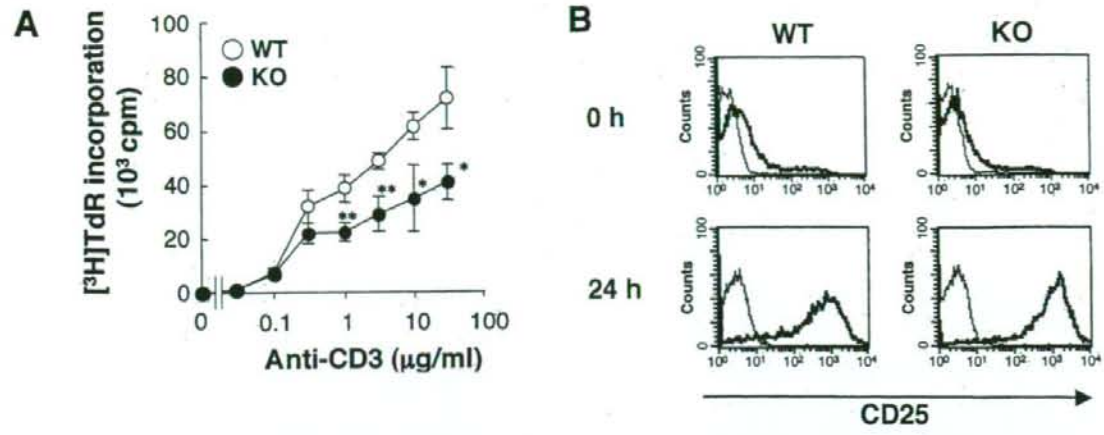

Fig. 2 Impaired proliferation of CD4⁺ T cells from SHPS-1 mutant mice. (A) Purified CD4⁺ T cells from WT or SHPS-1 mutant mice were cultured for 48 h on plates coated with various concentrations of a mAb to CD3. The cells were exposed to [³H] TdR during the final 14 h of culture, and the cell-associated radioactivity was subsequently measured with a scintillation spectrometer. Data are means ± SE of values from triplicate determinations and are representative of three separate experiments. *$p<0.05$, **$p<0.01$ versus corresponding value for WT cells (Student's t test). (B) The expression level of CD25 on the surface of purified CD4⁺ T cells from WT or SHPS-1 mutant mice was determined by flow cytometry at 0 and 24 h after stimulation by TCR cross-linking with a mAb to CD3 (10μg/ml). Data are representative of three separate experiments.

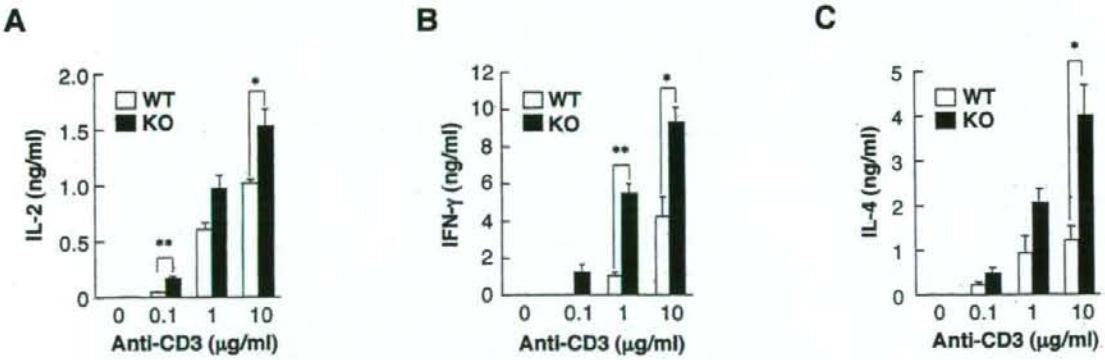

Fig. 3 Enhanced cytokine production by CD4⁺ T cells from SHPS-1 mutant mice. Purified CD4⁺ T cells from WT or SHPS-1 mutant mice were cultured for 48 h on plates coated with various concentrations of a mAb to CD3, after which the concentrations of IL-2 (A), IFN-γ (B), and IL-4 (C) in culture supernatants were determined. Data are means ± SE of values from triplicate determinations and are representative of three separate experiments. *$p<0.05$, **$p<0.01$ for the indicated comparisons (Student's t test).

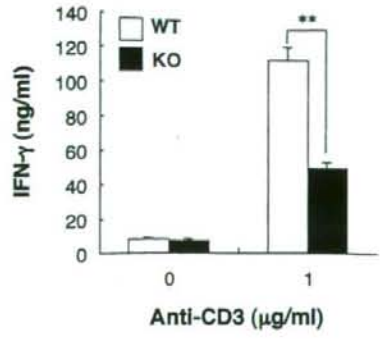

Fig. 4 Impaired Th1 differentiation of CD4⁺ T cells from SHPS-1 mutant mice. Purified CD4⁺ T cells from WT or SHPS-1 mutant mice were cultured for 6 days in medium containing IL-12 (10ng/ml) and a mAb to IL-4 (10μg/ml) and on culture plates coated with a mAb to CD3 (2μg/ml). The cells were then stimulated with a mAb to CD3 (1μg/ml) for 24 h, after which culture supernatants were assayed for IFN-γ. Data are means ± SE of values from triplicate determinations and are representative of three separate experiments. **$p<0.01$ (Student's t test).

of EAE as well as in that of other experimental autoimmune diseases.[23,24] SHPS-1 might thus also play a regulatory role in development of Th17 cells, and a defect in this function also might contribute to the resistance of SHPS-1 mutant mice to EAE.

