IL-22 secreting CD4+ T cells in the patients with neuromyelitis optica and multiple sclerosis

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Interleukin (IL)-22 secreting CD4+ T (Th22) cells and IL-22 are involved in the pathogenesis of autoimmune disease, but their role in neuromyelitis optica (NMO) and multiple sclerosis (MS) is unclear. We measured the proportion of Th22, Th17, CD4+IL-22+IL-17A+ T cells and serum IL-22 in NMO and MS patients. The proportion of Th22 cells, Th17 cells and serum IL-22 were increased in patients with NMO and MS. Our findings suggest that increased Th22 cells may play an important role in the pathogenesis of NMO and MS.

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1. Introduction

Neuromyelitis optica (NMO) and multiple sclerosis (MS) are prototypic autoimmune inflammatory diseases of the central nervous system (CNS). Most NMO and MS patients present with relapsing–remitting (RR) clinical courses (Fazio et al., 2011). Anti aquaporin 4 antibodies (AQP4-Ab) has been proposed to be specific biomarker for distinguishing NMO from conventional MS (Lennon et al., 2004).

NMO and MS have different pathology and clinical symptoms, although the pathogenesis of both disorders is not completely understood. The role of T cells in the pathogenesis of NMO and MS is important. Previously, we showed that interleukin (IL)-17 secreting CD4+ T (Th17) cells in the peripheral blood of NMO and MS patients during relapse have a higher activation status compared with healthy control (Li et al., 2011; Wang et al., 2011), suggesting an important pathogenic role of Th17 cells in NMO and MS patients. Th22 cells subset is a recently identified CD4+ T helper group distinct from Th17 and Th1 cells, which is characterized by the secretion of IL-22 (Duhen et al., 2009; Trifári et al., 2009). IL-22 is a member of the IL-10 family, which also includes IL-19, IL-20, IL-24, IL-26, IL-28 (α and β) and IL-29 (Wolk et al., 2010). IL-22 signals through a receptor complex consisting of the IL-10R β chain and IL-22R (Kotenko et al., 2001). IL-6, IL-21, and IL-23 can induce Th17 cell differentiation, but IFN-γ and IL-27 suppress Th17 cell differentiation. IL-6 and tumor necrosis factor (TNF) can promote Th22 cells differentiation (Duhen et al., 2009). Recently, Th22 cells have been shown to be important in the pathogenesis of many autoimmune diseases. Th22 cells were significantly elevated in rheumatoid arthritis (RA) and ankylosing spondylitis (AS) (Zhang et al., 2011a, 2012), are increased in the peripheral blood of patients with systemic sclerosis (SSc) (Truchetet et al., 2011), and over-expressed in systemic lupus erythematosus (SLE) and psoriasis (Kagami et al., 2010; Qin et al., 2011). IL-22 is also involved in the pathogenesis of experiment autoimmune encephalomyelitis (EAE), a murine model of human MS (Grigorian et al., 2011). IL-22 activates Jak1 and Tyk2, and induces tyrosine phosphorylation of STAT3 (Lejeune et al., 2002). It is speculated that activation of the STAT3 pathway is the key and cross point of the activation of Th22 cells and Th17 cells. However, the role of Th22 cells in NMO and MS are rarely reported.

In this study, we investigated the proportions of Th22 cells (CD4+IL-22+IL-17A+ T cells), Th17 cells (CD4+IL-22-IL-17A+ T cells) and CD4+IL-22-IL-17A+ T cells in the peripheral blood, and the serum concentrations of IL-22, IFN-γ, IL-6, IL-21 and IL-27 in patients with NMO and MS during relapse. We aimed to evaluate whether Th22 cells are involved in the pathogenesis of NMO and MS by analyzing the correlation between Th22 cells, Th17 cells.

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2. Subjects and methods

2.1. Patients and controls

Twenty one patients with relapsing NMO and 15 patients with RRMS in the relapsing stage were enrolled from the Neurology Department of the Third Affiliated Hospital of Sun Yat-sen University between May 2011 and April 2012. Twelve age- and gender-matched healthy volunteers were recruited as controls (CTLs). NMO patients fulfilled the Wingerchuk diagnostic criteria (Wingerchuk et al., 2006) and RRMS patients were diagnosed based on the 2005 McDonald criteria (Polman et al., 2005). Relapses were defined as the appearance of new symptoms and signs, or worsening of existing symptoms, lasting for at least 24 h, with an increase in Expanded Disability Status Scale (EDSS) scores over 1.0 before sampling. No patients were receiving immunomodulatory therapies during remission. Indirect immunofluorescence test systems for human AQP4-Ab detection from EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika, Lubeck, Germany) were used. AQP4-Ab was assessed following the manufacturer’s instructions. The study protocol has been approved by the Ethics Committee of Sun Yat-sen University.

