological appearance similar to that of the original lesions.

Histologically all the original lesions (e.g. Fig. 1a) showed the typical changes associated with SK [10]. The grafted lesions (e.g. Fig. 1b — this lesion had been present on the mouse for 4 months) maintained histological appearances quite similar to the original lesions, possessing the typical histological and cytological features of SK referenced above. There was a distinct junction between the relatively thick human epidermis and the thinner mouse epidermis at either margin. Furthermore, the dermis of the grafts was observed to be quite different in overall morphology from that of the host. The same was true for the small number of SCCs examined and no major difference was noted in the appearance of the grafted lesions as compared to that of the original lesions.

Measurement of mean epidermal thickness (MET) and mean keratinocyte height (MKH) indicated that the grafted lesions in general had a slightly thinner epidermis than the original lesions and also that the epidermal cells are smaller in the grafts.

Simple observation of the site grafted showed that the grafted lesions were quite distinct from the mouse skin (Fig. 2). However, to further validate the human origin of the tissue we used an antibody specific for human involucrin [11].

In sections incubated with involucrin antibodies the human epidermis stained well but the stratum corneum (SC) did not stain (Fig. 3). The junction between mouse and human skin was always quite distinct and easily identified.

Frozen sections stained for HLA showed no staining of the epidermis either in the grafted

