**Lymphocyte Subsets And Cytokine Production In Patients With Neuromyelitis Optica**

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**Introduction**

Neuromyelitis optica (NMO) is an inflammatory disease of central nervous system (CNS) with production of antibodies against aquaporin-4 that play a crucial role in the pathogenesis triggering complement dependent cytotoxicity. However, the exact role of cellular mechanisms and cytokine crosstalk in NMO remains elusive. It has been shown in vivo that neutrophils could play a role in early NMO lesion development followed by macrophages and lymphocytes.

In our work we focused on basic lymphocyte subpopulations and intracellular cytokines production in patients with NMO. Results data were compared with control group and in group between rituximab treated individuals vs. other form of treatment and between relapse and remission of the disease. Tested groups consisted of 20 patients with NMO and 23 healthy controls (see Table 1). Patients were tested for lymphocyte subpopulations CD3+, CD3-CD4+ , CD3-CD8+, NK cells, B-cells and intracellular cytokine production (in T-lymphocytes: IL-2, IL-4, TNFa, IFNγ; in mocytes: IL-10, IL-12, TNFα). NMO patients were treated with usual immunosuppressive therapy or with rituximab. Follow-up period was approximately 41 months (from 17 to 60).

**RESULTS**

The total lymphocyte count and the absolute counts of CD3+CD4+, CD3-CD8+, CD19+ lymphocytes and NK cells NMO patients were significantly lower compared to controls. However, the relative count of CD3+CD8+ lymphocytes was higher and for CD19+ lower in NMO patients (p = 0.02 and 0.002 respectively) (Figure 1).

During the relapse of the disease, the total lymphocyte count as well as the absolute counts of CD3+CD8+ , CD19+ and NK cells in NMO patients were not changed with the exception of CD3+CD4+, where we observed lower absolute and relative count (p = 0.05 and 0.0001 respectively). However, there was a significant relative increase of CD3-CD8+ lymphocytes (p = 0.001). CD19+ lymphocytes and NK cells did not show any changes in relative count depending on disease activity (Figure 2).

![Figure 1: Lymphocyte subpopulations in patients with neuromyelitis optica (NMO) at baseline and controls (C) (relative count).](image)

![Figure 2: Lymphocyte subpopulations in patients with NMO in remission and relapse (relative count).](image)

Production of IL-4 and TNFa by CD3+ lymphocytes was significantly lower in patients with NMO compared with controls (p = 0.04 and p = 0.001 respectively). There was no significant difference in production of other cytokines.

Rituximab administration lead to a different profile of cytokines production compared to other immunosuppressive treatment. We observed higher production of TNFa and IFNγ by CD3+ lymphocytes (both p = 0.002) and decreased production of IL-12 by monocytes (p = 0.04) (Figure 3).

Higher production of IFNγ by CD3+ lymphocytes was present also during the relapse of the disease compared to NMO in remission (p = 0.02) (Figure 4). The production of other cytokines was not significantly changed depending on disease activity or treatment.

![Figure 3: Lymphocytic and monocytic cytokine production in rituximab treated NMO and NMO treated with other drugs.](image)

![Figure 4: Production of IFNγ by T lymphocytes in remission NMO and NMO in remission.](image)

**CONCLUSIONS**

Even if humoral mechanisms are fundamental in NMO pathogenesis, there are possible mechanisms participating in disease exacerbation that involve cellular response and specific cytokines production. Increased production of IFNγ and changes in CD3+CD4+ and CD3-CD8+ counts could be involved. IFNγ might change the production of CNS chemokines by astrocytes and microglia resulting in recruitment of proinflammatory leukocytes. This could explain the lower total and relative count of CD3+CD4+ lymphocytes that could be associated with recruitment of T or B cells to the CNS. Increased numbers of CD3-CD8+ T-cells can play an important role in initiation of disruption of blood-brain barrier tight junction proteins with increased vascular permeability, thus allowing AQP4-IgG to enter the CNS.

We have shown that rituximab treatment is associated with significant effects on the T lymphocyte pool and cytokine production in NMO modifying the immune response perhaps by limiting macrophages activation through reduced monocyic IL-12 production and by shifting the Th1/Th2 ratio to Th1 response. Predictive value of lymphocyte subpopulation and intracellular cytokine production tests needs, however, a more precise evaluation of lymphocyte activation markers and perhaps taking other cytokines into account.

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