2.2. Flow cytometry

Peripheral blood was collected in sodium heparin tubes. Peripheral blood mononuclear cells (PBMCs) were purified using Ficoll–Hypaque gradient centrifugation (Tianjin Hao yang Biological Manufacture, Tianjin, China). PBMCs were adjusted to a final concentration of 10^6/ml in 1640 medium supplemented with 10% heated inactivated fetal calf serum. The PBMCs were stimulated with phosphor-12-myristate 13-acetate (50 ng/ml; Sigma, ST Louis, MO, USA) and ionomycin (1 μg/ml) for 4 h, in 5% CO2 incubation at 37 °C. These stimulated PBMCs were surface stained with CD3-FITC, CD4-PE-Cy 5.5 (eBioscience, San Diego, CA, USA) and CD8-FITC or PerCP-Cy 5.5 (eBioscience) and their isotype controls to enable correct compensation. After intracellular staining, the PBMCs were washed and resuspended for analysis by flow cytometry (FACS Calibur; BD Bioscience, San Jose, CA, USA).

2.3. Cytokines enzyme-linked immunosorbent assay (ELISA)

Serum samples were isolated within 1 h of blood sample collection and stored at -70 °C until use. The concentrations of IL-22, IFN-γ, IL-6, IL-21 and IL-27 were assessed by ELISA (Bender, MedSystems, Vienna, Austria) in accordance with the manufacturer’s instructions. Optical density was measured by microtiter plate reader at 450 nm. The sensitivities of each ELISA were: 5 pg/ml for IL-22, 0.99 pg/ml for IFN-γ, 0.92 pg/ml for IL-6, 20.0 pg/ml for IL-21 and 9.5 pg/ml for IL-27. The inter- and intra-coefficients of variabilities were 7.3% and 4.6%, 5.2% and 4.3%, 5.5% and 4.6%, 7.8% and 6.9%, 5.7% and 6.5%, respectively, for IL-22, IFN-γ, IL-6, IL-21 and IL-27.

3. Results

3.1. Demographic and clinical features of NMO and MS patients

As shown in Table 2, there was no difference in the age or gender of each group. The body mass index (BMI), EDSS, disease duration, and annualized relapse rate scores in the patients with NMO and MS were not statistically different.

3.2. The proportion of Th22 cells, Th17 cells both are elevated in PBMCs from patients with NMO and MS

A representative plot of the proportion of Th22 cells, Th17 cells and CD4+IL-22^+IL-17A^+ T cells in a typical patient with NMO, MS and healthy control is shown in Fig. 1. The proportion of Th22 cells was significantly higher in NMO than in MS (1.37% ± 0.87% VS. 1.13% ± 0.72%, P < 0.05) and CTLs (1.37% ± 0.87% VS. 0.55% ± 0.51%, P < 0.01). Th22 cells in MS patients were also higher than in CTLs (1.13% ± 0.72% VS. 0.55% ± 0.51%, P < 0.01). The percentage of Th17 cells in NMO was similar to that in MS (1.21% ± 0.74% VS. 1.04% ± 0.71%, P = 0.347) but were markedly higher than in CTLs (1.21% ± 0.74% VS. 0.79% ± 0.57%, P < 0.05). The percentage of Th17 cells in MS patients was also higher than CTLs (1.04% ± 0.71% VS. 0.79% ± 0.57%, P < 0.05). The proportion of CD4^+ IL-22^+IL-17A^+ T cells in patients of NMO, MS and CTLs was not statistically different (Fig. 2).

3.3. Serum levels of cytokines in patients with NMO, MS and CTLs

Serum concentrations of cytokines are shown in Fig. 2. Serum IL-22 levels were significantly higher in patients with NMO (43.54 ± 32.90 pg/ml VS. 18.92 ± 6.52 pg/ml, P < 0.05) and MS (41.55 ± 13.51 pg/ml VS. 18.92 ± 6.52 pg/ml, P < 0.05) than CTLs. Serum levels of IFN-γ in MS were higher than NMO (64.20 ± 12.57 pg/ml VS. 42.09 ± 17.01 pg/ml, P < 0.01) and CTLs (64.20 ± 12.57 pg/ml VS. 30.92 ± 12.23 pg/ml, P < 0.01). The IL-21 concentration in NMO was higher than CTLs (94.81 ± 37.16 pg/ml VS. 75.08 ± 24.85 pg/ml, P < 0.01). No significant differences were found in serum IL-6 and IL-27 levels between the three groups.