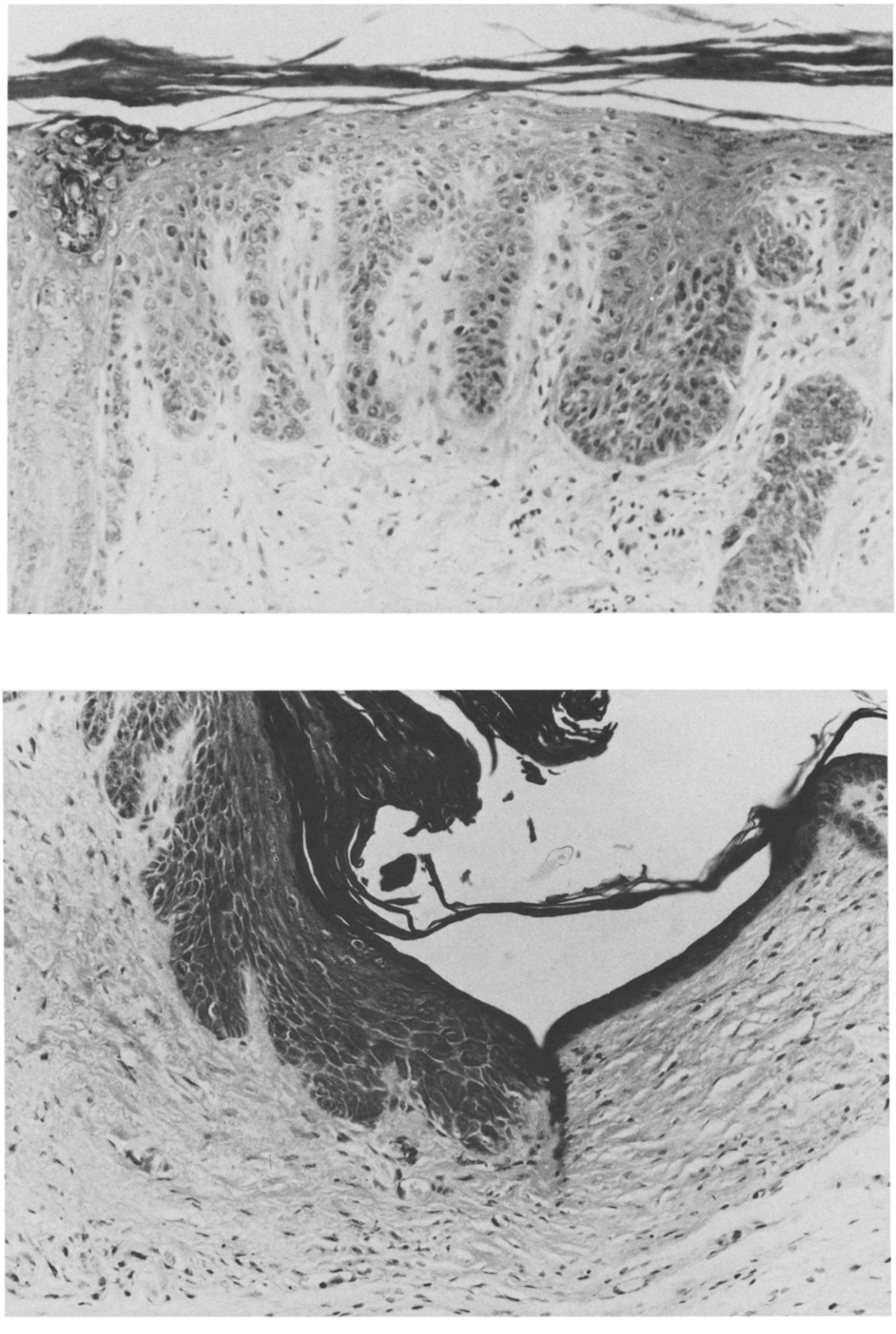

Fig. 1. (*a*) *Original lesion of a solar kerotosis* (*H* & *E*, ×90). (*b*) *Histological appearance of grafted lesion present on a nude mouse for* 4 months (*H* & *E*, ×90).

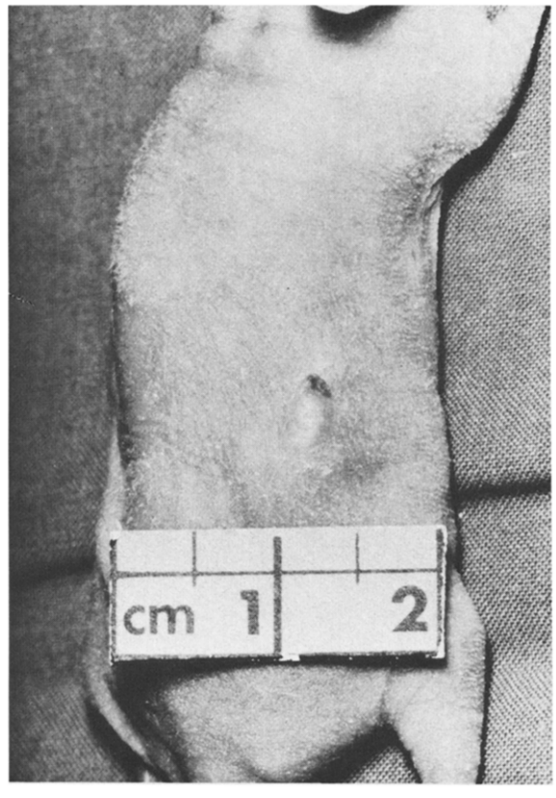

Fig. 2. Solar keratosis in place on nude mouse host.

