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# UV-related Skin Conditions and Langerhans' Cell Populations in Human Skin

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**Chronic sun exposure and cumulative exposures to PUVA are associated with a risk of development of non-melanoma skin cancer. Acute UV irradiation reduces the number of epidermal antigen-presenting Langerhans' cells in the skin. Alterations in the Langerhans' cell population could be relevant to a disturbance in the cutaneous immune response in UV-exposed skin. Therefore the density of CDla+ and ATPase+ Langerhans' cells in epidermal sheets from the hand, buttock and in some cases face was examined in PUVA-treated patients ($n = 8$) and in patients with actinic keratosis ($n = 13$) or basal cell carcinoma ($n = 16$). The number of Langerhans' cells was significantly lower on the hand compared with that on the face and buttock ($p < 0.05$). There was no difference in Langerhans' cell distribution between the different diagnostic groups and controls, and there was no age-related change in Langerhans' cell density. These results indicate that patients who develop actinic keratosis or multiple basal cell carcinomas do not differ in Langerhans' cell density from healthy controls and that cumulative sunlight or PUVA exposure does not lead to a persistent reduction in Langerhans' cell numbers.** ***Key words: Sun exposure; PUVA; Skin tumours; Age.***

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Langerhans' cells (LC) participate in cell-mediated immune reactions within the skin and are important for the induction of contact hypersensitivity (1). LC may also be important in immunosurveillance against tumour antigens in the skin (2). Ultraviolet irradiation is known to decrease the number of LC. A depletion of LC has been shown after UVB (290–320 nm) as well as PUVA = psoralen + UVA (320–400 nm) treatments (3, 4). This effect on LC has been reported as an acute result of UV radiation, and the LC numbers return to normal 2–3 weeks after irradiation (3, 5). Sun exposure induces actinic keratosis and squamous cell carcinoma and is an important factor associated with the induction of basal cell carcinoma (6). Long-term PUVA treatment with high cumulative doses also increases the risk of developing skin cancer (7–9). To investigate if chronic sun exposure or cumulative exposure to PUVA treatment leads to a permanent reduction of LC on exposed skin and if a depletion of LC is associated with the development of skin tumours, the density of LC in chronically sun-exposed and covered skin was examined. Skin biopsies were obtained from patients with actinic keratosis, basal cell carcinoma (BCC) of the face or multiple BCC of the trunk. Biopsies were also obtained from PUVA-treated psoriasis patients, who had received an average cumulative UVA dose of 2,300 J/cm$^2$, and from healthy controls.

## MATERIAL AND METHODS

### *Subjects*

Thirteen patients, 10 males and 3 females, with a mean age of 67 years (range 55–86) with actinic keratosis of the face and hands and 16 patients, 9 males and 7 females, mean age 67 years (range 41–83) with multiple superficial BCC (more than 3 BCC) on the trunk participated in the study. Eight PUVA-treated psoriasis patients, 5 males and 3 females, mean age 58 years (range 38–73), and 19 healthy controls, 6 males and 13 females, mean age 53 years (range 20–89), were also included in the study. In the PUVA group, patients had been given 8-methoxypsoralen orally and had received an average of 220 (161–330) treatments of PUVA or an average UVA dose of 2,300 J/cm$^2$ (920 J/cm$^2$–3,248 J/cm$^2$) but had only used emollients for the last year, as their psoriasis was at present in a mild stage. None of the PUVA-treated patients had had skin cancers. Biopsies were taken from tumour-free skin on the back of one hand and buttock. All participants were Caucasians with skin types I–III according to Fitzpatrick (10). From 16 patients, 7 males and 9 females, with a mean age of 75 years (range 57–89) with BCC of the face, biopsies were obtained from perilesional skin. These patients had participated in a different study reported elsewhere (11), and the LC density on the face in these patients was compared with that on the hands in the 16 patients with multiple BCC of the trunk. The study was performed between October and mid-April, and none of the participants had had UV exposure during 6 weeks prior to the study. After intradermal injection of lidocain, 3 mm punch biopsies were obtained from the back of one hand and buttock or from the face. Informed consent was obtained and the study was approved by the local ethical committee.

