Intrathecal synthesis of complement components C3c and C4 in the central nervous system infections with signs of the acute serous meningitis syndrome

Željka Tatomirović*, Radojka Bokun*, Dubravko Bokonjić

Military Medical Academy, *Institute of Pathology, *Poison Control Centre, Belgrade

Two hundred and ten patients with meningismus and the infections of the central nervous system (CNS) with the clinical symptoms and signs of the acute serous meningitis syndrome, were divided into groups according to etiology (enterovirus meningitis-ENTERO, serous meningitis various etiology-SM and tuberculous meningitis-TBC). Intrathecal synthesis (ITS) of C3c and C4 complement components and IgG were determined by the method of cerebrospinal indexes (I), to examine their role in differential diagnosis of this syndrome. Correlative study between the CSF/serum ratio (Q) for albumin (Alb) and QC3c and QC4 in patients with no proven ITS of this two complement proteins, and the comparative study of the increased value of C3cI and C4I (and IgGI) between the examined groups of the patients was done. Highly significant correlations were found between QA1b and QC3c (r = 0.89, p < 0.001) and QC4 (r = 0.85, p < 0.001). In 22.4% of the examined patients ITS of C3c and C4 were found. There was no difference in frequency of ITS of the two complement proteins between the examined groups, nor inside any particular group. TBC group had significantly lower (p < 0.05) intensity of ITS of C3c and C4 than MNG and ENTERO, and significantly higher intensity of ITS of IgG (p < 0.05) than the other tested groups. CSF index was confirmed as a valid method to detect intrathecal C3c and C4 production. Determination of ITS C3c and C4 could not be of great help in differential diagnosis in the acute serous meningitis syndrome. The intensity of ITS of C3c and C4, related to the intensity of ITS of IgG, could be of help in the determination of the duration of the disease.

K e y w o r d s : meningitis; meningism; cerebrospinal fluid; cerebrospinal fluid proteins; complement; immunoglobulin G; diagnosis, differential.

Introduction

The main mediator of humoral immunity and inflammation is the complement system. It consists of nine proteins and many of their proteolytic cleavage products with important biologic effects like opsonization, activation of inflammation and solubilization of immune complexes. The final effect of the activation of this system was the destruction of microorganisms, but it might also have led to the host tissue damage. According to the nature of the activator, this system was divided into classical, where the main activators were immunoglobulins (Ig), and alternative, which was activated in the absence of antibody; thus, this pathway of complement activation plays a role in the natural immunity. Determination of complement components C3c and C4 in serum was widely used in routine analysis which covered both pathways of activation of this system.

There were no many systematic investigations of the complement system in the cerebrospinal fluid (CSF) in various neurological diseases, particularly in infections of the central nervous system (CNS). The reasons were the need for sufficiently precise methods for determinations of
normally low concentrations of complement components and their proteolytic products in CSF, difficulties in simultaneous determinations of various different components and their derivatives in routine use and complexity of interpretation of results in the light of the blood-brain barrier (BBB), pathological process in CNS and in mutual influence of complement components.

In the last decade several reports particularly pointed to the probability of diagnostic significance of the complement components determinations in CSF. It was shown that CSF levels of C4d or C4d index had served as the basis for differentiating progressive supranuclear paralyses from Parkinson's disease (1), and that CSF levels of C4d had served as a sensitive indicator for the radicular involvement in demyelinating polyneuropathy (2). In one of the recent reports it was found that intrathecal synthesis (ITS) of C3 or C4 contributed a little to the differential diagnosis of immunological CNS disorders (multiple sclerosis, systemic lupus erythematosus and human immunodeficiency virus infection) (3).

Examinations of the complement components C3c and C4 in CSF in inflammatory CNS diseases of infections nature were restricted only to frequency presentation of their activation and ITS (4, 5). We presumed that determination of C3c and C4 in CSF in the patients with CNS infections, which covered the classical, as well as alternative pathway of complement activation, could be of diagnostic use, or could indirectly point to the nature of the activator.

