Immune Cell Subtyping in the Cerebrospinal Fluid of Patients with Neurological Diseases at Rīga Eastern Clinical University Hospital "Gaiļezers"

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Abstract

Cerebrospinal fluid (CSF) analysis is very important in differential diagnosis of CNS diseases. Normally the number of cells is increased in patients with inflammatory CNS diseases. In addition to the standard analysis of CSF, in this study we analysed the distribution of immunocompetent cells subtypes in various neurological diseases. $CD4^+$, $CD8^+$ lymphocytes (ly) prevails in healthy individual CSF, whereas the number of NK cells and B lymphocytes (Bly) is negligible.

In total, 15 patients were recruited. We analysed immune cells subtypes in the distribution of blood and cerebrospinal fluid. The study patient group was divided into four subgroups according to CNS diseases. The first group included 5 patients with a primary diagnosed multiple sclerosis, second group – 3 patients with viral type meningitis, third group – 3 patients who were diagnosed with Parkinson's disease and the control group consisted of 4 patients with non-inflammatory orthopaedic diseases.

In routine CSF test, we observed that elevated WBC count in patients with neuroinfections and MS, but WBC count in Parkinson's disease patients was normal or slightly elevated. The percentage of $CD4^+$ T cells and NK cells was higher in MS patients ($p < 0.05$). Higher percentage of T helper cells was observed in MS and neuroinfection groups, significantly higher than in healthy control group ($p < 0.05$).

The percentage difference of $CD8^+$ Tly subtype in all groups was not observed, but the percentage of activated T lymphocytes ($CD38^+$ cells) was significantly higher than in healthy controls ($p < 0.05$).

Patients with MS had significantly higher percentage of NK cells; that is significantly higher than in healthy controls ($p < 0.05$). Fraction of Bly was higher in all patient groups, comparing with healthy control group ($p < 0.05$).

There was no significant difference in mean percentage of apoptosis receptor $CD95^+$ bearing cells and CD4/CD8 ratio in all groups.

The analysis of immune cell subsets in the CSF adds valuable information to clinicians and is a promising tool for the differential diagnosis of neurological diseases. Additional studies are necessary in order to differentiate CSF cell populations in more detail.

Keywords: immunocompetent cell subtypes, CD3, CD4, CD8, CD38, CD16, CD19, CD95, index CD4/CD8, multiple sclerosis, neuroinfection, Parkinson's disease.

Introduction

Recently more and more attention is paid to neurodegenerative and inflammatory processes in the central nervous system. It is known that the immune system is highly organised and plays a key role in both inflammatory and in nerve cell degeneration [15, *216*]. It was believed that the CNS is immune privileged system, mainly because of a relative lack of antigen-presenting cells and "safe" blood-brain barrier that protects brain from periphery circulating cells. Recently, the views have changed due to the fact of a close and two-way communication between the central nervous system and the immune system [3, *89*]. Antigen presentation – the main process of immune response – takes place at the periphery with the help of the specific antigen-presenting cells (APC), such as dendritic cells (DC), macrophages and B cells. APC initiate the immune response by collecting antigen peptides, then processing and presenting them on their surface. As a result, T cells receive information about the antigen and become able to provide defence against foreign substances [6, *57*; 7, *72*; 19, *45*]. There is evidence that activated immune cells can migrate to the CNS and induce various processes with the help of neurotransmitters and immune mediators [21, *103*]. Microglia and astrocytes are immune cells in CNS that mainly interact with T cells [12, *1*]. As a result of such interaction, various diseases can develop, such as multiple sclerosis (MS), Parkinson's disease (PD), autoimmune encephalitis, and others [8, *1*; 9, *55*]. Activated T cells induce blood-brain barrier damage and facilitate other peripheral immune cell entry into the CNS [19, *47*].

Cerebrospinal fluid (CSF) analysis is very important in differential diagnosis of CNS diseases. Routine analysis includes cell count, total protein levels, and glucose levels. Normally the number of cells is increased in patients with inflammatory CNS diseases. In addition to standard analysis of CSF, in this study the distribution of immunocompetent cells subtypes in various neurological diseases was analysed. $CD4^+$, $CD8^+$ lymphocytes (ly) prevail in healthy individual CSF, whereas the number of NK cells and B lymphocytes (Bly) is negligible [5, *45*; 22, *516*]. According to literature, $CD4^+$ and Bly level can be increased in patients with MS [4, *1668*; 20, *95*].

