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Effect of Simulated Sunlight on Langerhans' Cells in Malignant Melanoma Patients

A. V. POWLES,[1] G. M. MURPHY,[4] A. J. RUTMAN,[1] G. HAFFENDEN,[2] R. D. ROSIN,[3] J. L. M. HAWK[4] and LIONEL FRY[1].

Departments of [1]Dermatology, [2]Histopathology & [3]Surgery, St. Mary's Hospital, London W2, and [4]Department of Photobiology, Institute of Dermatology, St. Thomas' Hospital, London, England

The effect of artificial sunlight on the number and HLA class II expression of Langerhans' cells was studied in 10 patients with malignant melanoma and 10 control volunteers. The total number of Langerhans' cell decreased in both groups but at 96 h there was a greater and significant decrease ($p < 0.01$) in the number of Langerhans' cells in the melanoma group, compared with controls. This decrease persisted and was still greater in the melanoma group ($p < 0.02$) at one week post-irradiation. There was a rise in Langerhans' cell count over the following 3 weeks in both groups. Unexpectedly, during this period in the melanoma group—but not controls—there was a significant median peak rise above pre-irradiation levels ($p < 0.001$). Alteration in the response of Langerhans' cells to sunlight may play a part in the aetiology of malignant melanoma. ***Key words: Immunopathology; Antigen-presenting cells; Ultraviolet light.***

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A. V. Powles, Department of Dermatology, St. Mary's Hospital, London W2 1NY, England.

There is accumulating evidence that sunlight plays an important aetiological role in malignant melanoma (1). It appears that the rising incidence of malignant melanoma is not due to long-term sun exposure, but to short intermittent bursts of intense sunlight such as obtained on holiday (2). The Langerhans' cell (LC) is important in immune surveillance and therefore may play a role in the clearance of potentially dangerous neo-antigens. Exposure to either ultraviolet A (UVA) or ultraviolet B (UVB) irradiation in normal individuals has an injurious effect on LC, as they have been shown to decrease within 24–48 h, but return to pre-irradiation levels within 2 weeks (3). The purpose of this study was to examine and compare the effect of artificial sunlight on the number and HLA-DR expression of LC in malignant melanoma patients and control subjects.

METHODS

Patients

Ten patients (5 male and 5 female, median age 49 years), with previously excised malignant melanoma, were matched with 10 healthy control volunteers for skin type and sex (median age 35.5 years). Three of the patients had superficial spreading melanoma and 7 had nodular melanoma. Nine of the patients and 9 of the controls had skin types I or II and had experienced a sunburn during the last 7 years. None of the controls or patients reported a history of long-term sun exposure.

Light source

A xenon arc solar simulator (Kratos) was used as the source of artificial sunlight. The output at 7 cm from the exit of the lamp housing was UVB 750 $\mu W\ cm^{-2}$ and UVA 25 $mW\ cm^{-2}$. An area of approximately 2 cm^2 on the back was irradiated to three times the minimal erythema dose both in patients and in controls.

Skin biopsies

Two-millimetre punch biopsies were taken prior to irradiation and at 4, 24, 48, and 96 h, 1, 2, 3 and 4 weeks thereafter. The 2-mm punch biopsies were embedded in Tissue-Tek II OCT compound (Lamb, London) and immediately frozen in liquid nitrogen and stored at −80°C. 5-μm sections were cut on a cryostat, air-dried for at least 30 min and stored at −80°C.

Double-labelling immunofluorescence

LC were identified in the epidermis by staining of dendritic cells with monoclonal antibody OKT6 and their DR expression was measured with YE/2/36HLK which reacts with a non-polymorphic region of HLA-DR. Immunofluorescent staining of sections was carried out as previously documented (4). The specimens were coded and counted blind. The total number of LC (i.e. T6 positive dendritic cells) per 50 high-power fields and the proportion expressing DR antigen were determined.

Statistics

Statistical significance of differences between medians was assessed using the Mann-Whitney 'U' test for independent samples and the Wilcoxon matched pairs signed rank test, as appropriate. Correlation analysis of LC count with age was performed using the Spearman rank correlation test.

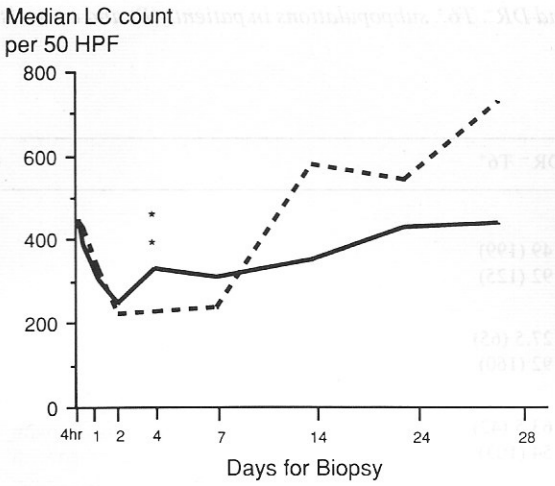

Fig. 1. Changes in Langerhans' cell numbers following exposure to artificial sunlight. ..., Patients; —, controls, $^{**}p<0.01$.