### *Staining technique*

The skin samples were immediately incubated for 2 h in EDTA. After incubation, the epidermis was separated from the dermis and divided into halves. One half was fixed in acetone for 20 min. The other half was fixed in formaldehyde-cacadylic acid solution at 4°C over night and then stained for ATPase activity (12). Acetone-fixed specimens were washed in PBS and incubated in CDla (DAKO-T6, code M 721, Dakopatts a/s Denmark) in PBS (1:50 dilution) at 4°C over night. After incubation with the primary antibody, the epidermal sheets were washed in PBS and incubated with peroxidase-conjugated rabbit anti-mouse antibody (P161 Dakopatts) for 30 min at 37°C. After incubation, the specimens were washed in PBS and developed with 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma Mo.) 6 mg in 10 ml 0.05 M Tris buffer with addition of 3% hydrogen peroxide for 5 min. and mounted dermal side up in Mountex mounting medium (13).

### *Counting of LC*

ATPase-positive and CDla-positive cells were examined by light microscopy. The mean number of LC/mm$^2$ was determined by counting 5–15 random fields at 400× magnification using an optical grid.

### *Statistical analysis*

Differences in the distribution of LC between the diagnostic groups, age groups, skin types or sexes were investigated by analysis of variance. The significance of differences of paired data for LC counts on the hand and buttock was calculated using the paired Student's *t*-test. The LC density in skin samples from the face of 16 patients with BCC of the face was compared with the LC density on the hand in 16 patients with multiple BCC of the trunk, and the significance of this difference was calculated using Student's *t*-test.

Table I. *The number of Langerhans' cells/mm² on the hand and buttock in different diagnostic groups*

The numbers show mean ± SD. The difference between the locations is significant ($p < 0.05$) for each group.

| | ATPase-positive LC | | | |
|---|---|---|---|---|
| | Hand | N | Buttock | N |
| Actinic keratosis | 438±130 | 13 | 643± 75 | 13 |
| Basal cell carcinoma* | 434± 67 | 16 | 644± 50 | 16 |
| PUVA | 438±141 | 8 | 637±155 | 8 |
| Control | 396±105 | 19 | 644± 85 | 19 |
| | CDla-positive LC | | | |
| | Hand | N | Buttock | N |
| Actinic keratosis | 390±107 | 13 | 524± 99 | 13 |
| Basal cell carcinoma* | 371±104 | 13 | 511± 83 | 12 |
| PUVA | 394±122 | 8 | 475± 79 | 8 |
| Control | 392±101 | 19 | 560± 70 | 19 |

* Patients with multiple BCC of the trunk.

## RESULTS

Analysis of variance demonstrated that there were no significant differences in LC density between the different diagnostic groups or in relation to age, sex or skin type. The mean LC density was significantly lower on the hand than on the buttock in each diagnostic group, as shown in Table I. There was no difference in the morphology of LC between the different diagnostic groups, nor between the different locations. There was a lower number of LC on the hand compared with the buttock for both LC markers in each age group ($p < 0.05$), with the exception of CDla + LC in the age group 20–39 (Table II). In this group, the CDla staining tended to give lower values on the hand compared with the buttock, but the difference was not statistically significant. There was no significant age-dependent reduction in LC numbers on exposed or covered skin.

There was no significant difference in LC distribution between males and females (Hand; ATP: males 430 ± 100, females 475 ± 115. CDla: males 403 ± 108, females 368 ± 98, Buttock; ATP: males 633 ± 63, females 650 ± 105, CDla: males 503 ± 80, females 555 ± 83). The mean LC density was higher in the face of patients with BCC of the face (ATPase 658 ± 110, CDla 652 ± 83) as compared with the LC density of the hand (ATPase 434 ± 67, CDla371 ± 104) in patients with multiple BCC of the trunk ($p < 0.05$). The number of CDla+ cells was slightly but significantly lower than the number of ATPase+ cells ($p < 0.05$).