3.4. Correlation of Th22 cells, Th17 cells and serum IL-22 levels in the three groups

As shown in Fig. 3, the proportion of Th22 cells and Th17 cells had a positive correlation in patients with NMO (r = 0.458, P = 0.037) (Fig. 3 A). However, the proportion of Th22 cells and serum IL-22 was not positively correlated in patients with NMO (r = 0.204, P = 0.375) (Fig. 3 B). MS patients showed no correlation between the percentage of Th22 cells and Th17 cells, or between the proportion of Th22 cells and serum IL-22 levels (Fig. 3 C, D). There was also no correlation between the Th22 cells and Th17 cells, or Th22 cells and serum IL-22 in CTLs (Fig. 3 E, F).

Table 1

<table>
<thead>
<tr>
<th>Correlation</th>
<th>R value</th>
<th>P value</th>
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<tbody>
<tr>
<td>MS</td>
<td>0.362</td>
<td>0.139</td>
</tr>
<tr>
<td>Disease duration and EDSS scores</td>
<td>0.428</td>
<td>0.072</td>
</tr>
<tr>
<td>Disease duration and Th22cells</td>
<td>0.542</td>
<td>0.024</td>
</tr>
<tr>
<td>NMO</td>
<td>0.732</td>
<td>0.074</td>
</tr>
<tr>
<td>Disease duration and EDSS scores</td>
<td>0.685</td>
<td>0.065</td>
</tr>
<tr>
<td>Disease duration and Th22cells</td>
<td>0.572</td>
<td>0.109</td>
</tr>
</tbody>
</table>

Disease duration (in months) refers to the number of months from disease onset to interview; EDSS: Expanded Disability Status Scale; MS: multiple sclerosis; NMO: neuromyelitis optica.
4. Discussion

In this study, we revealed that the proportion of Th22 cells and Th17 cells in NMO and MS patients was higher than in CTLs. Serum IL-22 levels were also increased in NMO and MS. The proportion of Th22 cells significantly correlated with Th17 cells in patients with NMO.

MS is considered a T cell-mediated autoimmune disease and both Th1 and Th17 cells play an important role in the pathogenesis of MS and EAE (Grigorian et al., 2011; Jadidi-Niaragh and Mirshafiey, 2011). However, Th22 cells have been shown to have a complicated and important role in many inflammatory and autoimmune diseases (Qin et al., 2011; Zhang et al., 2012).

There is evidence that IL-22 is produced by Th17 cells regulated by RORγt (Miller and Weinmann, 2009). IL-22 activates Jak1 and Tyk2, which also induces tyrosine phosphorylation of STAT1, STAT3 and STAT5 (Lejeune et al., 2002). A single nucleotide polymorphism of IL-22 receptor (IL-22RA2) is a risk gene in EAE and in patients with MS (Beyeen et al., 2010). IL-22 was decreased following N-acetylglucosamine inhibition of Th1 and Th17 cell responses in EAE (Grigorian et al., 2011). In MS patients, simvastatin was found to inhibit the secretion of IL-22 and IL-17A and other inflammatory cytokines (Zhang et al., 2011b). In an EAE study (Almolda et al., 2011), IL-22 concentrations were highest in the peak phases and sharply decreased during the recovery. These studies indicated that IL-22 may play an important role in the pathogenesis of both MS and EAE.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>NMO (n = 21)</th>
<th>MS (n = 15)</th>
<th>CTL (n = 12)</th>
<th>P values</th>
</tr>
</thead>
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<tr>
<td>Gender, female/male</td>
<td>17/4</td>
<td>10/5</td>
<td>9/3</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>22.4 ± 4.4</td>
<td>21.8 ± 4.2</td>
<td>21.2 ± 2.6</td>
<td>—</td>
</tr>
<tr>
<td>New symptoms/signs (%)</td>
<td>14/21</td>
<td>7/15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Worsening (%)</td>
<td>7/21</td>
<td>8/15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.14 ± 13.42</td>
<td>35.33 ± 7.87</td>
<td>35.25 ± 14.57</td>
<td>NS</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>47.21 ± 46.43</td>
<td>46.38 ± 44.24</td>
<td>—</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Annualized relapse rate</td>
<td>1.2 ± 0.9</td>
<td>0.9 ± 0.6</td>
<td>—</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>EDSS score</td>
<td>3.47 ± 1.06</td>
<td>3.43 ± 1.33</td>
<td>—</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Seropositive number of AQP4-Ab</td>
<td>18</td>
<td>2</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Disease duration (in months) refers to the number of months from disease onset to interview; EDSS: Expanded Disability Status Scale; MS: multiple sclerosis; NMO: neuromyelitis optica. BMI: body mass index; NS: no statistical difference; Values are means ± SD.

