Compositional Changes of B and T Cell Subtypes during Fingolimod Treatment in Multiple Sclerosis Patients: A 12-Month Follow-Up Study

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Keyword(s): Multiple Sclerosis, Fingolimod, B cells, T cells

1. BACKGROUND AND OBJECTIVE
A complex interplay between T and B cells drives the disease course of multiple sclerosis (MS). Thereby, non class-switched (CD19+IgD+CD27+) and class-switched (CD19+IgD-CD27+) memory B cells are generally considered to be the main pathogenic B cell subtypes, whereas, conventional (autoreactive) T cells (CD4+CD25-CD127+) can drive the disease and regulatory T cells (CD4+CD25hiCD127lo) control immune homeostasis1-3. Fingolimod is a FDA approved oral treatment for MS and has shown efficacy in relapsing remitting (RR) MS4-7. It is an immunomodulator that interferes with the signaling of the sphingosine-1-phosphate receptor 1 (S1PR1), present on lymphocytes, and causes the internalization and degradation of this receptor8. Consequently lymphocytes cannot exit the lymph nodes into the circulation, leading to the entrapment of lymphocytes in lymphatic systems. MS patients treated with fingolimod show a general lymphopenia, therefore it is assumed that the number of inflammatory cells migrating to the central nervous system (CNS) are reduced8-11. The long-term effects of fingolimod on blood circulating B and T cell subtypes in MS patients are not completely understood. This study describes for the first time the longitudinal effects of fingolimod treatment on B and T cell subtypes in MS patients in a 12 month follow-up study.
2. METHODS

Using flow cytometry, B and T cell subtypes were measured during a 12 month follow-up in the peripheral blood of MS patients. Data of fingolimod-treated MS patients (n=49) were compared to those from treatment-naive (n=47) and interferon-treated (IFN-β; n=27) MS patients.

3. RESULTS

The cohort of 49 fingolimod-treated MS patients was compared at baseline with 47 treatment-naive and 27 IFN-β treated MS patients (together referred to as controls). Fingolimod-treated MS patients at baseline and controls were comparable in terms of age, gender distribution and median expanded disability status scale (EDSS) score (table 1). Furthermore, no significant difference was observed in numbers of total lymphocytes, B cells or T cells (figure 1) between patients at start of fingolimod treatment and controls. For the MS patients receiving fingolimod treatment, pretreatment (baseline) values were used as reference to assess the effects of treatment.

Total lymphocyte numbers were decreased after 1 month (1m) of fingolimod treatment compared with baseline and controls for the total duration of the study (12m) (p<0.001; figure 1). Furthermore, total CD19+ B cell and CD4+ T cell numbers were decreased at 1m and reached a steady state at 3m (p<0.001; figure 1). Similar results were observed for the percentage of CD19+ and CD4+ cells within the lymphocyte population (p<0.001; data not shown).

Table 1 Study population

<table>
<thead>
<tr>
<th>Age (range)</th>
<th>Gender F/M</th>
<th>Classification</th>
<th>EDSS (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n=123)</td>
<td>44 (17-79)</td>
<td>90/33</td>
<td>RR 29</td>
</tr>
<tr>
<td>Treatment naive (n=47)</td>
<td>48 (17-79)</td>
<td>33/14</td>
<td>RR 22</td>
</tr>
<tr>
<td>Interferon (n=27)</td>
<td>42 (17-66)</td>
<td>19/8</td>
<td>RR 43</td>
</tr>
<tr>
<td>Fingolimod (n=49)</td>
<td>44 (18-69)</td>
<td>38/11</td>
<td>RR 49</td>
</tr>
<tr>
<td>Non-responders (n=7)</td>
<td>44 (34-54)</td>
<td>5/2</td>
<td>RR 41</td>
</tr>
<tr>
<td>Drop outs (n=5)</td>
<td>41 (32-56)</td>
<td>5/0</td>
<td>RR 41</td>
</tr>
<tr>
<td>0 m (n=28)</td>
<td>43 (18-67)</td>
<td>21/7</td>
<td>RR 43</td>
</tr>
<tr>
<td>1 m (n=24)</td>
<td>41 (18-67)</td>
<td>18/6</td>
<td>RR 43</td>
</tr>
<tr>
<td>3 m (n=29)</td>
<td>43 (18-67)</td>
<td>22/7</td>
<td>RR 43</td>
</tr>
<tr>
<td>6 m (n=26)</td>
<td>43 (18-69)</td>
<td>20/6</td>
<td>RR 43</td>
</tr>
<tr>
<td>9 m (n=27)</td>
<td>45 (18-69)</td>
<td>23/4</td>
<td>RR 45</td>
</tr>
<tr>
<td>12 m (n=13)</td>
<td>45 (29-69)</td>
<td>11/2</td>
<td>RR 45</td>
</tr>
</tbody>
</table>