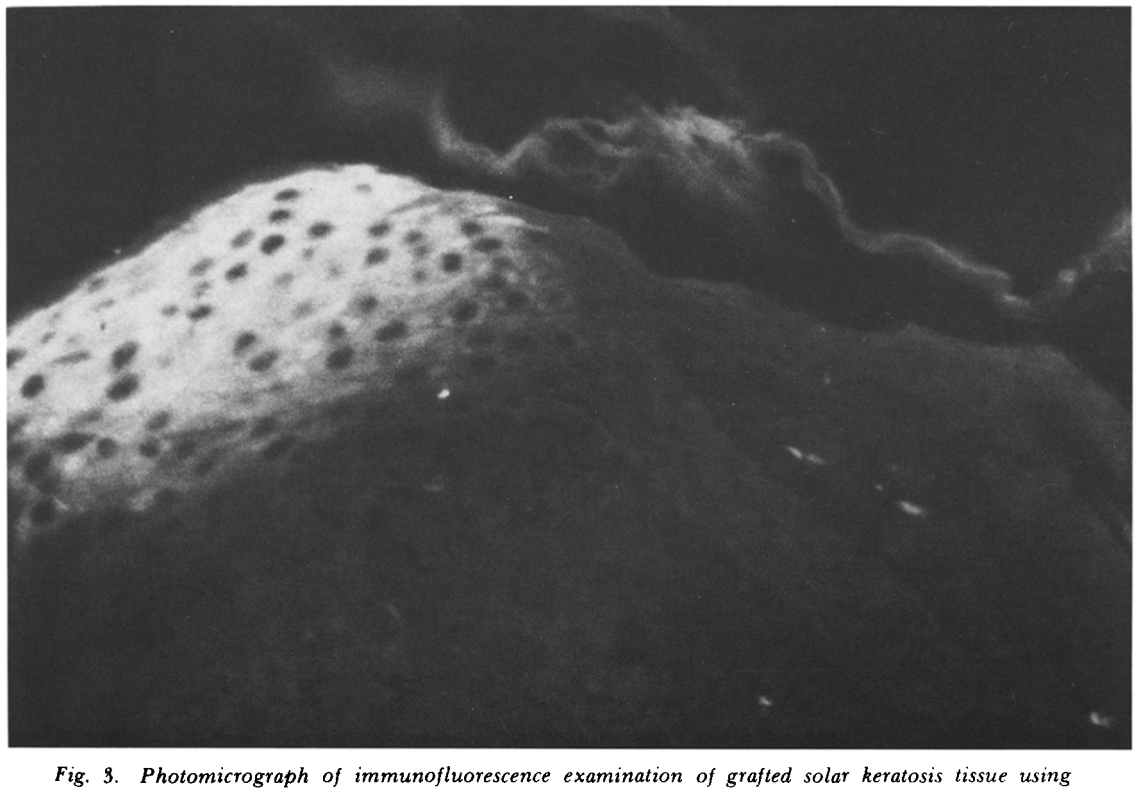

Fig. 3. Photomicrograph of immunofluorescence examination of grafted solar keratosis tissue using involucrin antiserum.

lesions or in the normal mouse skin. Normal human buttock skin demonstrated a bright membrane fluorescence of all epidermal cells.

The mean LI of the grafted lesions was higher ($11.2 \pm 1.9\%$) than that usually observed in normal human skin (4-8%, depending on age, site and technique [12, 13]). The LI of the lesions was also higher than that of the adjacent nude mouse epidermis ($3.6 \pm 1.9\%$).

DISCUSSION

These results indicate that grafts of solar keratoses on to nude mice are maintained as *in vivo* in man and may be used as a model for the study of human premalignant lesions of epidermis. Grafted lesions showed no degenerative changes or significant morphological alterations as stated to occur with other skin lesions [7, 14]. Both epidermis and dermis of the grafts survived and it is not possible to determine from these experiments whether transplantation of both is obligatory for graft success.

The acceptance rate of 73% for the grafted keratoses compares well with those of other studies in which skin lesions were grafted to nude mice. Briggaman and Wheeler [15] reported a 75% success for transplantation of psoriatic epithelium and a 93% success for lamellar ichthyosis. Kondo and Azo [16] were able to grow only one tumour after numerous attempts at implanting squamous cell carcinoma cells into nude mice.

Our results indicate that the epidermal thickness and epidermal cell size are in general slightly decreased in the grafted lesions; however, all the features of the original lesion are maintained. The enhanced epidermal cell production usually associated with SK [12, 17] persisted in the grafted epidermis of SKs.

The immunological identity of the grafted lesion is preserved, at least with regard to the envelope precursor protein involucrin, which stains only the human epidermis of the grafted lesions. However, the HLA A, B and C surface antigens were absent in grafted lesions and gave a patchy distribution in non-grafted solar keratoses. These results are similar to those found by Turbitt and Mackie [18], who looked at B2 microglobulin and found a loss of this antigen from the surface of malignant cells. Our group have also found loss of intercellular staining in SCC using pemphigus antibody [19]. Clearly, the transformation to epidermal malignancy involves extensive changes in cell membranes with loss of several antigenic components. No inflammatory infiltrates were seen in the human dermis of the grafted lesions, which was also found by Haftek *et al.* [20]; once grafted, the lesions are cut off from any immunological influence of the human host and the mouse is not able to mount a cell-mediated response to the SK.

These studies indicate that human SKs maintain their histologic appearance, increased LI and immunological identity after grafting to nude mice. No progression of the grafted lesion to frank malignancy was observed. The lesions did not disappear or revert to normal, but remained as intact SK. Thus, interestingly, the grafts remained static on the host and did not either regress or progress. This may indicate that they are not predominantly under the influence of immunological or other systemic influences when *in situ*. Further studies are in progress to characterize the determinants of progression of premalignant keratoses to squamous cell carcinoma and will be reported later.

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