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CASE REPORT

Aseptic meningitis in mixed connective tissue disease: cytokine and anti-U1RNP antibodies in cerebrospinal fluids from two different cases

Yoshimasa Fujita · Takao Fujii · Ran Nakashima · Masao Tanaka · Tsuneyo Mimori

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Abstract In this paper, we report two patients with mixed connective tissue disease (MCTD) who developed aseptic meningitis. In both cases, the concentrations of IFN-γ and IL-6 in cerebrospinal fluid (CSF) were increased. In the first case, with non-steroidal anti-inflammatory drugs (NSAIDs)-induced meningitis, where anti-U1RNP antibodies (Abs) were not detected in CSF, NSAIDs induced both IFN-γ and IL-6 secretion from peripheral blood mononuclear cells in vitro. In the second case, with disease-associated meningitis, anti-U1RNP Abs were detected also in CSF. Of note, anti-U1RNP Abs appeared to be more concentrated in CSF than in serum and CSF-anti-U1RNP Ab titer was correlated with disease activity. We suggest that IFN-γ and IL-6 may be involved in both disease-associated and drug-induced aseptic meningitis, whereas CSF-anti-U1RNP Abs is detected only in a patient with MCTD-associated aseptic meningitis.

Keywords Anti-U1RNP antibodies · Aseptic meningitis · IFN-γ · IL-6 · Mixed connective tissue disease (MCTD)

Y. Fujita
Department of Rheumatology, Utano National Hospital, Kyoto, Japan

T. Fujii (✉) · R. Nakashima · T. Mimori
Department of Rheumatology and Clinical Immunology, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan
e-mail: takfujii@kuhp.kyoto-u.ac.jp

M. Tanaka
Division of Hematology and Immunology, Department of Internal Medicine, Kanazawa Medical University, Ishikawa, Japan

Introduction

Aseptic meningitis is one of the serious neurological manifestations of connective tissue diseases (CTD) [1]. In sera from Japanese aseptic meningitis patients with CTD, anti-U1RNP Abs are frequently recognized [2]. Several NSAIDs and other drugs induce aseptic meningitis, especially in patients with systemic lupus erythematosus (SLE) and MCTD [3]. It is, therefore, sometimes difficult to distinguish disease-associated aseptic meningitis from drug-induced meningitis when NSAIDs are used in patients with SLE and MCTD. We experienced two different patients with MCTD and aseptic meningitis, and examined cytokine concentrations and anti-U1RNP Ab titer in CSF.

Case reports

Case 1

According to the preliminary diagnostic criteria for the classification of mixed connective tissue disease (MCTD) [4], a 43-year-old woman was diagnosed as having MCTD in 2001, since she had Raynaud's phenomenon, sausage-like fingers, proximal muscle weakness with an elevated creatinine kinase (CK) level, leukocytopenia, and serum anti-U1RNP Abs. She developed trigeminal neuralgia in March 2002 and carbamazepine (200 mg daily) was initiated. Two weeks later, high fever and systemic rash were observed, along with severe liver dysfunction (aspartate aminotransferase [AST] 1,105 IU/L, alanine aminotransferase [ALT] 766 IU/L). She was admitted to our hospital because carbamazepine-associated hepatotoxicity was strongly suggested. Because of her high fever, she took loxoprofen (total dose = 60 mg), diclofenac sodium (total

dose = 50 mg), and acetaminophen (total dose = 3.66 g). The serum titer of anti-U1RNP antibodies (Abs) determined by enzyme-linked immunosorbent assay (ELISA) was 44.1 index (positive, normal <15.0). Hypocomplementemia and a false-positive serologic test for syphilis were not recognized. Three days later, she suddenly developed a severe headache and revealed a stiff neck. Anti-dsDNA, β_2GPI, and cardiolipin Abs were all negative, and magnetic resonance imaging (MRI) tomography showed no evidence of cerebrovascular disease. Cerebrospinal fluid (CSF) examination showed pleocytosis (29/3 μl^{-1}), elevated levels of protein (53.7 mg/dl), and normal levels of glucose (46 mg/dl). The total IgG concentration in serum and CSF were 1,634 and 11.4 mg/dl, respectively. The IgG index of CSF was 0.57 (normal). Because bacterial growth in CSF was not detected and no other manifestations associated with MCTD were recognized, non-steroidal anti-inflammatory drugs (NSAIDs)-induced aseptic meningitis was strongly suspected. After she discontinued drugs, her symptoms completely disappeared. The second CSF examination showed decreased levels of proteins (41 mg/dl). While she had no serious symptoms other than trigeminal neuralgia, she was discharged from our hospital without steroid use.

