ACKNOWLEDGEMENTS

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The Epidermal Langerhans' Cell Population in Psoriasis during Topical Coal Tar Therapy

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We have studied the effect on the Langerhans' cell (LC) population of topical 3% coal tar therapy. Biopsies were taken from psoriatic plaques and from controls with no skin disease before and after the application of 3% coal tar for one week; LC were identified by immunofluorescence using monoclonal antibody. LC counts expressed per unit epidermal surface length were similar in untreated psoriasis plaques and in normal skin. Differences in the LC population in paired biopsies from both patients and controls showed considerable variation following coal tar treatment but no consistent effect could be demonstrated. (Received January 8, 1986.)

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The Langerhans' cell (LC) is a bone marrow derived dendritic cell found almost exclusively in the epidermis and responsible for antigen presentation. There are conflicting views on the nature of immune involvement in the pathology of psoriasis (1). A marked reduction in the LC population has been reported following PUVA (2) therapy used in the treatment of psoriasis. It is therefore of interest to determine whether other forms of treatment for psoriasis have this effect.

We have compared the LC population in untreated psoriasis plaques with that in normal skin and after initiation of healing with topical coal tar. This treatment produces a
therapeutic effect observable within a few days of commencing application. We therefore
looked for changes in the number of LCs after one week's treatment in order to determine
whether the early healing response is also associated with a fall in the LC count.
Volunteers with normal skin acted as controls for both untreated and coal tar-treated
values.
LC were identified by monoclonal antibody in cryostat sections of punch biopsies of
skin, since it is very difficult to obtain epidermal sheets of psoriatic plaque by the suction
blister technique (3).

MATERIALS AND METHODS
Biopsies were taken from involved skin from 10 patients with chronic plaque psoriasis, and from 4
volunteers with normal skin both prior to and after one week of treatment with 6% coal tar in zinc
paste diluted 50% in soft paraffin. All subjects were strictly instructed to avoid all topical treatments
including UVL for at least a week prior to the trial. The second biopsy was taken from a correspond­
ing site on the opposite side of the body or from a site several centimetres distant from the first, using
1% lignocaine local anaesthesia and a 4 mm punch biopsy. Other normal skin was also obtained from
3 patients undergoing surgery.

Immunofluorescence procedure
The biopsies were orientated in O.C.T. compound (Lab-Tek Products, Miles Laboratories Inc.) and
snap-frozen in isopentane chilled with liquid nitrogen (4). Sections (6 µm) perpendicular to the skin
surface were air-dried and fixed in acetone at room temperature for 5 min. The standard immuno-
fluorescence procedure was performed as previously described (4). Sections were treated with OKT6
(Ortho) diluted 1 in 10 in phosphate buffered saline containing 10% foetal calf serum, followed by
fluorescent sheep anti-mouse Ig conjugate. After the final wash nuclei were counterstained with
propidium iodide (1 µg per ml) and the slides mounted in 90% glycerol containing diazabicyclo-octane
to retard fading during microscopy (5). The fluorescence microscope was equipped for incident
illumination with the HBO 50 mercury burner; a dry apochromatic ×40 objective was employed with
×10 oculars incorporating a calibrated graticule. All specimens were read by one observer (LP)
without knowledge of their identity.

Cells were counted in adjacent fields excluding severely convoluted or damaged areas, and avoiding
pilosebaceous follicles where LC are increased. Langerhans' cell counts were based on the identifica­
tion of characteristic dendritic cells showing a clearly visible nucleus; isolated dendrites were
excluded. One graticule length corresponded to 0.2 mm of the epidermis. Replicate sections were cut
60 µ distant in order to avoid recounting the same cells. Eleven or more fields (>2.2 mm) suitable for
counting were available in 22 samples, but sections from the affected skin of 2 patients were
persistently folded and yielded only 7 and 10 fields respectively. The number of LC per mm surface
length of epidermis is shown in Table I. The graticule was also used to determine mean epidermal
thickness.

In preliminary studies, consecutive sections were stained with OKT6, a monoclonal antibody with
similar specificity, NA134 (Dr A. J. McMichael, Oxford) and DA6231 which is a monoclonal antibody
to HLA class II DR antigens (Dr C. M. Steel, Edinburgh).