Immune cells are not uniform in the body, depending on their function and receptors on their surface lymphocytes could be divided into different subtypes. Similar to peripheral blood, immune cell distribution in CNS may imply a pathological process and help identify the nature of pathology, especially in marginal cases.

The analysis of studies about immune pathogenesis of neurological diseases performed in Latvia demonstrated that the CD4/CD8 index, IL-12 and TNF-α is increased in patients with exacerbation of multiple sclerosis and correlate with CNS focus activity. All parameters were studied only in peripheral blood [1, *74*]. Immunocompetent cells distribution in different types of neurological abnormalities in CSF has been analysed in Latvia for the first time.

The aim

The aim of the respective study was to evaluate immunocompetent cell subtypes in CSF in patients with CNS diseases.

Material and methods

The study was conducted from 1st January 2014 to 1st June 2014 at Rīga Eastern Clinical University Hospital "Gaiļezers". In total, 15 patients were recruited. We studied immune cells subtypes in the distribution of blood and cerebrospinal fluid. The research patient group was divided into four subgroups according to CNS diseases (Table 1). The first group included 5 patients with primary diagnosed multiple sclerosis; three women and two men, mean age – 32.8 years. Inclusion criteria – patients with MS that was diagnosed and confirmed by McDonald criteria, and patients did not receive any immunomodulatory or immunosuppressive therapy before spinal tap was performed The second group, 3 patients with viral type meningitis, one man and two women, mean age – 36.3 years. The third group, 3 patients who were diagnosed with Parkinson's disease, two men and one woman, mean age – 52 years. All these patients were undergoing standard diagnostic procedure for their neurological disease.

Exclusion criteria for the study were: patients with MS who received any immunomodulatory or immunosuppressive therapy, patients with other aetiology for parkinsonism (as vascular or toxic) and patients with bacterial or unknown aetiology of meningitis.

Control group consisted of 4 patients with non-inflammatory orthopaedic diseases, one male and three female with a mean age of 38.5 years; healthy control group workup did not show any changes in CSF (WBC count, protein level, glucose level).

Lumbar puncture was performed using non-traumatic needle and 10 ml of cerebrospinal fluid was taken for analysis. T-helper ($CD3/CD4^+$), T cytotoxic cells ($CD3/CD8^+$), activated lymphocytes (CD38), natural killer cells ($CD16^+56^+$), Bly ($CD19^+$) and lymphocytes with receptor of ($CD95^+$). CD4/CD8 ratio was calculated. Immunocompetent cells were determined with laser flow cytometer (Becton Dickinson, USA) at Rīga Eastern Clinical University Hospital Centre of Laboratory Medicine.

Further data was analysed using IBM SPSS 20 statistical program and χ-test.

Table 1. Patient groups and characteristics

Group	Number of patients, n	Sex, m/f, n	Age, years	Phase of the disease	Treatment before analysis
Multiple sclerosis	5	2 / 3	18–45, mean – 32.8	Acute	No
Neuroinfection	3	1 / 2	18–52, mean – 36.3	Acute	No
Parkinson's disease	3	2 / 1	48–53, mean – 51.1	Firstly diagnosed	No
Controls	4	1 / 3	18–52, mean – 38.5	Acute	No

Results

In routine CSF test, it was observed that elevated WBC count persisted in patients with neuroinfections and MS, but WBC count in Parkinson's disease patients was normal or slightly elevated (Table 2) The results were demonstrated in percentage because of different lymphocyte count in all groups.

Percentage of $CD4^+$ T cells and NK cells was higher in MS patients ($p < 0.05$). Higher percentage of T helper cells was observed in MS and neuroinfection groups, significantly higher than in healthy control group ($p < 0.05$).

The percentage difference of $CD8^+$ T lymphocyte subtype in all groups was not observed, but the percentage of activated T lymphocytes ($CD38^+$ cells) was significantly higher than in healthy controls ($p < 0.05$).

Patients with MS had significantly higher percentage of NK cells; that is significantly higher than in healthy controls ($p < 0.05$). Fraction of Bly was higher in all patient groups, comparing with the healthy control group ($p < 0.05$)

There was no significant difference in mean percentage of apoptosis receptor $CD95^+$ bearing cells and CD4/CD8 ratio in all groups.

