Monitoring Peripheral Blood CD4+ Intracellular Adenosine Triphosphate Concentration in Patients with Psoriasis Treated with Fumaric Acid Esters

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Several lines of evidence have demonstrated immunomodulatory and immunosuppressive effects for fumaric acid esters (FAE). Short- and long-term clinical studies in psoriasis have shown a marked reduction in peripheral and lesional CD4+ and CD8+ T-lymphocytes; yet FAE therapy does not appear to be related to an increased risk of infections (1–7). Lymphocytes depend on sufficient import of energy-carrying metabolites and enhanced oxidative phosphorylation. Intracellular adenosine triphosphate (iATP) concentration in CD4+ cells correlates with cytokine secretion and T-cell proliferation and serves as a marker of T-cell activation (8, 9). In patients with autoimmune diseases undergoing different immunosuppressive therapies, decreased iATP correlates with the risk of developing opportunistic infections (10). Hence, we aimed to monitor CD4+ iATP activity in patients with psoriasis undergoing FAE treatment.

MATERIALS AND METHODS
In the present pilot-study, 21 adult patients (mean ± standard deviation (SD) age 54.7 ± 14.9 years; 13 males, 8 females) with chronic plaque psoriasis (Psoriasis Area and Severity Index (PASI) > 10) were recruited. The study followed a protocol approved by our institutional review board. Fumaderm® (Biogen Idec GmbH, Ismaning, Germany) was initiated and the dose incremented over 9 weeks according to the standard dosing schedule (1). If side-effects occurred, the patient’s dosage was reduced to the previously tolerated dose. The efficacy of Fumaderm® was assessed primarily using the PASI (1). All patients were devoid of acute and chronic infections (clinically, white blood cell count, C-reactive protein). Adverse events, in particular infections, were routinely identified monthly by clinical history and routine blood investigations (full blood count, renal and liver function, C-reactive protein). The duration of FAE treatment was 4 months. Blood flow cytometry and iATP analysis were performed at least at baseline (t0), 2 months (t2) and 4 months (t4).

Blood flow cytometry for lymphocyte subpopulations was performed as described in detail previously (11). For investigation of CD4+ iATP, we used a FDA-approved assay for the detection of cell-mediated immunity in an immunosuppressed population (FDA no. k013169). The procedure for CD4+ iATP determination is described in detail elsewhere (10). The patient’s level of immune response in immunosuppressed transplant patients corresponds with absolute iATP concentrations (8). Healthy controls (n = 59) exhibited iATP concentrations of 479.9 ± 19.8 ng/ml (mean ± standard error of the mean (SEM)) with between day variability. However, no infections were observed in these patients. The iATP concentration did not significantly correlate with CD4 cell count, C-reactive protein, PASI or cumulative FAE dosage (r < 0.19; p > 0.05).

DISCUSSION
Our flow cytometry data confirm the results of previous studies on lymphocyte subpopulations under FAE therapy (3, 5, 6), even though we observed that CD4+ cells, as well as B lymphocyte numbers, were most strongly reduced by FAE treatment. Lehmann et al. (5) have suggested that dimethylfumarate acts as an active compound within the FAE mixture and, at least partially, mediates its immunomodulatory/immunosuppressive activity by the induction of the anti-inflammatory stress protein heme oxygenase 1, ascribed to the functional depletion of reduced glutathione (5). Quantification of iATP serves as a surrogate marker of global cell-mediated immunity. As observed in many studies in transplantation medicine, low levels of iATP (<225 ng/ml), are predictive of recurrent infections, relapse and poor prognosis due to the suboptimal immunologic function of the graft independently from leukocyte numbers assessed (8). We have demonstrated very low
Table I. Clinical improvement, intracellular CD4+ adenosine triphosphate (iATP) concentration, and lymphocyte counts in patients with psoriasis who underwent therapy with fumaric acid esters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>t0 (baseline) median (range)</th>
<th>t2 (2 months) median (range)</th>
<th>t4 (4 months) median (range)</th>
<th>p-value (Friedman test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASI</td>
<td>26.7 (10.6–56.2)</td>
<td>12.7 (4–28.2)</td>
<td>8.8 (1.2–16.4)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>iATP, ng/ml</td>
<td>474.4 (210.7–713.4)</td>
<td>489.7 (133.2–781)</td>
<td>405.3 (95.2–601.2)</td>
<td>0.11</td>
</tr>
<tr>
<td>Lymphocytes, cells/µl</td>
<td>1,578 (669–2,729)</td>
<td>1,216 (551–2,362)</td>
<td>1,088 (499–1,988)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CD3+, cells/µl</td>
<td>1,069 (314–2,101)</td>
<td>959 (335–1,593)</td>
<td>757 (239–1,344)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CD4+, cells/µl</td>
<td>752 (274–1,584)</td>
<td>657 (251–1,096)</td>
<td>412 (97–974)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CD8+, cells/µl</td>
<td>322 (35–685)</td>
<td>315 (37–779)</td>
<td>228 (35–454)</td>
<td>0.012*</td>
</tr>
<tr>
<td>CD4/CD8, cells/µl</td>
<td>2.3 (1.2–7.8)</td>
<td>2.2 (0.9–7.9)</td>
<td>2 (0.5–6.1)</td>
<td>0.32</td>
</tr>
<tr>
<td>CD56+, cells/µl</td>
<td>249 (51–623)</td>
<td>216 (77–515)</td>
<td>167 (89–528)</td>
<td>0.17</td>
</tr>
<tr>
<td>CD19+, cells/µl</td>
<td>167 (56–486)</td>
<td>145 (23–392)</td>
<td>71 (8–287)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

PASI: Psoriasis Area and Severity Index.

Fig. 1. Individual course of the intracellular CD4+ adenosine triphosphate (iATP) concentration during a 4-month therapy with fumaric acid esters. Statistical analysis confirmed that the median CD4+ iATP activity did not significantly differ between baseline, 2 and 4 months therapy (p=0.11).

REFERENCES