Supplemental Information

EBI2 Expression and Function: Robust in Memory Lymphocytes and Increased by Natalizumab in Multiple Sclerosis

Aurélie S. Clottu, Amandine Mathias, Andreas W. Sailer, Myriam Schluep, Jörg D. Seebach, Renaud Du Pasquier, and Caroline Pot
Figure S1. Related to Figure 1.

A

B

Live/Dead stain

CD3

CD4

CD56

CD8

CD45RA

CD45RO

CD19

CD27

CXCR3

CCR4

CCR6
Figure S1. Related to Figure 1. Flow cytometry gating strategy of lymphocyte subpopulations. (A) Naive and memory (defined as CD45RA⁺ and CD45RA⁻, respectively) CD4⁺ and CD8⁺ T cells, central memory (CCR7⁺) and effector memory (CCR7⁻) CD4⁺ T cells, naive and memory (defined as CD27⁻ and CD27⁺, respectively) CD19⁺ B cells, NK (CD3⁻CD56⁺) and NKT (CD3⁺CD56⁺) cells. (B) Th1 (CXCR3⁺CCR4⁻), Th2 (CXCR3⁺CCR4⁺) and Th17 (CXCR3⁻CCR6⁺) memory CD4⁺ T cell subsets. FS means forward scatter, with A for Area or W for Width for doublets exclusion; SS means side scatter. Histograms show EBI2 in dark grey and isotype in light grey.
Figure S2. Related to Figure 4.

A

B

CD4 T cell subsets

RRMS patients

DMF T0

DMF T9-12

r = -0.1867
p = 0.3611

r = -0.1416
p = 0.4903
Figure S2. Related to Figure 4. EBI2 expression is not increased with disease duration, elevated EDSS score or dimethyl fumarate treatment. (A) EBI2 expression with corresponding disease duration in years (left panel) or EDSS score (right panel) of RRMS patients (n=26) with or without disease modifying treatment (except natalizumab). EDSS means expanded disability status scale. RRMS means relapsing remitting multiple sclerosis patients. (B) Flow cytometric analysis of EBI2 expression on naive (CD45RA−) and memory (CD45RA+) CD4+ T cells before (T0) and after 9-12 months (T9-12) of dimethyl fumarate (DMF) treatment (n=5). EBI2 expression shown as mean fluorescence intensity ratios (MFIR).
Table S1. Related to Figure 4. Dimethyl fumarate treated patients.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Mean Age (± SEM)</th>
<th>n</th>
<th>Mean EDSS at T0 (±SEM)</th>
<th>Mean EDSS at T9-12 (±SEM)</th>
<th>Disease mean duration at blood draw (± SEM)</th>
<th>Previous DMT</th>
<th>Mean EDSS at T0 (±SEM)</th>
<th>Mean EDSS at T9-12 (±SEM)</th>
<th>Disease mean duration at blood draw (± SEM)</th>
<th>Previous DMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF patients</td>
<td>female</td>
<td>32.33 (5.78)</td>
<td>3</td>
<td>1.5 (0.00)</td>
<td>2.00 (0.5)</td>
<td>4.67 (1.20)</td>
<td>1 Glatiramer acetate</td>
<td>1 Fingolimod (stopped since two months)</td>
<td>1 untreated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>40.5 (7.5)</td>
<td>2</td>
<td>2.25 (0.75)</td>
<td>2.00 (0.5)</td>
<td>16.5 (3.5)</td>
<td>2 Interferon-beta</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RRMS: relapsing remitting MS patients
DMF: dimethyl fumarate treated patients
Age in years. Disease duration in years.
EDSS: expanded disability status scale
Figure S3. Related to Figure 4
Figure S3. Related to Figure 4, NTZ is not able to promote EBI2 expression in vitro. Human PBMC were kept after an overnight resting in culture medium for 40 minutes at 37°C with either only culture medium or additioned (A) with NTZ or (B) 7α,25-OHC at the indicated concentrations (n=3). The T cells were analyzed by flow cytometry. EBI2 expression is shown as mean fluorescence intensity ratios (MFIR) in memory CD3⁺CD4⁻ T cells, gated after dead cell and doublet exclusion. Statistical analysis using unpaired t test with a p value ≤0.05 considered significant.