SHORT REPORTS

Langerhans' Cell Population Studies with OKT6 and HLA-DR Monoclonal Antibodies in Vitiligo Patients Treated with Oral Phenylalanine Loading and UVA Irradiation

W. WESTERHOF, I. GROOT, S. R. KRIEG, J. D. BOS and R. H. CORMANE

Department of Dermatology and Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands


In vitiligo patients, treated with oral phenylalanine loading combined with UVA irradiation (Phe-UVA), Langerhans' cells (LC) were counted in pigmented and depigmented skin. The LC, which were labelled with OKT6 and HLA-DR monoclonal antibodies, were expressed per linear mm epidermis. Before treatment the number of OKT6 (+) cells was significantly increased in vitiliginous skin especially in the basal layer. Under treatment the number went down and was comparable to normal skin. When using HLA-DR labelling the number of LC increased in vitiliginous skin which had been treated with Phe-UVA. The influence of Phe-UVA on the shift of LC subpopulations is discussed. (Received November 5, 1985.)

W. Westerhof, Department of Dermatology, Academisch Medisch Centrum, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

Langerhans' cells (LC) have proved to be markedly sensitive to ultraviolet irradiation (1). The apparent decrease in number of epidermal LC after UVB irradiation may be due to changes in cell surface determinants since Aberer et al. (2) have demonstrated by electron microscopy that significant numbers of Langerhans' cells remained intact after a single dose UVB, whereas cytomembrane markers like ATPase and Ia were absent. Friedmann (3) showed that 90% of ATPase expressing cells lost their staining capacity during PUVA treatment. Tjernlund & Juhlin (4) demonstrated that under PUVA treatment Ia and OKT6 labels were strongly reduced as well.

By ATPase staining and electron microscopy it has been shown that LC disappear from the epidermis during PUVA treatment and reappear only 15 days after cessation of the treatment (5). This depletion occurs within the dose range normally used for PUVA therapy. However, in the case of UVB Koula & Jansén (6), who studied normal human skin, found that the decrease of ATPase expressing cells was less prominent. In man UVB has no effect at all on LC markers (ATPase, OKT6 and Ia) in slightly erythematogenic doses (4).

Whether the reduction of LC surface markers or the depletion of LC due to UVB irradiation and PUVA treatment are also responsible for the reduction in contact sensitivity (7) and the development of photocarcinogenesis (8) remains to be elucidated. It is also unclear what role, if any, the LC depletion plays in the process of healing dermatoses.

Recently good results have been reported for oral L-phenylalanine loading combined with UVA irradiation in the treatment of vitiligo (9, 10). In the present study the quantity and distribution of LC in pigmented and vitiliginous skin was estimated before phenylalanine loading and UVA irradiation and then again in repigmented skin during therapy.
Table I. Mean number of LC in the epidermis per mm epidermis length

<table>
<thead>
<tr>
<th></th>
<th>Before Phe-UVA</th>
<th>During Phe-UVA</th>
<th>During Phe-UVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pigmented</td>
<td>Depigmented</td>
<td>Pigmented</td>
</tr>
<tr>
<td>Basal +</td>
<td>34.00±7.51</td>
<td>44.08±9.59</td>
<td>36.39±3.60</td>
</tr>
<tr>
<td>suprabasal OKT-6</td>
<td>7.88±3.88</td>
<td>15.24±2.86</td>
<td>9.25±2.84</td>
</tr>
<tr>
<td>Basal</td>
<td>16.69±5.99</td>
<td>15.70±6.21</td>
<td>20.18±4.69</td>
</tr>
</tbody>
</table>

PATIENTS AND METHODS

A total of 17 patients with common vitiligo were selected for this study. Their ages ranged from 22 to 56 years (mean 38.5 years). The duration of vitiligo varied from 2 to 17 years (mean 7.06 years). In 3 patients autoantibodies directed against thyroid cell and gastric parietal cells were found without functional disease. Two patients had been treated with PUVA previously, both with little success. Another patient had been taking beta carotene without improvement. Normal and vitiliginous skin of 8 patients was biopsied before and during Phe-UVA. From 9 patients undergoing Phe-UVA normal and repigmented skin was taken for microscopical analysis.

Fresh frozen skin sections were stained by a two stage immunoperoxidase technique using two monoclonal antibodies as first stage reagents, followed by incubation with rabbit-anti-mouse peroxidase conjugated antiserum (Dakopatts, Copenhagen, Denmark) counterstained with methylgreen. Monoclonal antibodies in this study included OKT 6 (Ortho Pharmaceuticals) and anti-HLA-DR (Beckton and Dickington). The sections were examined with a light microscope furnished with an ocular micrometer. The length of an epidermal section was measured and the number of LC (cell bodies) in the entire epidermis and in the basal layer was counted. The LC counts were expressed as the number per linear mm length of epidermis.

RESULTS

We found a significant difference between the number of OKT6-positive LC in normal pigmented skin and vitiliginous skin before starting the Phe-UVA therapy. The LC counts are summarized in Table I.

During Phe-UVA therapy no significant difference was discovered between the normal pigmented skin, the repigmented skin and the remaining depigmented skin when counting the number of OKT6-positive LC in the total epidermis or in the basal layer of the epidermis.