To test this possibility, we measured the CSF and plasma concentrations of C3c and C4 in patients with the CNS infections with signs of the acute serous meningitis syndrome, to show the activation and the production of this two components of complement within the CSF compartment and to examine whether there were any differences in frequency and intensity of ITS of this two components of complement, according to the etiology of disease.

Methods

Two hundred and ten patients were examined in this study: 178 with infections of CNS with the clinical symptoms and signs of the acute serous meningitis syndrome (ASM), and 32 with meningismus which appeared during the acute diseases of various etiology. Patients with the ASM syndrome were divided into groups according to etiology, clinical picture, cytochemical findings in CSF and courses of the disease: enterovirus meningitis (ENTERO, n = 120), acute serous meningitis of various, probably viral and nonviral etiology (SM, n = 44) and tuberculous meningitis (TBC, n = 14). Control group consisted of 59 patients (36 women and 23 men, aged 5–82 years) with clinical and laboratory findings without objective signs of organic neurological diseases.

Peripheral venous blood and CSF were taken simultaneously in all examined patients according to standard procedures. Cytological analysis and total protein measurements were done in fresh samples of CSF as a part of routine laboratory investigations, and part of CSF and serum was stored at -70°C until assayed. The concentrations of C3c, C4, albumin (Alb) and IgG were determined simultaneously by laser immunonephelometry (Behring laser Neelofar) in unfrozen samples of CSF and serum. Serum dilutions were adopted for CSF calibration curves (5, 6).

CSF/serum ratio (Q) for Alb (QAlb = Alb CSF/Alb serum x 103) was used as an indicator of BBB function, and the CSF index (I) was used as an indicator of intrathecal synthesis of IgG and complement components C3c and C4 (I = Q protein/QAlb) (7). The upper reference values were established by the analysis of the control group: IgGI<0.7; C3cI<0.7; C4I to 15 years <1.6; from 16 years <1.2; and the upper reference values QAlb were determined according to Reiber (8) (to 15 years <5; from 16 years <6.5; from 40 years <8).

The Student’s t-test and Mann-Whitney test were used to compare the groups of patients and control group, and the regression analysis was done to determine the significance of correlations between various parameters.

Results

The concentrations of C3c and C4 (x and SD) in sera of the patients with MNG and the syndrome of ASM are shown in Fig. 1. All patients groups had significantly higher mean concentrations of C4 (p<0.001), and significantly lower mean concentrations of C3c (except ENTERO) in serum (MNG, SM p<0.001; TBC p<0.001), compared to the control group. Among the patients groups significantly higher mean concentrations of C3c in serum were in ENTERO than in MNG and SM groups (p<0.05), while among the other groups there were no differences, which were also not found for the mean concentrations of C4.

![Fig. 1 – Concentrations of the complement C3c and C4 in sera of the patients with the syndrome of ASM, divided in groups according to etiology of the disease (x ± SD)](chart)

Looking at C3c and C4 quotients (x and SD) (Fig. 2), higher values of QC4 then QC3c were seen, as well as a gradual increase in the values of the both Q from MNG to ENTERO, SM to TBC group which had significantly higher mean values of the both Q then the other groups (p<0.05), among which there were no significant differences. Com-
pared to the control group, MNG group had significantly lower values of QC3c and QC4, and significantly higher TBC (p<0.05), while ENTERO group had significantly lower value of QC4 (p<0.001), and SM significantly higher value of QC3c (p<0.001).

![Fig. 2 – CSF/serum concentration ratios (Q) of the complement C3c and C4 in the patients with the ASM syndrome, divided into groups according to the etiology of the disease (x ± SD)](image)

The values of QAlb are shown at the Fig. 3 (x and SD). Here also the mean values of QAlb were increasing from MNG, over the ENTERO, SM to TBC group. The significantly higher mean values of QAlb were in TBC than in the other groups (p<0.05), and the significantly lower mean values of QAlb had MNG comparing to ENTERO and SM groups (p<0.05). In relation to the control group, MNG had significantly lower, and SM and TBC groups significantly higher values of QAlb (p<0.001), while among the ENTERO and control group there were no significant differences.

In patients in whom the ITS of C3c and C4 was not proved, the high positive correlation between QAlb and QC3c (τ = 0.89, p<0.001) and QC4 (τ = 0.85, p<0.001) was found.

