Leukopheresis for Treatment of Psoriasis: Is Therapeutical Benefit Related to Reduced Activities of Neutral Proteinases of Polymorphonuclear Leukocytes?


1 Department of Dermatology, Academy of Medicine, Warsaw, Poland
2 Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Summary. Ten patients were treated with repeated leukophereses performed one to three times per week for 2—5 weeks. Two of the patients was cleared completely, four exhibited regression of more than one-half of the lesions, and four showed only a slight improvement. The therapy did not markedly affect the granulocyte count in peripheral blood, and the beneficial clinical response was not related to the number of polymorphonuclear leukocytes (PMNs) removed by leukophereses. During therapy, the activities of elastase, cathepsin G, lysozyme, and myeloperoxidase in PMNs were determined by spectrophotometry. PMNs isolated using a Haemonetics 30 blood-cell separator were about 50% deficient in these activities in comparison to cells obtained directly from peripheral blood. Thus, leukopheresis induces a marked degranulation of PMNs. Repeated leukophereses were found to generate significant variations in the activities of circulating PMN granule enzymes and in the levels of acid-soluble proteins. Remission or great improvement were observed in patients who, during therapy, exhibited decreased PMN elastase and cathepsin G activities, whereas a poor clinical response was accompanied by high enzymatic activities.

Key words: Leukopheresis — Psoriasis — Neutrophils — Serine neutral proteinases

Introduction

Polymorphonuclear leukocytes (PMNs) appear to play an important role in the pathogenesis of psoriasis [13]. Hyperactive and hyperresponsive PMNs infiltrate the epidermis and may be of importance in the maintenance of psoriatic lesions [17]. The chemotaxis of these cells towards various stimuli has been found to be increased in psoriatics [20, 23, 29]. Other functions of PMNs are also stimulated, since their adherence [25], respiratory burst [16, 26], phagocytic activity [29], and cytolysis [16] have been found to be markedly enhanced.

In previous studies, we have established that the activities of neutral proteinases of PMNs undergo changes during the course of psoriasis. Unselected patients exhibited normal activities of these granule enzymes [7, 10], whereas an approximately twofold increase of elastase and cathepsin G activities was demonstrated in patients with active plaque lesions, while a reduction of these activities to levels below normal was found in patients with stationary disease or in remission [11].

These findings, as well as the regression of psoriatic lesions during peritoneal dialysis which is related to the depletion of numerous PMNs [8], provided the rationale for using repeated leukophereses as a therapeutic treatment for widespread psoriasis [9]. Apart from the direct elimination of white blood cells (WBC) along with a large fraction of PMNs, other systemic effects of leukophereses which may induce the regression of psoriatic lesions are not known.

Since the beneficial effect of peritoneal dialysis is also related to the significant reduction in the neutral proteinase activity of PMNs, and reduced activities of their elastase and cathepsin G seem to be characteristic of symptom-free psoriatics [8, 11], the present study was confined to neutrophils and their neutral proteinases. The purpose of our study was to establish optimal conditions for the clearing of psoriatic lesions by repeated leukophereses and to find out whether there is a relationship between the clinical response to the therapy, the PMN elimination, and the activities of some granule enzymes of neutrophils.
Table 1. Clinical effect of leukophereses in psoriatic patients in relation to the number of procedures, the duration of therapy, and the number of eliminated PMNs