Fig. 1. Proportion of Th22 cells, Th17 cells and CD4+IL-22+IL-17A+ T cells in NMO, MS and CTLs. CD3+ T cells (A); CD3+CD4+ T cells (B); Isotype control staining of IL-22 and IL-17A (C). Th22 cells, Th17 cells and CD4+IL-22+IL-17A+ T cells from a representative patient of NMO (D), MS (E) and healthy control (F) groups.

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Whether Th22 cells are involved in the pathogenesis of NMO is still unclear.

In our study, we first showed that the proportion of Th22 cells in NMO and MS patients was higher than in CTLs, and that serum IL-22 levels were also increased in NMO and MS. It is suggested that Th22 cells were activated in patients of NMO and MS during relapse. Th22 cells correlated with Th17 cells, suggesting that Th22 cells and Th17 cells may play a synergistic role in NMO and MS. As IL-23 promotes the secretion of IL-17A and IL-22 (Zheng et al., 2007; Volpe et al., 2008), we speculate that activation of the STAT3 pathway is a key and cross point in the activation of Th22 cells and Th17 cells. In contrast to the results for Th22 cells and Th17 cells, no statistical difference was detected in the proportion of CD4+IL-22+IL-17A+ T cells between each group. The results showed that Th22 cells in patients with NMO are higher than in patients with MS similar to Th17 cells, which has been shown in our previous study (Wang et al., 2011). One study has found that aging has been associated with IL-22 and IL-17 (Ouyang et al., 2011). We did not found this, perhaps because we selected age-matched patients and controls. However we found that the disease duration positively correlated with Th22 cells in MS, and that Th22 cells are increased in both MS and NMO. Unexpectedly, we did not observe a correlation between serum IL-22 concentrations and Th22 cells in patients with NMO and MS. It is could be because serum IL-22 may originate from CD8+ T cells, NK cells, B cells and myeloid cells, in addition to CD4+ T cells. It was reported that IL-21, IL-23 and IL-6 promote Th22 differentiation, but IFN-γ and IL-27 suppress Th17 cell differentiation (Duhen et al., 2009). We also found that serum IL-21 levels were higher in the NMO, and

Fig. 2. Th22 cells, Th17 cells, CD4+IL-22+IL-17A+ T cells and relative cytokines levels in each group. Th22 cells (A), Th17 cells (B), CD4+IL-22+IL-17A+ T cells (C), serum IL-22 (D), IL-6 (E), IFN-γ (F), IL-21 (G) and IL-27 (H) in NMO, MS and control groups.

Fig. 3. Correlation between Th22 cells and Th17 cells, Th22 cells and serum IL-22 concentrations in the three groups. Correlation between Th22 cells and Th17 cells in NMO patients (A), and MS patients (C). Correlation between Th22 cells and serum IL-22 concentrations in NMO patients (B), and MS patients (D).
IFN-γ levels were high in NMO and MS, similar to previous studies (Wu et al., 2012). Serum and cerebrospinal fluid IL-6 concentrations have been found to be higher in patients with NMO (Icöz et al., 2010), however, in our study there were no differences in serum IL-6 or IL-27 levels between NMO, MS and CTLs. This may be a result of the small sample size used.

5. Conclusion

In conclusion, our study revealed for the first time that the proportion of Th22 cells, Th17 cells and serum IL-22 levels was increased in patients with NMO and MS. Our findings suggest that increased Th22 cells and serum IL-22 may play an important role in patients with NMO and MS, similar to Th17 cells.

Abbreviations

NMO: neuromyelitis optica
MS: multiple sclerosis
CNS: central nervous system
CTLs: controls
EDSS: Expanded Disability Status Scale
PBMC: peripheral blood mononuclear cell
TNF: tumor necrosis factor
RA: rheumatoid arthritis
AS: ankylosing spondylitis
SSc: systemic sclerosis
SLE: systemic lupus erythematosus
EAE: experimental autoimmune encephalomyelitis
ELISA: Enzyme-Linked Immunosorbent Assay.

Conflict of interest

These authors have no conflict of interest to declare.

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References