The number and the percent of the patients in whom ITS of C3c and C4 was proved is shown in Table 1. ITS of C3c and C4 was found in 22.4% of the examined patients. Almost equal frequency of ITS of this two protein complements was found, and there were neither statistically significant differences between the groups nor inside of the particular group compared to the found combinations of ITS of C3c and C4.

![Fig. 3 – CSF/serum concentration ratios (Q) of albumin in the patients with the ASM syndrome, divided into groups according to the etiology of the disease (x ± SD)](image)

Intensity of the ITS of C3c and C4 is shown in Fig. 4 (x and SD). All groups had the higher mean value of C4I then C3cI. A slight increase of the mean values of C3cI from MNG, over the ENTERO to SM group was shown. On the contrary, the mean values of C4I gradually fell in the same way. The lowest mean values of C3cI and C4I were in TBC group, which reached the statistical significance for C3cI (p<0.05) with reference to ENTERO and SM, and for C4I with reference to MNG and ENTERO.

![Fig. 4 – Intrathecal synthesis of the complement C3c and C4 in the patients with the ASM syndrome, divided into groups according to the etiology of the disease, proved with the CSF indexes (x ± SD)](image)

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>C3c n (%)</th>
<th>C4 n (%)</th>
<th>C3c + C4 n (%)</th>
<th>Total C3c and/or C4 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNG n=32</td>
<td>2 (6.3)</td>
<td>2 (6.3)</td>
<td>3 (9.4)</td>
<td>7 (21.9)</td>
</tr>
<tr>
<td>ENTERO n=120</td>
<td>10 (8.3)</td>
<td>8 (6.7)</td>
<td>7 (5.8)</td>
<td>25 (20.8)</td>
</tr>
<tr>
<td>SM n=44</td>
<td>4 (9.1)</td>
<td>4 (9.1)</td>
<td>3 (6.8)</td>
<td>11 (25.0)</td>
</tr>
<tr>
<td>TBC n=14</td>
<td>–</td>
<td>2 (14.3)</td>
<td>2 (14.3)</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>Total 210</td>
<td>16 (7.6)</td>
<td>16 (7.6)</td>
<td>15 (7.1)</td>
<td>47 (22.4)</td>
</tr>
</tbody>
</table>

* x, (SD)
Intensity of the ITS of IgG is shown in Fig. 5 (x and SD). The highest mean value of IgGI was found in TBC group, which was statistically significant in reference to the other groups of patients (p<0.05).

![Graph](image)

Fig. 5 – Intrathecal synthesis of IgG in the patients with the ASM syndrome, divided into groups according to the etiology of the disease, proven with the CSF indexes (x ± SD)

The positive correlation between IgGI and C3cI (r = 0.45, p<0.01) and a little lesser positive correlation between IgGI and C4I (r = 0.19, p<0.05), and also positive correlation between C3cl and C4I (r = 0.45, p<0.01) was found in ENTERO group. In TBC group, there was neither correlation between IgGI and C3c (r = -0.40), nor between C3cl and C4 (r = -0.09)

Discussion

Some anatomical and ultrastructural characteristics of the CNS – the unique cellular environment, contributed to the observed differences in immunological function between the CNS and other organs (9). The low, nonconstitutive expression of the major histocompatibility complex molecules, the low concentrations of antibodies and proteins of complement were the cause of the relative immunological brain isolation, respectively implying a suppressive environment for immune reactivity in this organ, so the one of the main questions was the mode for starting the inflammation in the CNS, especially when the BBB is intact (10). It was found that complement-derived polypeptide C3adesArg increased the permeability of BBB and produced an accumulation of leucocytes in the CSF when injected into the cisterna magna or intraventricularly. This peptide acted as a mediator of inflammation in the CNS (11, 12). The complement system could be one of the important elements of the brain primary defense from infections, especially in the early stage, when the specific antibodies were not yet created. It was not only the alternative pathway of complement activation, like evolutionary ancient defense system against infections which could be activated without the antibodies, but also the classical pathway and could also be activated very early, even before the appearance of antibodies by other factors such as trypsin-like enzymes, myelin and vira, as well (13).

