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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 coefficient of variation (CV) of 9.3% (10). In addition, changes in mitochondrial transmembrane potential ($\Delta\Psi_m$) in PMA-treated (2.5 µg/ml, 30 min; Sigma-Aldrich, Taufkirchen, Germany) $CD4^+$ cells (anti-CD4-APC, Becton Dickinson, Heidelberg, Germany) of six psoriasis patients were measured by flow cytometry (tetramethylrhodamine methyl and ethyl esters, 2 µM, 10 min; Invitrogen, Darmstadt, Germany). Mean fluorescence intensity (Canto II, Becton Dickinson) was assessed, with a minimum of 10,000 $CD4^+$ cells analysed (10). Non-parametric statistics, including the Friedman test and Spearman's rank correlation procedure, were used. A p-value < 0.05 was regarded as statistically significant.

RESULTS

As shown in Table I, FAE therapy resulted in a significant reduction in the median PASI from t0 to t2, and to t4. Lymphocyte counts of $CD3^+$, $CD4^+$ and $CD19^+$ cells decreased significantly from t0 to t2 and t4. $CD8^+$ lymphocytes at t4 were decreased significantly compared with t0 and t2. CD4/CD8 ratio and $CD56^+$ lymphocytes did not significantly differ between t0, t2 and t4. The median $CD4^+$ iATP concentration did not differ significantly between t0, t2 and t4 (Fig. 1). As shown by flow cytometry, $CD4^+$ iATP and mitochondrial tetramethyl rhodamine methylester proved to be overall stable bioenergetic parameters. Under FAE therapy, two patients developed iATP levels below 225 ng/ml (patient number 13: 133.2 ng/ml ($CD4^+$ count 1,092/µl); patient number 12: 95.2 ng/ml ($CD4^+$ count 251/µl)). 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 immunological 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*

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

[a] t0–t2 and t4, t2–t0 and t4, t4–t0 and t2. [b] t0–t4, t2–t4, t4–t0 and t2.
PASI: Psoriasis Area and Severity Index.

iATP concentrations in patients receiving monoclonal antibody therapy for the treatment of autoimmune diseases, who developed opportunistic central nervous system infections (10). We demonstrate here, for the first time, that T-cell-mediated immunity, as expressed by the $CD4^+$ iATP concentration, does not significantly differ in patients with psoriasis during FAE therapy, although a significant decline in the number of circulating lymphocytes is observed. Two patients transiently developed low $CD4^+$ iATP concentrations, but there was no evidence for infections.

Mrowietz et al.'s data (12) indicate that oral administration of dimethylfumarate probably does not modulate free fumaric acid and subsequently alter the citrate cycle and ATP metabolism (12, 13). A recall study indicated that killing of bacteria or fungi is not impaired by FAE therapy (14). As is also indicated by the present data, $CD4^+$ lymphocyte counts and their functional activity, as measured by the degree of induced ATP production, are independent variables in healthy subjects, immunosuppressed patients and patients with autoimmune diseases (10, 15).

Limitations of the present pilot-study include small sample size and short-time observation. Hence, $CD4^+$ iATP activity should also be investigated in a large cohort of patients who undergo long-term FAE therapy.

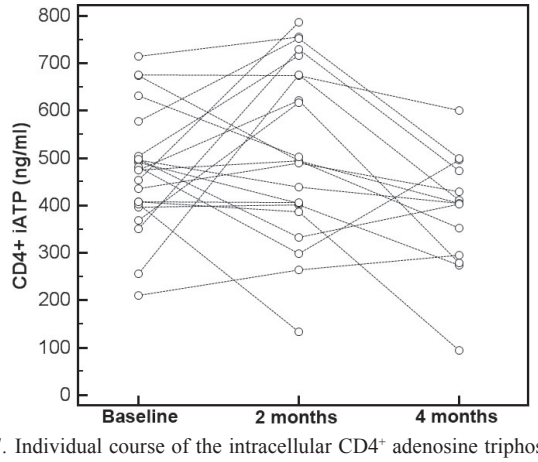

Fig. 1. Individual course of the intracellular $CD4^+$ adenosine triphosphate (iATP) concentration during a 4-month therapy with fumaric acid esters. Statistical analysis confirmed that the median $CD4^+$ iATP activity did not significantly differ between baseline, 2 and 4 months therapy ($p = 0.11$).

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