Case 2

According to the preliminary diagnostic criteria for the classification of MCTD [4], a 29-year-old woman was diagnosed as having MCTD in 1991, since she had Raynaud's phenomenon, polyarthritis, proximal muscle weakness with an elevated CK level, leukocytopenia, and serum anti-U1RNP Abs. She was admitted to our hospital in May 2002 because she had high fever, headache, nausea, and systemic erythema. Meningeal irritation was also recognized. Before and after admission, she had taken diclofenac sodium (total dose = 25 mg), loxoprofen (total dose = 60 mg), and acetaminophen (total dose = 200 mg), since severe headache with high fever continued. The serum titer of anti-U1RNP Abs determined by ELISA was 69.2 index (positive). Hypocomplementemia was also recognized. Although all of the drugs, including NSAIDs, were discontinued, her symptoms and laboratory data did not improve. On the third hospitalized day, she developed disseminated intravascular coagulation (platelets 8.0 × 10^4 μl^{-1}, D-dimer 82.8 μg/ml). Anti-dsDNA, β_2GPI, and cardiolipin Abs were all negative and a false-positive serologic test for syphilis were not recognized. MRI tomography showed no evidence of cerebrovascular disease. CSF examination showed pleocytosis (33/3 μl^{-1}) and an elevated level of protein (81 mg/dl). The total IgG concentration in serum and CSF were 1,845 and 20.7 mg/dl, respectively. The IgG index of CSF was 0.72 (normal upper limit). Because of the negative results in CSF culture, aseptic meningitis was thought to be attributable to the disease activity. After a high dose of prednisolone (1 mg/kg) was initiated, her clinical symptoms completely disappeared. The data was improved in the second CSF examination performed on the 16th day after the first CSF examination (cell number 13/3 μl, protein 51 mg/ml).

Immune complex was not detected in CSF derived from both patients. Recently, MCTD-associated aseptic meningitis after a herpes virus infection was reported [5]. Our patients had no evidence of herpes virus infection before the development of meningitis.

Determination of cytokine levels and anti-U1RNP Abs in CSF

Drug-induced lymphocyte stimulation test (DLST) was performed with peripheral blood mononuclear cells (PBMC), as described previously [6]. Briefly, the PBMC in blood were isolated from both patients by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) and 2×10^5 of PBMC in 200 μl of RPMI1640 containing 20% autologous plasma were stimulated with each drug. Carbamazepine, dicrofenac sodium, loxoprofen, or acetaminophen was dissolved in 5 ml of RPMI1640 (Sigma, St. Louis, MO) and sonicated for 30 s. The suspension was centrifuged at 200g for 5 min and 20 μl of supernatant was used as an antigen. To determine the appropriate concentration for PBMC stimulation, a serial dilution of each drug was prepared for proliferation assay. Lymphocyte stimulation was measured as the incorporation of ^{3}H-thymidine. Concentrations of IFN-γ, IL-4, and IL-6 in culture supernatants (100 μl) were determined in duplicates by EIA kits (e-Bioscience, San Diego, CA). In both patients, the concentrations of IL-6 and IFN-γ, but not IL-4, in CSF were increased in their acute phase (Table 1). In the convalescent phase, the concentrations of IL-6 and IFN-γ were

Table 1 Cytokine concentrations in cerebrospinal fluid (CSF) of patients with mixed connective tissue disease (MCTD)

	Acute phase	Convalescent phase
Case 1		
IL-6 (pg/ml)	59.1	0.8
IFN-γ (pg/ml)	87.3	16.1
IL-4 (pg/ml)	<2	<2
Case 2		
IL-6 (pg/ml)	3,240	20.8
IFN-γ (pg/ml)	163.7	<4
IL-4 (pg/ml)	<2	<2

Table 2 Drug-induced lymphocyte stimulation test (DLST) in patients with MCTD

	Stimulation index (%)[a]	IFN-γ (pg/ml)	IL-6 (pg/ml)	IL-4 (pg/ml)
Case 1				
Carbamazepine	250	44.9	1,420	<2
Acetaminophen	331	97.3	8,595	<2
Diclofenac sodium	203	47.6	880	<2
Loxoprofen	184	43.7	64,530	<2
Control (vehicle)	100	34.2	671	<2
Case 2				
Diclofenac sodium	136	16.6	65	<2
Loxoprofen	172	38.6	267	<2
Control (vehicle)	100	28.9	66	<2

[a] Stimulation index (SI) = ([3H]-thymidine uptake of experimental wells/[3H]-thymidine uptake of control wells) × 100. SI > 180% is significant

decreased. DLST demonstrated that acetaminophen markedly stimulated the proliferation of PBMC derived from Case 1 (Table 2). In addition, the IFN-γ concentration in the culture supernatant from Case 1 was markedly increased in the presence of acetaminophen. The IL-6 concentration in the culture supernatant from Case 1 was increased in the presence of carbamazepine, acetaminophen, and loxoprofen. IL-4 was not detected in the culture supernatant, nor in CSF. On the other hand, PBMC derived from Case 2 failed to proliferate in response to dicrofenac sodium and loxoprofen, while IL-6 secretion from the PBMC from Case 2 was induced by loxoprofen.