Some author’s considerations that the specific identification of complement factors in CSF promised to be diagnostic not only for complement activation but also for the mode of activation, i.e., the nature of the activator (5), induced us to test it by determination of C3c and C4 in the CSF of patients with the CNS infection with the signs of ASM syndrome.

The abnormal complement values were found in both serum and CSF in the examined patient groups. Significantly higher values of C4 and significantly lower of C3c (except in the ENTERO group) in serum, could demonstrate the strong activation of the main complement pathway in serum of the patients with the ASM syndrome, with probable simultaneous increase of C3 consumption. Between the groups there were no significant differences in concentrations of this two complement proteins in serum. In the patients with similar etiology, Trbojević-Cepe (5) found significantly higher concentrations both in C3c and C4 sera, in reference to the control group.

Most of the complement proteins present in the serum are synthesized by hepatocytes and mononuclear phagocytes, but some other cell types, like epithelial and endothelial cells, have this ability, too. Like immunoglobulins, the complement proteins traverse BBB by mechanism of diffusion, so their concentrations in CSF depend upon the concentration in serum. The higher CSF/serum concentration ratio (Q) for C4, compared to QC3c, not only in examined patients but also in the control group, could be explained by additional ITS of C4. In contrast to immunoglobulins which ITS was found only in the pathological conditions (in the inflammatory diseases of CNS, and malignant CNS tumors) (14), this findings pointed out that ITS of C4 was most probably continuously present in the normal brain. This could be supported by other studies in which the production of two proteins of the alternative pathway of complement activation (C3 and factor B) in primary cultures of mouse astroglial cells (15), some proteins of the main pathway (Clq, C3 and C4) in normal and pathological human postmortem brain tissues (16) were reported, and Gasque, et al (17–19) found that astrocytes could synthesize a complete, functional complement system under appropriate cytokine drive.

Significantly lower values of QC3c and QC4 in patients with meningismus with respect to the control group were the consequence of significantly lower QAItb values, i.e., the patients younger than the control group. The gradual increase of the quotients of this two complement proteins from MNG, over the ENTERO and SM to TBC group, entirely followed the increase of QAItb, i.e., the height of the quotients depended upon the degree of the BBB permeability, which was approved with the highly positive correlation found between QAItb and QC3c and QC4 in patients in whom ITS of this two complement proteins was not proved.

Thus, in patients with the infection of CNS the elevated concentrations of C3c and C4 in CSF, i.e., the ele-
vated values of QC3c and QC4, were the consequence of the increased permeability of the BBB and/or ITS, where this two proteins could be synthesized by monocyte/macrophages, which could traverse the BBB from circulation and also some cellular types inside the brain, particularly astrocytes.

In the examined patients not only the total frequency of ITS of C3c and C4 was almost equal in all groups, but there also was almost identical frequency of ITS only C3c, and only C4, and at the same time both of this two proteins were found not only in examined groups, but in between every particularly group. It pointed out that both ways of complement activation, a classical and alternative one, were equally represented, in relation to various etiology of the disease. Yet, differences between groups were present when the intensity of ITS was observed. A significantly lower intensity of ITS, and both C3c and C4 in the TBC group related to the other groups was an unexpected finding. The groups were arranged by the height of the middle values of examined protein parameters, and completely corresponded with the clinical picture and the rate of path anatomical changes in CNS during these infections. Alb quotients, QC3c and QC4, but also ITS IgG were highest in the TBC group which was expected, because it was very serious CNS infection in which there was an intensive activation of both complement pathways, and also an intensive immunological response and consequently a heavy inflammatory response of the CNS. It seemed that the explanation of this finding should be sought for in the phase of the disease. The examination was conducted in the acute phase and this was in average during the first 72 hours from the appearance of clinical symptoms of CNS irritation in the MNG and ENTERO groups, (in SM group it was the fourth day, and in the TBC the tenth day). TBC meningitis is a disease that starts slowly and unobtrusively, with nonspecific symptoms, so the beginning of this disease is very hard to determine. By the time the symptoms of CNS infection were clear enough, the disease was in high progression, so the information about the duration of the condition and the obtaining of material for analysis was not reliable. Thus, a significantly higher ITS of these two proteins of complement in MNG, ENTERO and SM referred to the TBC group was most probably the reflection of an early phase of disease in which there was the most intensive activation of the complement system, in distinction from the TBC meningitis where the lower values are the result of gradual reduction of complement activation in the later phases of the disease. In behalf of such explanation there was the research of Stahl, et al (20) who, studying ITS of the complement protein of the alternative pathway of activation in the brain of mice with Lysteria monocytogenes meningitis, found a higher expression of C3 and mRNA factor B in the pyramidal neurons and Purkinje's cells within 6 hours, with maximum within 12 hours, and after that period they registered gradual fall of this expression within the next 72 hours after the infection.

