Dysregulation of T and B cells in Myalgic encephalomyelitis/Chronic Fatigue Syndrome
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Results

Methods

Results

Background

- ME/CFS is a severely debilitating disease of unknown etiology
- Immune system has been considered to be involved in the pathogenesis of ME/CFS (ex. NK cell dysfunction, increased level of inflammatory cytokines, Th2 shift etc.)
- Recently, rituximab studies showed therapeutic benefit to ME/CFS patients
- However, underlying mechanism of the therapeutic effect of rituximab on ME/CFS patients is unclear

Objective
Evaluation of 1) T and B cell subsets 2) B cell receptor repertoire 3) gene expression of B cells in ME/CFS patients

Results

- Plasmablasts were decreased in ME/CFS patients
- High expression of CD86 on mB in ME/CFS
- Treg cells were decreased in ME/CFS patients
- Tcm cells were activated in ME/CFS patients
- IFN inducible genes were up-regulated in PB of ME/CFS

Findings

- High clonality of BCRs in ME/CFS are observed

Patients and Healthy controls (HC) demography

<table>
<thead>
<tr>
<th>P</th>
<th>ME/CFS (n = 46)</th>
<th>HC (n = 21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD)</td>
<td>39.4 (± 11.7)</td>
<td>39.0 (± 9.45)</td>
<td>0.81</td>
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<tr>
<td>Age at onset (mean ± SD)</td>
<td>28.9 (± 11.1)</td>
<td>26.5 (± 10.5)</td>
<td>0.52</td>
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<tr>
<td>Sex (Male:Female)</td>
<td>9:37</td>
<td>6:15</td>
<td>0.52</td>
</tr>
<tr>
<td>Disease duration (mean years (range))</td>
<td>10.7 (1-33)</td>
<td>10.0 (1-33)</td>
<td></td>
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<tr>
<td>Flu-like symptoms* prior to onset</td>
<td>27 (57%)</td>
<td>6 (29%)</td>
<td></td>
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<tr>
<td>Immune associated complications*</td>
<td>27</td>
<td>6</td>
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</tr>
</tbody>
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*Flu-like symptom; fever, sore throat, lymph node swelling.

P-values were determined by Mann-Whitney U-test or Fisher’s exact test.

Flowcytometer analysis

B cell populations were defined as follows: total B cells, CD19+; naïve B cells (nB), CD19+CD27–; memory B cells (mB), CD19+CD27+CD180+; plasmablasts (PB), CD19+CD27+CD180–CD38++; transitional B cell (TrB), CD19+CD27–CD24+ Mito tracker green ++
The expression of CD40, 80 and 86 on each B cell subset was analysed

T cell populations were defined as follows: naïve T cells (Tn), CD3+CD4+CCR7+CD45RA+; central memory T cells (Tcm), CD3+CD4+CCR7+CD45RA–; effector T cells (Teff), CD3+CD4+CCR7–CD45RA–; Regulatory T cells (Treg), CD3+CD4+CD45RA–CD127+CD25++
The expression of HLA-DR on each T cell subset was analysed

Gene expression analysis of sorted plasmablasts was performed using the Nanospring Counter technology with the nCounter GX Human Immunology V2 kit. Gene expressions were analyzed if expression levels were more than 10 reads and more than 1.25-fold compared to HC.

B cell receptor (BCR) repertoire analysis

Total RNA isolation and sequencing of the BCR were performed at Repertoire Genesis Incorporation (Osaka, Japan) using the unbiased gene amplification method with Adaptor-Ligation PCR.
The diversity and clonality of BCR were evaluated by calculating indices including normalized Shannon index, inverse Simpson’s index and diversity evenness 50 (DE50)