## DISCUSSION

The distribution of LC has previously been investigated, and in some studies the highest density of LC was found in the face (14–16). In other studies, a lower number of LC was found in chronically sun-damaged skin than in non-exposed locations, and it has therefore been suggested that non-melanoma skin cancer arises in areas deficient in LC due to chronic sun exposure (17, 18). A reduction in LC numbers within hours after acute UV irradiation is well documented, and the LC density is normalized over a 2–3 week period after short-time UVB as well as PUVA exposures (3, 5, 13). The effect on LC after chronic sun exposure or high cumulative PUVA doses is less well known. To evaluate if skin conditions such as actinic keratosis and BCC are associated with a depletion of LC and whether high cumulative PUVA doses lead to a permanent reduction in LC, the distribution of LC was examined in patients with actinic keratosis, nodular or superficial BCC, PUVA-treated patients and healthy controls. The LC density in two different anatomical locations was compared in the same individuals, chronically sun-exposed skin (hand) and covered skin (buttock). Two different markers, ATPase and CDla, were used to visualize the epidermal LC. There was a consistently lower number of CDla+ cells than ATPase+ cells, which could be explained by the fact that CDla is a more specific marker for LC, whereas ATPase can stain inflammatory cells as well. The number of LC was significantly reduced on the hand compared with the buttock in each diagnostic group. There was no difference in LC counts between the different diagnostic groups for either covered or sun-exposed skin, and the morphology of LC was similar.

Taking into consideration the small number of subjects in each diagnostic group, this study indicates that neither long-term exposure to sunlight, resulting in actinic keratosis, nor high cumulative PUVA exposure reduces LC density permanently; patients who develop multiple BCC do not have a depletion of LC compared to age-matched controls. When the LC density in paired samples from the hand and buttock in all individuals was examined by age group, there was also a significant difference in LC number between the two locations for both staining techniques with the exception of CDla+LC in the youngest age group. However, in this group (age 20–39)

Table II. *The number of Langerhans' cells/mm² on the hand and buttock in different age groups*

The numbers show mean ± SD. Significance between the locations in each group, $*p < 0.05$, $**p < 0.01$. NS, not significant

| Age | ATPase-positive LC | | | |
|---|---|---|---|---|
| | Hand | N | Buttock | N |
| 20–39 | 417±123 | 6 | 620*±87 | 6 |
| 40–59 | 403± 98 | 16 | 658*±97 | 16 |
| 60–79 | 434±111 | 25 | 646*±90 | 25 |
| 80– | 430±113 | 10 | 625*±55 | 10 |
| Age | CDla-positive LC | | | |
| | Hand | N | Buttock | N |
| 20–39 | 413±118 | 5 | 562 NS± 82 | 5 |
| 40–59 | 381±112 | 16 | 543** ± 79 | 15 |
| 60–79 | 380±100 | 23 | 520** ±102 | 23 |
| 80– | 390±103 | 9 | 500** ± 43 | 9 |

there was a small number of patients and a large variation in the number of CDla+ LC on the hand, making the significance of this finding uncertain. There was no age-related reduction in LC counts within each diagnostic group or when the groups were pooled (56 subjects). This is in agreement with a study by Czernielewski et al., who examined 95 healthy individuals and found a lower LC density on sun-exposed skin (hand) compared with buttock skin, but no age-related differences (19). In the present study, the LC density on the face and hand was also compared. This comparison of LC distribution was made between different individuals, but from two locations rather equally exposed to UV light. Alterations in LC due to age and/or chronic UV exposure observed in previous reports (17, 18) could not be demonstrated in this study. However, in the work by Gilchrest et al. the number of patients was small, and in only 3 of 7 patients was there a significant difference between the paired sun-protected and sun-exposed specimens (17).

The present data show that the mean number of LC was higher on both the chronically sunexposed face and the sun-protected buttock as compared with the chronically sun-exposed hand. It therefore seems reasonable to suggest that this disparity in LC distribution reflects anatomical differences rather than UV-related differences (19).

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