The results of this research confirmed the findings of other authors that the CSF index was a very good method for detection of ITS C3 and C4 (3–5, 21). The determination of ITS C3c and C4 was not of great help in differential diagnosis of patients with syndrome of ASM. Remarkable was the displayed difference in intensity of ITS C3c and C4 between the TBC and the other groups, which might be able to refer to the phase of the disease under the condition to simultaneously examine the intensity of ITS IgG, which should be confirmed by further examination of the dynamic of ITS C3c and C4 in CNS infections.

REFERENCES


Apstrakt

INTRATEKALNA SINTEZA KOMPONENTI KOMPLEMENTA C3c I C4 U INFECIJIMA CENTRALNOG NERVNOG SISTEMA KOJE SE MANIFESTUJU SINDROMOM AKUTNOG SEROZNOG MENINGITISA

Kod 210 bolesnika s meningizmom i infekcijama centralnog nervnog sistema koje se manifestuju sindromom akutnog seroznog meningitisa (ASM), podeljenih u grupe na temelju etiologije (enterovirusni meningitis-ENTERO, serozni meningitis rezličite etiologije-SM i tuberkulozni meningitis-TBC) određena je intratekalna sinteza (ITS) komponenta komplementa C3c i C4 (kao i IgG) metodom likvorskih indeksa (l), da bi se istražila njihova uloga u diferencijalnoj dijagnozi ovoga sindroma. Urađena je korelativna studija između albuminskih kvocijenata (QAlb) i QC3c i QC4 kod bolesnika kod kojih nije dokazana ITS ova dva proteina komplementa, kao i komparativna studija povišenih vrednosti C3cI i C4I (kao i IgGI) između ispitivanih grupa bolesnika. Nađena je statistički visoka značajna korelacija između QAlb i QC3c (r = 0,89, p<0,001) i QC4 (r = 0,85, p<0,001). ITS C3c i C4 nađena je ukupno u 22,4% ispitivanih bolesnika. Nije nađena razlika u učestalosti ITS ova dva proteina komplementa, kao i u raspodji li povišenih vrednosti C3cI i C4I (kao i IgGI) u ispitivanih grupa bolesnika. Nađena je statistički visoka, značajna korelacija između QAlb i QC3c (r = 0,89, p<0,001) i QC4 (r = 0,85, p<0,001). ITS C3c i C4 nađena je visoka u 22,4% ispitivanih bolesnika. Nije nađena razlika u učestalosti ITS ova dva proteina komplementa između ispitivanih grupa, niti unutar pojedine grupe. Nađen je statistički značajni niz intenzitit ITS C3c i C4 u TBC grupi (p<0,05) i u odnosu na ENTERO i SM za C3c, a u MNG i ENTERO za C4, a značajno viši intenzitet ITS IgG (p<0,05) u odnosu na ostale ispitivane grupe. Likvorski indeks je veoma dobra metoda za detekciju ITS C3c i C4. Određivanje ITS C3c i C4 ne može biti od veće pomoći u diferencijalnoj dijagnozi bolesnika sa sindromom ASM. Intenzitet ITS C3c i C4, ukoliko se stavi u odnos sa intenzitetom ITS IgG, možda bi mogao govoriti o fazi bolesti.

Ključne reči: meningitis; meningizam; cerebrospinalna tečnost; cerebrospinalna tečnost, protein; komplement; IGG; dijagnoza, diferencijalna.