RESULTS

The median total number of LC for the melanoma group prior to irradiation was 441, and for the control group, 452 (Figs. 1, 3). Following irradiation there was a decrease in LC count in both groups which began at 24 h and was maximal at 48 h. However, at 96 h the LC counts were significantly lower in the melanoma group (226.5) than in the controls (330.5) ($p<0.01$) (Figs. 2, 4). This difference persisted and was still significant at one week (melanoma group 239, controls 307.5, $p<0.02$).

Following this decrease there was a rise in the LC counts in both groups over the subsequent 3 weeks, but there was a highly significant rise above pre-irradiation levels in the melanoma group (median peak rise 761, $p<0.001$) but not for the controls (490, $p>0.4$) (Figs. 2, 5).

Prior to irradiation, the DR^+ $T6^+$ subpopulation formed 66% and 70% of the total LC numbers in the melanoma and control groups, respectively. The DR^- $T6^+$ subpopulation formed the remaining 34% and 30% of the total LC numbers in the melanoma patients and controls respectively. DR^+ $T6^-$ dendritic cells were only occasionally seen and the numbers

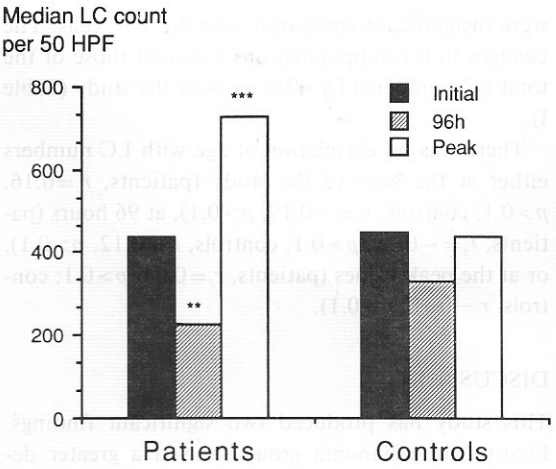

Fig. 2. Peak rise in Langerhans' cell count after irradiation. $^{**}p<0.01$; $^{***}p<0.001$.

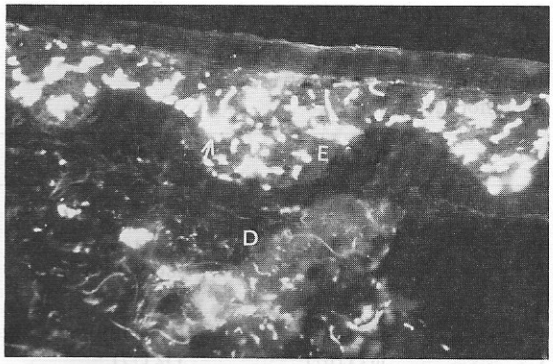

Fig. 3. Immunofluorescent photomircrograph of T6-stained Langerhans' cells pre-irradiation (melanoma patient). *E*, Epidermis; *D*, Dermis; →, Langerhans' cell.

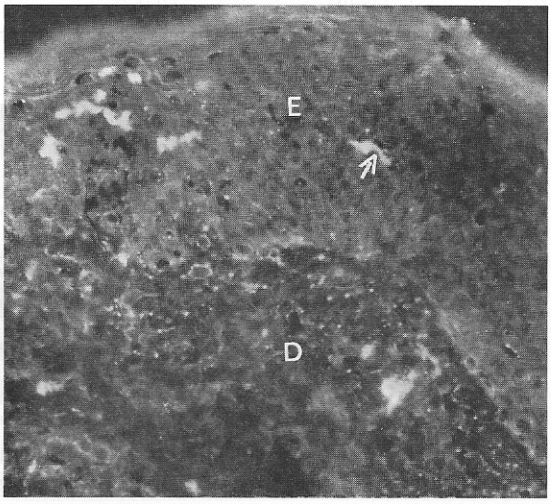

Fig. 4. Immunofluorescent photomicrograph of T6-stained Langerhans' cells at 96 h, showing a significant decrease (melanoma patient). *E*, Epidermis; *D*, Dermis; →, Langerhans' cell.

Table I. *Median total Langerhans' cells and DR$^+$ T6$^+$ and DR$^-$ T6$^+$ subpopulations in patients (P) and controls (C)*

Interquartile range in parentheses
$n=10$ for each group

	Total LC	DR$^+$T6$^+$	DR$^-$ T6$^+$
0 h			
P	441.5 (180)	294 (109)	149 (199)
C	451 (118)	386.5 (140)	92 (125)
4 h			
P	427 (134)	283 (113)	127.5 (65)
C	387 (167)	332 (77)	92 (160)
24 h			
P	331.5 (107)	232.5 (71)	63.5 (42)
C	303.5 (68)	221 (92)	54 (103)
48 h			
P	221.5 (88)	148.5 (64)	52 (75)
C	246 (128)	215 (108)	16.5 (119)
96 h			
P	226.5 (82)	127 (58.5)	30.5 (66)
C	330.5 (119)	241 (84)	56.5 (162)
1 week			
P	239 (87)	179.5 (67.5)	70.5 (42)
C	307.5 (46)	225 (114)	81 (126)
2 weeks			
P	580 (369)	470.5 (236)	130 (129)
C	350.5 (39)	295.5 (57)	93 (168)
3 weeks			
P	543 (139)	324 (85)	165 (101)
C	430.5 (362)	376.5 (104)	108.5 (156)
4 weeks			
P	730 (505)	452.5 (376)	214.5 (178)
C	438 (93)	360 (63)	84 (104)