Comparing the normal pigmented skin before and during Phe-UVA therapy, there is no significant variation between their numbers of OKT6-positive LC, neither in the total epidermis nor in the basal layer.

In addition we subdivided each group of patients into those who had suffered from vitiligo for 7 years or less and those who had had the disease for more than 7 years. We did not find any significant differences between these two groups of patients, neither when counting the LC in the total epidermis, nor in the basal layer.

The LC counts performed with HLA-DR labelling demonstrated approximately half the number of LC found with OKT6 labelling (Table I). The difference in LC count seen with OKT6 labelling between normal pigmented and vitiliginous skin before treatment was not significant with HLA-DR labelling. Basal layer counts of LC were not performed with
HLA-DR labelling. The number of HLA-DR positive cells during Phe-UVA appeared to rise in vitiliginous skin and the numbers in normal pigmented skin and in repigmented skin remained unchanged.

The number of LC during treatment increased in vitiliginous skin when applying HLA-DR labelling, whereas with OKT6 labelling the number of LC in vitiliginous skin went down and remained the same as in normal pigmented skin during Phe-UVA therapy.

DISCUSSION

The reason for cutting the biopsies in cross sections and expressing the number of LC per linear mm epidermis was to give a better impression of the relation between the number of suprabasal and basal LC.

Our results confirm the findings of Zelickson (11), and further those of Mishima (12), who, using an electron microscope, found an increase of LC in the basal layer but an equal number in the total epidermis of vitiliginous skin as compared to normal skin. However, he also found an increase in the number of indeterminate cells in vitiliginous skin. Furthermore, OKT6 and HLA-DR antibodies are markers for indeterminate cells as well and might explain why we discovered an increase of OKT6 positive cells in vitiliginous skin.

In contrast to PUVA therapy the treatment of vitiligo patients with oral phenylalanine loading and UVA irradiation does not result in LC depletion, nor is any drastic reduction in cell surface markers noticed. Therefore the beneficial effects of Phe-UVA in the treatment of vitiligo are apparently not the result of the destruction of LC but merely the change in the population of subsets of LC.

According to Harrist et al. (13) epidermal LC or indeterminate cells can be divided into HLA-DR (+)/T6(+) and HLA-DR(-)/T6(+) cells in which the former represents an activated state and a functionally distinct subtype.

Our results indicate that in the course of Phe-UVA treatment of vitiligo the number of OKT6 positive cells decreases, whereas the number of HLA-DR positive cells increases in vitiliginous skin meaning a change in the ratio between two subtypes of LC. So the increase in HLA-DR(+)/T6(+) cells and the decrease in HLA-DR(-)/T6(+) cells might be related to the treatment with Phe-UVA as similar shifts were not noticed in normal pigmented skin, nor in repigmented skin before and during treatment. It may indicate an altered control of immune responsiveness to certain antigens as well as a susceptibility to the therapeutic modality applied.

REFERENCES


Association between HLA-B16 and Psoriatic Spondylitis

E. CRIVELLATO and T. ZACCHI

Department of Dermatology, University of Trieste, and Tissue-Typing Laboratory, General Hospital, Trieste, Italy


The frequency distribution of HLA antigens was studied in a group of 40 patients with psoriatic arthritis from a region in North-eastern Italy. Our results indicate: 1) a strong association, in this population, between HLA-B16 and inflammatory spinal involvement; 2) a failure to confirm previous reports on HLA-B27 increased frequency in psoriatic spondylitis and/or sacroiliitis.

Key words: Psoriatic arthritis; HLA-B16; HLA-B27.

(Received September 7, 1985.)

E. Crivellato, Department of Dermatology, University of Trieste, Trieste, Italy.

In Caucasians, the HLA-B27 antigen has been closely linked to susceptibility to seronegative spondyloarthropathies, particularly to ankylosing spondylitis and Reiter’s syndrome (1). A statistically significant association has also been reported between HLA-B27 and the axial type of arthritis often seen in psoriatic patients (2, 3, 4). In this study we were unable to find any significant increase of HLA-B27 in a group of Italian patients with psoriatic arthritis. Conversely results indicate a significantly increased frequency of HLA-B16, even more marked in the group with spondylitic involvement.

MATERIAL AND METHODS

A total of 40 unrelated psoriatic arthritis patients (24 males, 16 females) from the Trieste area (North-eastern part of Italy) were studied. The Moll & Wright (5) criteria for the diagnosis of psoriatic arthritis and the New York criteria (6) for spondylitis were used. Twenty-four (60%) patients had peripheral arthropathy alone (PA); ten (25%) had radiographically proved spondylitis and sacroiliitis (SS) while the remaining six (15%) had sacroiliitis without spondylitis (SA).

HLA typing was performed by microlymphocytotoxicity test following N.I.H. technique (7). 32 antigens were studied for A, B and C loci. Antisera were provided by the Italian Cooperative Group for Tissue Typing, the Behring-Werke and Biotest. Antigen frequencies were compared with 341 controls.

The statistical analysis was made by using the Fisher’s exact test. The corrected p-values were obtained by multiplying the p-values by the number of HLA antigenic specificities tested for.