Anti-U1RNP IgG Abs in serum and CSF were measured by RNA-immunoprecipitation (RNA-IPP) and ELISA (MBL, Nagoya, Japan). RNA-IPP was performed as previously described [7]. A total of 10 μl of patient sera or CSF was mixed with 2 mg of protein A-sepharose CL-4B in 500 μl of IPP buffer and incubated with end-over-end rotation for 2 h at 4°C. The IgG-coated sepharose was washed four times in 500 μl of IPP buffer and then resuspended in 400 μl of NET-2 buffer. This suspension was incubated with 100 μl of HeLa cell extracts, derived from 6×10^6 cells, on the rotator for 2 h at 4°C. The antigen-bound sepharose was then collected, washed four times with NET-2 buffer, and then resuspended in 300 μl of NET-2 buffer. To extract bound RNAs, 30 μl of 3.0 M sodium acetate, 30 μl of 10% SDS, 2 μl of carrier yeast tRNA (10 mg/ml; Sigma), and 300 μl of phenol/chloroform/isoamyl alcohol were added to the sepharose beads. After agitation in a vortex mixer and spinning for 1 min, RNAs were recovered in the aqueous phase after ethanol precipitation and dissolved in 20 μl of electrophoresis sample buffer in TBE buffer. The RNA samples were denatured at 65°C for 5 min and then resolved in

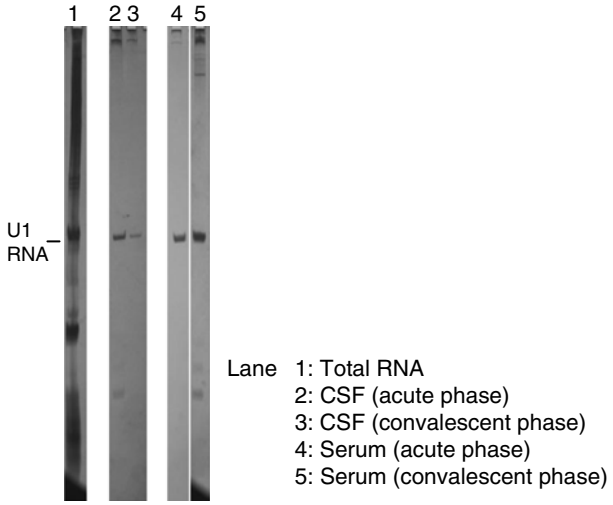

Fig. 1 Anti-U1RNP Abs in CSF derived from Case 2. Anti-U1RNP Abs in her serum and CSF were examined by RNA-IPP with HeLa cells. In CSF, anti-U1RNP Abs appeared to be increased in the acute phase (*lane 2*, 144.0 index determined by ELISA) compared to the convalescent phase (*lane 3*, 43.0 index). Serum titers of U1-RNP Abs were not significantly different between the acute (69.2 index) and convalescent phases (84.0 index)

7 M urea-10% polyacrylamide gel, which was stained with silver. In ELISA, the serum and CSF were diluted to 1:100 and 1:5, respectively. Appropriate dilution of CSF for ELISA was determined by comparison between the results of RNA-IPP and ELISA with sequentially diluted CSF derived from some CSF-anti-U1RNP Ab-positive patients. It is of note that anti-U1RNP Abs was detected not only in serum, but also in CSF of Case 2 (Fig. 1). Interestingly, the anti-U1RNP Ab index/mg/dl total IgG were more increased in CSF (=34.8) than in serum (=3.7) (Table 3). In the convalescent phase, the anti-U1RNP antibody level appeared to be decreased (=19.5 index/mg/dl total IgG) compared with that in the acute phase. In CSF derived from Case 1, however, anti-U1RNP Abs were not detected by RNA-IPP and ELISA, even in the active phase (data not shown).