a. Mean age in years
b. For 6 treatment-naive patients, MS type was not specified
c. Median EDSS score; this information was not available for 7 treatment-naive patients and 6 IFN-β-treated patients

Abbreviations: F = female; M = male; RR = relapsing-remitting MS; CP = chronic progressive MS; EDSS = expanded disability status scale, m = month
Figure 1. Total number of lymphocytes, CD4+ T cells and CD19+ B cells in the PB. Total number (x10^3 cells/µl blood) of lymphocytes, T cells and B cells in treatment-naive, IFN-β treated MS patients at baseline and fingolimod-treated MS patients during 12 months follow-up. Mean and standard error of the mean are presented. ● lymphocytes; ■ CD4+ T cells; ▲ CD19+ B cells

In the B cell population, we observed a decrease in the proportion of non class-switched and class-switched memory B cells (figure 2 in appendix; p<0.001), both implicated in MS pathogenesis, while the proportion of naïve B cells was increased during fingolimod treatment in the peripheral blood (PB) of MS patients (figure 2; p<0.05). The remaining T cell population, in contrast, showed elevated proportions of memory conventional (Tconv) and regulatory (Treg) T cells (figure 2 in appendix; p<0.01) and declined proportions of naïve conventional and regulatory cells (p<0.05). These naïve T cell subtypes are main drivers of MS pathogenesis.

4. CONCLUSION

In this study, we elucidate the effects of fingolimod, approved as therapy for RR-MS, on different B and T cell subtypes during a 12 month follow-up study. Under fingolimod treatment, the B cell subtype distribution changed, resulting in a decreased proportion of memory B cells and an increased proportion of naïve and double negative B cells in the PB. In contrast, the proportions of T cell subtypes changed towards less naïve Tconv and naïve Treg in the PB, while the proportions of memory Tconv and memory Treg increased. To conclude, this study shows that fingolimod induces compositional changes of B and T cell subtypes that are potentially implicated in MS pathogenesis and may explain the therapeutic efficacy of the treatment. With this descriptive study we provide additional longitudinal immunological proof for the diverse mechanisms of action of fingolimod in MS patients.
5. REFERENCES
Figure 2: Proportional B cell and T cell subtype changes in MS patients during fingolimod treatment. (A) CD19+ B cell subtype proportion and (B) CD4+ T cell subtype proportion within the PB of treatment-naive, IFN-β and fingolimod-treated MS patients. Results are presented as relative values within the CD19+ B cell or CD4+ T cell population. Subtype proportions were calculated as follows: (% subtype/100) x % CD19+ or CD4+ within the total lymphocyte population. Statistically significant differences compared to 0m are shown in bold. For B cells: naive B cells; NCSM B cells = non class-switched memory B cells; CSM B cells = class-switched memory B cells and DN B cells = double negative B cells. For T cells: nTreg = naive Treg; mTreg = memory Treg; TransTreg = transitional Treg; nTconv = naive Tconv; mTconv = memory Tconv; TransTconv = transitional Tconv.