Increased frequencies of IgD+CD27- double negative (DN) B cells with a pro-inflammatory phenotype and function in multiple sclerosis patients

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Introduction

B cells are important players in multiple sclerosis (MS) pathogenesis:
1) Production of (auto)antibodies
2) Cytokine production
3) Antigen presentation & costimulation
4) Formation of ectopic lymphoid follicles

Immune aging

Changes in the immune system when people grow older:

Age-associated IgD+CD27- double negative (DN) B cells

Immune response development, decline:

Present in a proportion of MS patients

Aim of the study
To investigate the prevalence and functional characteristics of DN B cells in MS patients

Patient sampling and methods

Study population

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Age</th>
<th>% F</th>
<th>MS type</th>
<th>Treatment</th>
<th>Previous treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of DN B cells</td>
<td></td>
<td></td>
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<tr>
<td>HC</td>
<td>42</td>
<td>41.8 ± 11.1</td>
<td>69.1</td>
<td>NA</td>
<td>NA</td>
<td>UT: 77; TRT: 11</td>
</tr>
<tr>
<td>MS</td>
<td>88</td>
<td>41.9 ± 11.5</td>
<td>71.8</td>
<td>0</td>
<td>63</td>
<td>14</td>
</tr>
</tbody>
</table>

Expression of costimulatory and antigen presentation molecules

CD31:31 ± 11.2:5
CD47: 48 ± 13.3

Chemokine receptor expression

HC | 25 | 0 ± 10.0 | 6.1 | NA | NA | UT: 47 |
MS | 49 | 44 ± 15.0 | 7.3 | 0 | 31 | 12 | 6 | 5.2 | UT: 47 |

T-cell expression

HC | 24 | 41 ± 12.4 | 7.0 | NA | NA | UT: 39; TRT: 8 |
MS | 47 | 44 ± 15.0 | 7.4 | 0 | 30 | 11 | 6 | 5.7 | UT: 39; TRT: 8 |

Cytokine production in vitro B cell stimulation

In vitro chemotaxis assay

MS untreated: n = 7
MS treated: n = 6
HC: n = 10 (with similar age/gender)

B cells express functional molecules

Antigen presentation and costimulatory molecules

HLA-DR/DQ

Expression levels (mean fluorescence intensity, MFI) of HLA-DR/DQ, CD80, and CD86 for naive, DN and CSM B cells from HC (n = 31) and MS patients (n = 47). Mean ± SD is depicted. * p < 0.05; ** p < 0.01; *** p < 0.001

DN B cells showed similar or increased HLA-DR/DQ expression as CSM B cells

DN B cells showed CD80/CD86 expression in between that of naive and CSM B cells

Pro-inflammatory chemokine receptors

CXCR3

Expression levels (mean fluorescence intensity, MFI) of CXCR3 in naive, DN and CSM B cells from HC (n = 31) and MS patients (n = 47). Mean ± SD is depicted. * p < 0.05; ** p < 0.01; *** p < 0.001

DN B cells showed CXCR3 and CXCR5 expression similar to naive and CSM B cells, respectively

DN B cells produce pro-inflammatory cytokines in vitro

LT-α

The percentage of LT-α+ and TNF-α+ cells after B cell stimulation using CD40 ligand for HC (n = 10) and MS patients (n = 12). Granulocyte-macrophage colony-stimulating factor (GM-CSF) was measured after B cell stimulation with 21 (LPS) or anti-human IgG, Mean ± SD is depicted. * p < 0.05; ** p < 0.01; *** p < 0.001

DN B cells showed a similar frequency of LT-α+ cells and a higher frequency of TNF-α+ and granocyte-B+ cells compared with the CSM B cell population

DN B cells migrate towards pro-inflammatory cytokines in vitro

CXCL10

Chemotactic index (CI) for naive, DN and CSM B cells of MS patients (n = 7) in the presence of CXCL10 or CXCL13. Mean ± SD is depicted. * p < 0.05; ** p < 0.01

MS DN B cells showed high migration capacity towards CXCL10 (CXCR3 ligand) and CXCL13 (CXCR5 ligand) that is similar to that of CSM B cells

A proportion of DN B cells express the transcription factor T-bet

A proportion of DN B cells express T-bet compared with naive and CSM B, with about 21.6% of DN B cells being T-bet+

Conclusions

- DN B cells are abnormally elevated in the peripheral blood and CSF of MS patients
- DN B cells could migrate into the central nervous system via chemokines involved in MS pathology
- DN B cells have pro-inflammatory functional characteristics
- DN B cells show highest expression of T-bet, that has been described in another pathological age-associated B cell subset
- Potential importance of DN B cells in MS pathology
- Could lead to novel targets for more specific MS therapy

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