Table 3 Anti-U1RNP antibodies (Abs) in serum and CSF of Case 2

	Acute phase		Convalescent phase
	Serum	CSF	CSF
Total IgG (mg/dl)	1,845	20.7	11
Anti-U1RNP antibodies			
Index	69.2 (×100)[a]	144.0 (×5)[a]	43.0 (×5)[a]
Index/mg/dl total IgG	3.7	34.8	19.5

[a] Dilution of samples

Discussion

In this paper, we presented two cases with MCTD who developed aseptic meningitis. Case 1 was clinically diagnosed as having drug-induced meningitis. In vitro proliferation and cytokine assays suggested that acetaminophen was the most likely cause of meningitis, as acetaminophen stimulated both IFN-γ and IL-6 with proliferation of PBMC. In patients with acute disseminated encephalomyelitis (ADEM), IFN-γ-producing $CD3^+$T cell number is markedly increased and significantly associated with disease activity [8]. IFN-γ in CSF, however, is not increased, suggesting that cytokine activation in PBMC is not associated with cytokine concentration in CSF [8]. We could not determine whether aseptic meningitis in Case 1 was induced by acetaminophen alone or not. The other NSAIDs (dicrofenac sodium and loxoprofen), as well as acetaminophen, may be considered as medication effects. The mechanism and etiology of such an association is not well understood; T cell hypersensitivity and the secreted cytokines might be involved in meningitis. Although loxoprofen, one of the propionic acid derivatives, appeared to stimulate larger amounts of IL-6 secretion than the other NSAIDs, it remained unclear whether this stimulation effect is specific to loxoprofen or not.

On the other hand, Case 2 was clinically diagnosed as having disease-associated aseptic meningitis, since she had disease activity and required a high dose of steroids. Recent reports have clearly indicated that anti-ribosomal P [9, 10] and anti-NR2 [11] Abs are linked to the depression-type psychiatric disorder and cognitive dysfunction, respectively. In addition, it has been reported that connective tissue disease (CTD) patients with aseptic meningitis tend to have anti-U1RNP Abs in the Japanese population [2]. CSF-anti-U1RNP Abs may have an important role in MCTD-associated aseptic meningitis in Case 2 because anti-U1RNP IgG Abs appeared to be more concentrated in CSF than in serum, especially in the acute phase. Duration between meningitis onset and CSF examination was similar (3–5 days), but disease severity was different in both cases. It is, therefore, possible that the development of CSF-anti-U1RNP Abs depends on the severity of aseptic meningitis. In our preliminary study, however, CSF-anti-U1RNP Ab development appeared not to be correlated to the total protein and IL-6 concentrations in CSF (data not shown). Also, CSF-anti-U1RNP Abs remain to be elevated, even in the convalescent phase, especially in patients with the other central nervous system (CNS) involvements (e.g., lupus psychosis). Cytokines other than IL-6 (e.g., IFN-α) may be involved in CSF-anti-U1RNP Ab development. Anti-Ro/SS-A Abs, which are frequently found in sera from patients with Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE), also appear in CSF, but CSF-anti-Ro/SS-A Abs were not associated with neuropsychiatric SLE (data not shown, manuscript in preparation). Additionally, in sera from anti-Ro/SS-A Ab-positive SLE patients with active transverse myelitis and SS with multiple sclerosis, anti-Ro/SS-A Abs were not detected in CSF by RNA-IPP. Because there is a single case report that anti-U1RNP Abs were detected in CSF [12], the present cases encounter the future study regarding the pathogenesis of CSF-anti-U1RNP Abs in serum-anti-U1RNP Ab-positive meningitis patients.

Oshima et al. [13] reported a Japanese patient with MCTD-associated pleuritis and showed more concentrated anti-U1RNP Abs in pleural fluid than that in serum. They suggested that the anti-U1RNP IgG Abs were secreted in the pleural cavity and pleuritis was induced mediated by immune complex. In our case, because immune complex in CSF was not detected, an anti-U1RNP Ab-dependent or autoreactive T cell-mediated mechanism is supposed. Autoantibodies gain access to the CSF of SLE patients by means of passive transfer from the circulation through a permeabilized blood–brain barrier [14]. The more increased anti-U1RNP Ab index/mg/dl total IgG in CSF than in serum, however, suggest that CSF-anti-U1RNP Abs are not attributable to the increased blood–brain barrier permeability alone.

In conclusion, IFN-γ and IL-6 appeared to be associated with drug-induced and disease-associated aseptic meningitis in patients with MCTD. In addition, anti-U1RNP Abs in CSF may be one of the important findings in MCTD-associated aseptic meningitis, whereas the determination of CSF-anti-U1RNP Abs in a larger number of patients should be required.

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