Effect of PUVA Treatment on the Locomotion of Polymorphonuclear Leukocytes and Mononuclear Cells in Psoriasis

W. Silny, M.D., H. Pehamberger, M.D., Ch. Zielinsky, M.D., and F. Schnait, M.D.

Department of Dermatology I. and Institute of Immunology (CZ) University of Vienna, Vienna, Austria

Polymorphonuclear leukocyte (PMN) and mononuclear cell (MNC) locomotion was investigated in patients with psoriasis vulgaris before and after treatment with oral photochemotherapy (PUVA) and was found to be significantly increased (PMN: $p < 0.001$; MNC: $p < 0.002$) when compared to healthy controls. No significant difference was observed in the locomotion of PMN or MNC before and after PUVA treatment. These results indicate that PMN and MNC locomotion is increased in psoriasis vulgaris and remains unaltered by PUVA treatment.

Psoriatic plaques contain increased amounts of a complement dependent chemotactic factor which might be responsible for the accumulation of polymorphonuclear leukocytes (PMN) in psoriatic skin lesions [1]. Wahba et al [2] demonstrated an enhanced chemotactic response of circulating PMN obtained from untreated patients with psoriasis. In contrast Krueger, Hill, and Jederberg failed to demonstrate increased locomotion activity of PMN [3] in psoriasis but reported an increased chemotactic response of circulating mononuclear cells (MNC) [4]. This study was performed in order to investigate whether photochemotherapy (PUVA) [5] affects the locomotion ability of circulating MNC and PMN in psoriatic patients.

MATERIALS AND METHODS

Subjects

A total of 98 individuals was studied, 44 patients with psoriasis vulgaris (stable plaque type) and 54 healthy volunteers. All of the psoriatic patients were untreated for at least 30 days prior to the investigations and had 30 to 50% of the body surface involved.

Mononuclear cell (MNC) locomotion was studied in a total of 21 psoriatic patients, 7 females (f), 14 males (m), age: 37.4 ± 4.1 years (yr); mean ± standard error of the mean (SEM) prior to the treatment. Ten patients out of this group (5 m, 5 f; £ age 38.1 ± 3.94 yr) were also investigated after complete clearing by oral photochemotherapy (PUVA) [6] in psoriatic patients. PMN locomotion was determined in an identical set up in the remaining 23 psoriatic patients, 10 f, 13 m; £ age 40.7 ± 13.7 yr; in 12 patients out of this group, 7 f, 5 m; £ age 29.5 ± 4.2 yr before PUVA and after PUVA clearing phase (total UVA dose: 72.9 ± 9.9 Joules/cm²) and in 29 healthy volunteers. Photochemotherapy (PUVA) was performed using a PUVA 4000 irradiation unit (Waldmann, Schwemningen, Germany). After phototesting [6] patients were treated aggressively 4 times per week. Treatment resulted in rapid clearing of the skin lesions accompanied by 1+ to 2+ erythematous reactions. There was an interval of at least 24 hr between the last PUVA exposure and the locomotion assays.

LOCOMOTION ASSAY

MNC were isolated from heparinized venous blood by Ficoll Hypaque separation [7] and PMN were gained by sedimentation of heparinized venous blood with 2% Dextran 500 (Pharmacia, Uppsala, Sweden) [8]. Contaminating erythrocytes were lysed by 0.84% NH₄Cl; cells were washed 3 times in medium RPMI 1640 (Flow Laboratories, Bonn, Germany) containing 100 IU penicillin per ml, 100 μg streptomycin (Gibco, Glasgow, Scotland) per ml, 2% bovine serum albumin (Fluka, Buchs, Switzerland) and were then adjusted in medium to a concentration of 2 × 10⁶ cells/ml. Trypan blue exclusion revealed more than 94% viable cells, in all experiments.

Locomotion was determined by a modification [9] of Boyden’s [10] micro pore filter technique. 0.5 ml of cell suspensions were placed in the upper compartment of the chemotaxis chamber; 1.5 ml of a pooled normal human serum, activated by incubation with zymosan (Koch Light Laboratories Colnbrook, Bucks UK) and diluted in gelatine veronal buffered saline (pH7.3) according to Clark and Kelbanoff [11], served as cytotaxin in the lower compartment. Filters, 5 μm and 3 μm pore size (Millipore Corporation, Bedford, Massachusetts, USA), were used for the determination of MNC and PMN locomotion, respectively. After incubation for 120 min at 37°C, filters were detached, stained with hemalun and the cells present on the lower side of the filters were counted in 20 oil immersion fields.

Assays were performed in triplicate and the results were expressed as locomotion index (LI) = mean number of cells/l immersion field ± SEM. The random mobility of the cells was evaluated by determining the LI using gelatine veronal buffered saline (without the cytotaxin)-in the lower compartment of the chemotaxis chamber.

Statistical analysis was performed by the Student’s t-test.

RESULTS

The results are listed in detail in the Table. The mean LI of MNC as well as PMN was significantly higher: (MNC: $p < 0.002$; PMN: $p < 0.001$) in patients with psoriasis when compared to the mean LI of the healthy controls. No significant difference was observed in the LI of MNC or PMN in psoriatic patients when determined before PUVA treatment and after complete clearing by PUVA ($p > 0.2$). The random mobility of MNC and PMN respectively was not statistically different ($p > 0.2$) when compared in patients versus controls, and in patients before and after PUVA treatment.

DISCUSSION

The significant increase in the response of psoriatic PMN to zymosan activated serum as chemoattractant in this study supports the finding of Wahba et al [2] and indicates that both an increased susceptibility of circulating PMN to chemoattractants in psoriasis plus an increased amount of chemotactic factors produced in psoriatic epidermis, as reported recently [1], are responsible for the presence of PMN in psoriatic stratum corneum. In contrast to Wahba et al and also in contrast to this...
Locomotion indices

<table>
<thead>
<tr>
<th>Normal individuals</th>
<th>Without treatment</th>
<th>Psoriatic patients</th>
<th>Before PUVA</th>
<th>After PUVA clearing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>ZAS</td>
<td>RM</td>
<td>N</td>
</tr>
<tr>
<td>PMN</td>
<td>29</td>
<td>3.3 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>23</td>
</tr>
<tr>
<td>MNC</td>
<td>25</td>
<td>3.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>21</td>
</tr>
</tbody>
</table>

N = number (of individuals).
PMN = polymorphonuclear cells (leukocytes).
MNC = mononuclear cells.
ZAS = zymosan activated serum (cytotaxin).
RM = random mobility.