Table 2. Distribution of immune cell subsets in the CSF of typical neurological diseases

Diagnosis	White blood cells, µl	$CD3^+$, %	$CD4^+$, %	$CD8^+$, %	$CD38^+$, %	$CD16^+$, %	$CD19^+$, %	$CD95^+$, %	CD4/CD8 ratio
Multiple sclerosis	7	87	35.50	18.00	3.60	13.80	29.00	3.4	3.36
Neuroinfection	98	98	28.30	30.00	4.00	1.33	24.33	3.7	3.00
Parkinson's disease	4	95	22.28	31.66	6.33	2.00	30.33	4.3	3.10
Healthy control group	2	81	13.25	20.00	28.00	5.25	2.00	6.8	4.70

Discussion

The aim of the respective study was to analyse the immune cell subtypes in patients with various neurological diseases in CSF, thus patients with diseases such as multiple sclerosis, Parkinson's disease and neuroinfection were included in it. Each group of diseases had its own different pathogenesis and the immune cells had a different role in each of them; however, that role is still not completely understood.

Multiple sclerosis is an inflammatory demyelinating CNS disease that mainly affects younger women. Syndromes depend on the CNS damage regions. Nowadays MS aetiopathogenesis is not completely clear, recent views consider it to be a multi factorial disease. Pathogenesis shows an autoimmune reaction against myelin and neurons [25, *172*]. The main pathogenic role in the development of MS assigned to cytotoxic $CD8^+$ T cell response against myelin and neuronal antigens [2, *402*]. Comparing multiple sclerosis and neuroinfection CSF analysis with the control group showed a significantly higher $CD4^+$ subtype cell percentage in MS and neurounfection group. $CD4^+$ T cells are well known for participation in antibody class switching process, cytotoxic T cell development process and in forming memory cells [13, *5*]. However, $CD4^+$ role in disease pathogenesis has not been fully understood, yet many authors believe that this cell subtype possesses an antiviral and a direct cytotoxic effect [24, *16*]. Taking into consideration the fact that in this study $CD4^+$ cell percentage was increased in neuroinfection, MS groups also support the popular theory that these diseases have a common immune response mechanism and in both cases an important role in pathogenesis belongs to viruses [16, *28*].

It is clear that in the case of neuroinfection main pathogenic process involves leukocytes, macrophages and microglia that release free radicals, cytokines and excretory amino acids, resulting in lack of energy and cell death. Vascular inflammatory reaction, oedema and focal ischemia promote blood-brain barrier damage [17, *3*; 23, *530*]. In case demyelinating diseases were activated, T lymphocytes migrate to the CNS, the main mechanism of pathogenesis is related to blood-brain barrier damage. In either case, the process-stimulating factor could be promoted by a virus as human herpesvirus 6, 7 (HHV6, HHV7) or Epstein-Barr virus (EBV) [16, *29*].

In turn, Parkinson's disease (PD) pathogenesis is described by the inflammatory process of $CD4^+$ and $CD8^+$ T cell infiltration to substantia nigra, although well aware that disease results in brain's extensive neurodegeneration [10, *83*]. In our study, Parkinson's disease group showed a higher proportion of $CD8^+$ cells than in other groups, indicating prevailing cytotoxic processes. $CD8^+$ cells massive cluster of brain tissue in patients with Parkinson's disease was already performed in study in 1988 [14, *575*].

Presently, a lot of debate is about multiple sclerosis and neurodegenerative diseases common mechanisms, immune cells subtypes that could play a major role in these processes and could influence the differential diagnosis for neurological diseases thus allowing to understand some steps in pathophysiology of these conditions and in its turn help to find new drugs for these diseases.

HHV6, HHV7 and EBV could be trigger factors for neuroinflammation and neurodegeneration, but still no specific claims can be proven. We are planning to test CSF for antibodies in these viruses in the future to understand if these viruses could be the stimulating factors.

Despite the small number of patients in our study, the first CSF immunocompetent cell analysis showed trends that are specific to patients with various neurological diseases. Further research is necessary with larger patient groups and, in addition, aetiopathogenic factor analysis (e.g., latent viruses or viral infections demonstration in CSF) to draw conclusions about the role of immune cells in neurological disease development.

Conclusions

1. The analysis of immune cell subsets in the cerebrospinal fluid adds valuable information to clinicians and is a promising tool for the differential diagnosis of neurological diseases.
2. Additional studies are necessary in order to differentiate cerebrospinal fluid cell populations in more detail.

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