RESULTS
Definite clinical improvement was seen in all psoriasis patients after application of coal tar
for 1 week. Slight local irritation was experienced by the normal subjects using coal tar.
Langerhans' cells were easily recognized and could be enumerated reproducibly. In
psoriatic plaques they were generally situated in the mid epidermis and showed a tendency
to clustering. Most biopsies contained a few LC in the upper dermis, but 1 patient and 1
control showed a large number in the upper dermis before and after coal tar. For the
purposes of this study only epidermal LC were counted.
Table I shows the number of LC present in the full thickness of the epidermis per mm
surface length. Patients and controls showed a similar incidence ranging from 10 to 28 and
11 to 24 per mm respectively. After coal tar both groups showed considerable variation
Table I. Epidermal Langerhans' cell numbers expressed per unit epidermal surface length (LC per mm)

<table>
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<th></th>
<th>1</th>
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<th>4</th>
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<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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</tr>
<tr>
<td>Pre-tar</td>
<td>17.5</td>
<td>10.5</td>
<td>12.2</td>
<td>24.5</td>
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<td>20.4</td>
<td>10.3</td>
<td>20.5</td>
<td>13</td>
<td>15.8</td>
<td>1.6</td>
<td>250</td>
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<tr>
<td>Post-tar</td>
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<td>12.8</td>
<td>10</td>
<td>23</td>
<td>21</td>
<td>15.3</td>
<td>12.9</td>
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<td>28</td>
<td>18.1</td>
<td>1.9</td>
<td>330</td>
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<td>-1.5</td>
<td>+7</td>
<td>-5</td>
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<td>+0.5</td>
<td>+15</td>
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<td>Pre-tar</td>
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<td>15.1</td>
<td>2.25</td>
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<tr>
<td>Post-par</td>
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* Only 7 suitable fields for counting.
ND = not done.

and neither a consistent trend. There was no correlation between the degree of individual clinical improvement and the change in LC count and there was no significant difference between mean counts before and after tar in either group.

Mean epidermal thickness was unchanged in psoriatic plaques, being 0.14±0.04 mm in both pre- and post-tar biopsies. Normal skin, however, was increased in thickness during tar therapy from 0.065±0.009 mm to 0.083±0.006 mm although this did not reach significance statistically. The number of dendritic epidermal cells stained by OKT6 and Nal34 was the same, but DA6231 (anti HLA class II) stained 10–80% more cells in the epidermis of both normal skin and psoriatic plaques pre-coal tar treatment. As no changes were seen with OKT6 staining after tar therapy the less specific HLA-DR staining was not repeated for post therapy estimations.

DISCUSSION

The number of epidermal LC per linear surface millimetre in untreated plaque psoriasis was found to be normal despite the increased thickness of the epidermis in this condition.

If we extrapolate from our results we obtain a mean figure of 225 LC per mm², slightly less than other workers (2, 6, 7). In this study cell nuclei were stained with propidium iodide and only LC containing a clearly-defined nucleus were included in the counts. The higher counts we obtained in epidermis stained with the HLA-DR-specific antibody presumably reflects the wide distribution of HLA class II antigens in tissues. The possible influence of subjective bias was eliminated by randomisation of the slides before microscopy.

Coal tar therapy for one week was sufficient to induce thickening in normal skin, and to show clinical improvement of psoriatic plaque. LC numbers, however, remained unaltered during this therapy. Both patients and controls showed differences in their individual counts following coal tar, but there was no apparent trend and no correlation with the degree of clinical effect. We therefore conclude that unlike the effect produced by exposure to ultra-violet light the early clinical response to coal tar in psoriasis is not associated with a change in the number of Langerhans' cells available in the epidermis.
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Effects of Retinoids on Type IV Collagenolytic
Activity in Melanoma Cells

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Oikarinen A, Salo T. Effects of retinoids on type IV collagenolytic activity in melanoma

The effects of retinol, all-trans-retinoic acid, isotretinoin and etretinate on the activity of
basement membrane collagen degrading enzyme was studied in melanoma cells. The
results indicated that retinoids at concentrations of up to $10^{-6}$ M did not significantly
affect type IV collagenolytic activity in these cells in vitro. Since type IV collagenolytic
enzyme may be involved in the metastatic potential of tumour cells, it appears that
retinoids do not affect the metastatic potential of melanoma cells by affecting type IV
collagenolytic activity. Key word: Type IV collagenase. (Received February 13, 1986.)

A. Oikarinen, Department of Dermatology, University of Oulu, SF-90220 Oulu, Finland.

Retinoids are used extensively for the treatment of various dermatological diseases (1).
New derivatives of vitamin A have also been suggested to have anti-tumour effects and
these retinoids would be particularly useful for the treatment of epithelial tumours (2, 3).
There are some studies indicating that retinoids decrease the number of certain types of
epitheliomas such as basal cell carcinomas, or premalignant lesions such as solar kerato­
sis. Retinoids have also been shown to decrease the proliferation rate of normal and
malignant cells (3). For tumour growth, the ability of tumours to invade and penetrate
basement membranes is essential (4). It has been shown that malignant tumours produce
specific proteolytic enzymes which can degrade basement membrane collagen (type IV)
(4–6). In some studies the production of type IV collagenase correlated well with the
metastatic potential of malignant cells (6). In the present study the effects of various
retinoids on type IV collagenolytic activity were studied in human melanoma cells (A
2058).

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