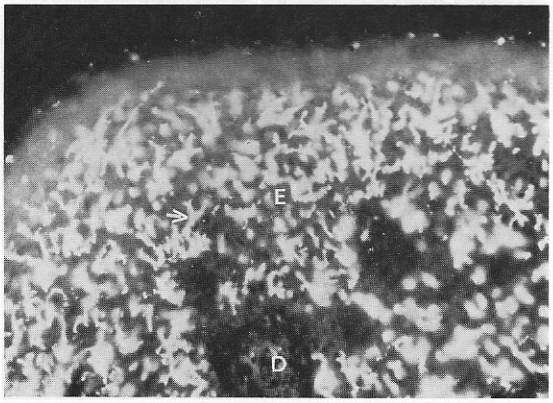

Fig. 5. Immunofluorescent photomicrograph of T6-stained Langerhans' cells at 2 weeks, showing a significant increase vis-à-vis pre-irradiated number (melanoma patient) *E*, Epidermis; *D*, Dermis; →, Langerhans' cell.

were insignificant compared with the T6$^+$ cells. The changes in the subpopulations followed those of the total LC numbers (T6$^+$) throughout the study (Table I).

There was no correlation of age with LC numbers either at the start of the study (patients, $r_s=0.16$, $p>0.1$; controls, $r_s=-0.19$, $p>0.1$), at 96 hours (patients, $r_s=-0.28$, $p>0.1$; controls, $r_s=0.12$, $p>0.1$), or at the peak values (patients, $r_s=0.04$, $p>0.1$; controls, $r_s=0.18$, $p>0.1$).

DISCUSSION

This study has produced two significant findings. Firstly, the melanoma group showed a greater decrease in LC numbers at 96 h after UV irradiation and secondly, a rise in LC count during the subse-

quent 3 weeks, compared with controls. The initial decrease in LC may result in an impaired ability to present antigen and contribute to impaired clearance of antigen including ultra-violet light-induced neo-antigens. The observation that there is a greater fall in LC numbers in the melanoma patients may therefore be important in predisposition to malignant change.

The decrease in LC expressing DR paralleled the total numbers of LC. Small numbers of DR^+ $T6^-$ cells were observed subsequent to UV irradiation, in contrast to the increased numbers reported in a previous study, but this may be a result of the wavelengths employed (5).

The significance of the subsequent rise in LC in the melanoma group is less clear. Possible explanations include a response to persistent antigens which may give rise to specific chemoattractants. Alternatively, it may merely represent a rebound homeostatic mechanism, or increased LC division (6). However, this was not a feature in controls, implying a possible further abnormality in LC behaviour in malignant melanoma patients.

Two studies (9, 10) have shown a decrease in LC numbers, which correlated with age. In one study (9) there were only 4 young subjects (aged 22–26 years) and 7 old subjects (aged 62–86 years). In the other study (10) there were 8 young subjects (aged under 24 years) and 12 males (older than 65 years). In this latter study the older group had evidence of chronic sun damage which may have accounted for the decreased numbers of LC. The inter-group age difference in both these studies was 40 years. Our groups had an age difference of only 13.5 years and we found no correlation of LC numbers with age.

Although it has been known for some time that UV radiation is carcinogenic, it has only recently been established that it has selective suppressive effects on the immune system in vivo (7, 8). This immunosuppression appears to be important in UV induced carcinogenesis and involves alteration in the function of LC in the skin.

Aberer et al. have shown that the LC is particularly susceptible to the effects of UV light in vivo and have been shown to be depleted by low doses of medium wavelength UV (UVB 280–320 nm) and high doses of UVA (11). Morphological signs of damage occurred with doses of UV that left other cells of the epidermis unaltered. There is a loss of surface markers, e.g. ATP-ase activity and T6 expression, within 24 h of irradiation. It is still not clear whether the loss of these surface markers represents a physical absence of the LC, but would nevertheless result in loss of functional activity.

Decreased LC numbers are seen during photochemotherapy and considerable attention has been directed to the possibility that this might produce immune incompetence (12). There has been no reported increase in malignant melanoma in patients with psoriasis undergoing long-term PUVA treatment—as yet. Possible reasons for this are: firstly, there is adaptation, as in chronic sun exposure; secondly, psoriatic patients may not have the required genetic predisposition to develop melanoma; finally, the part of the electromagnetic spectrum responsible for inducing malignant melanoma may not be included in the wavelengths used in PUVA.

In conclusion, this study has shown that there is a differential LC response to ultraviolet light between melanoma patients and controls and that this may be relevant to the pathogenesis of malignant melanoma.

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