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Number of procedures</th>
<th>Duration of therapy (days)</th>
<th>Frequency of procedures</th>
<th>Clinical effect*</th>
<th>Percentage of skin area affected</th>
<th>Number of PMNs eliminated ((\times 10^{-9}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.S.</td>
<td>M</td>
<td>6</td>
<td>30</td>
<td>Once a week</td>
<td>SI</td>
<td>90</td>
<td>28</td>
</tr>
<tr>
<td>J.T.</td>
<td>M</td>
<td>7</td>
<td>35</td>
<td>Once a week</td>
<td>SI</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>W.J.</td>
<td>M</td>
<td>9</td>
<td>37</td>
<td>Every 4 – 5 days</td>
<td>R</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>S.T.</td>
<td>F</td>
<td>5</td>
<td>14</td>
<td>Twice a week</td>
<td>R</td>
<td>60</td>
<td>26</td>
</tr>
<tr>
<td>L.P.</td>
<td>M</td>
<td>6</td>
<td>21</td>
<td>Twice a week</td>
<td>SI</td>
<td>70</td>
<td>60</td>
</tr>
<tr>
<td>T.G.</td>
<td>M</td>
<td>8</td>
<td>27</td>
<td>Twice a week</td>
<td>SI</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>W.M.</td>
<td>M</td>
<td>9</td>
<td>29</td>
<td>Twice a week</td>
<td>GI</td>
<td>45</td>
<td>15</td>
</tr>
<tr>
<td>E.N.</td>
<td>M</td>
<td>8</td>
<td>17</td>
<td>Three times a week</td>
<td>GI</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>S.D.</td>
<td>M</td>
<td>9</td>
<td>20</td>
<td>Three times a week</td>
<td>GI</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>K.P.</td>
<td>M</td>
<td>13</td>
<td>31</td>
<td>Three times a week</td>
<td>GI</td>
<td>60</td>
<td>25</td>
</tr>
</tbody>
</table>

* R, remission; GI, great improvement; SI, slight improvement

Materials and Methods

Chemicals
Materials were obtained as follows: Micrococcus lysodeicticus (dried cells), horseradish peroxidase, lysozyme (egg white), and N-tert-butoxycarbonyl-L-alanine p-nitrophenyl ester (BANE) from Sigma; N-benzoyl-tyrosine ethyl ester (BTEE) from Calbiochem; o-dianisidine from Fluka; Folin Ciocalteu reagent from Merck; Triton X-100 from BDH; Tris from Koch-Light Laboratories; gelatine from Loba Chemie Fischamend.

Selection of Patients
Ten patients with psoriasis vulgaris (mean age, 37 ± 12 years) were treated with repeated leukophereses. All of them had active widespread plaque psoriasis (no pinpoint lesions) involving more than 40% of the skin surface, and their lesions were resistant to routine external treatment. According to our previous classification [11], these patients were in group A1 of disease activity. The patients had not received any systemic medication for at least 3 months before leukopheresis, and during therapy, they were treated only with neutral ointment.

Leukopheresis Procedure
A Haemonetics 30 blood-cell separator processor was used throughout. Blood was mixed with anticoagulant (1.8% sodium citrate and 6% hydroxyethyl starch; Plasmasteril) and introduced into a rotor vol., 225 ml). After every six cycles, the leukocyte layer was sucked off for 5 min into a separate bag, and the remaining cells were sedimented for an additional 20 min, resuspended in the infusion fluid, and reinfused into the patient. The WBC loss varied from 8 to 12 x 10⁹ cells, 30% – 40% of which were PMNs. The residue was composed of mononuclear cells, i.e., monocytes (4%) and lymphocytes (over 50%).

PMN Separation and Elution
The cells were separated from the material recovered during each leukopheresis. In some patients, PMNs were also isolated from peripheral blood drawn out before, during, and after the therapy. PMNs from the peripheral blood of untreated psoriatics with the same disease activity and from that of healthy volunteers served as the controls. Details of cell separation can be found elsewhere [11].

Biochemical Measurements
Cathepsin G and elastase activities were tested against BTEE and BANE, respectively, according to procedures described previously [11].

Lysozyme activity was measured according to the Worthington Enzyme Manual [30], except that 0.066 M sodium-phosphate buffer (pH 6.6) was used to suspend the M. lysodeicticus cells.

Protein levels were determined using a microadaptation of the Lowry method with a 1% sodium-citrate/CuSO₄ solution and bovine serum albumin as a standard [27].

Results
Regression of Psoriatic Lesions During Leukophereses
Two of the ten patients exhibited complete remission during the therapy, four patients were cleared of more than one-half of their lesions (great improvement), and four showed a slight improvement, i.e., a decrease in erythema and desquamation as well as the disappearance of some skin changes (Table 1). However, after the therapy, the last group responded to conventional topical treatment with anthralin or tars, to which these patients had been unresponsive before. In one patient of this group (L.P.; Table 1), the leukophereses were stopped after six procedures due to the poor progress of the therapy and flare up of the lesions.