The molecular mechanisms by which SHPS-1 positively regulates the effect of IL-2 on T cell proliferation and that of IL-12 on Th1 differentiation remain unknown. Signaling by JAK1 or JAK3 and by STAT5 is thought to mediate responses to IL-2.[25,26] Similarly, the JAK2-STAT4 signaling pathway and the p38 isoform of MAPK are implicated in this effect of IL-12.[27,28] Given that SHP-2 positively regulates activation of the JAK-STAT pathway and MAPK signaling[25] and that association of SHP-2 with SHPS-1 is specifically defective in SHPS-1 mutant mice, it is possible that the SHPS-1-SHP-2 complex positively regulates activation of the JAK-STAT pathway or p38 MAPK by IL-2 or IL-12 in $CD4^+$ T cells. We have also found that the IL-12-induced production of IFN-γ by DCs of SHPS-1 mutant mice is impaired.[19] It is thus likely that SHPS-1, presumably through complex formation with SHP-2, positively regulates JAK-STAT signaling in general.

In contrast to the impaired responses to IL-2 or IL-12, the production of IL-2, IFN-γ, and IL-4 by $CD4^+$ T cells of SHPS-1 mutant mice in response to TCR activation was increased, compared with that apparent in WT cells. The production of IFN-γ in response to TCR activation is markedly enhanced in $CD4^+$ splenocytes of motheaten viable mice,[29] which harbor a mutation in the SHP-1 gene, suggesting that SHP-1 plays a negative role in regulation of this process. SHPS-1 may therefore negatively regulate the TCR-stimulated production of IFN-γ through its formation of a complex with SHP-1. Fcγ receptor (FcγR)-mediated phagocytosis is enhanced in macrophages from SHPS-1 mutant mice, and it is thought that the SHPS-1-SHP-1 complex negatively regulates such phagocytosis.[15,16] The signaling pathway downstream of FcγR is similar to that downstream of the TCR; the former includes the Src family kinase Lyn, which activates Syk, whereas the latter includes the Src family kinase Lck, which activates ZAP-70, a kinase structurally similar to Syk.[30,31] It is thus possible that the SHPS-1-SHP-1 complex negatively regulates processes mediated by Lyn/Lck and Syk/ZAP-70 signaling in immune and other hematopoietic cells.

In conclusion, our results indicate that SHPS-1 is essential for regulation of the proliferation and Th1 differentiation of $CD4^+$ T cells. Further studies are required to characterize the molecular mechanisms of such regulation.

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References

1. Fujioka Y, Matozaki T, Noguchi T, et al. A novel membrane glycoprotein, SHPS-1, that binds the SH2-domain-containing protein tyrosine phosphatase SHP-2 in response to mitogens and cell adhesion. Mol Cell Biol 1996; 16: 6887-6899.
2. Kharitonenkov A, Chen Z, Sures I, et al. A family of proteins that inhibit signalling through tyrosine kinase receptors. Nature 1997; 386: 181-186.
3. van Beek EM, Cochrane F, Barclay AN, et al. Signal regulatory proteins in the immune system. J Immunol 2005; 175: 7781-7787.
4. Ohnishi H, Kubota M, Ohtake A, et al. Activation of protein-tyrosine phosphatase SH-PTP2 by a tyrosine-based activation motif of a novel brain molecule. J Biol Chem 1996; 271: 25569-25574.
5. Timms JF, Carlberg K, Gu H, et al. Identification of major binding proteins and substrates for the SH2-containing protein tyrosine phosphatase SHP-1 in macrophages. Mol Cell Biol 1998; 18: 3838-3850.
6. Oshima K, Ruhul Amin AR, Suzuki A, et al. SHPS-1, a multifunctional transmembrane glycoprotein. FEBS Lett 2002; 519: 1-7.
7. Jiang P, Lagenaur CF, Narayanan V. Integrin-associated protein is a ligand for the P84 neural adhesion molecule. J Biol Chem 1999; 274: 559-562.
8. Seiffert M, Cant C, Chen Z, et al. Human signal-regulatory protein is expressed on normal, but not on subsets of leukemic myeloid cells and mediates cellular adhesion involving its counterreceptor CD47. Blood 1999; 94: 3633-3643.
9. Brown EJ, Frazier WA. Integrin-associated protein (CD47) and its ligands. Trends Cell Biol 2001; 11: 130-135.
10. Adams S, van der Laan LJ, Vernon-Wilson E, et al. Signal-regulatory protein is selectively expressed by myeloid and neuronal cells. J Immunol 1998; 161: 1853-1859.
11. Veillette A, Thibaudeau E, Latour S. High expression of inhibitory receptor SHPS-1 and its association with protein-tyrosine phosphatase SHP-1 in macrophages. J Biol Chem 1998; 273: 22719-22728.
12. Latour S, Tanaka H, Demeure C, et al. Bidirectional negative regulation of human T and dendritic cells by CD47 and its cognate receptor signal-regulator protein-α: down-regulation of IL-12 responsiveness and inhibition of dendritic cell activation. J Immunol 2001; 167: 2547-2554.
13. Seiffert M, Brossart P, Cant C, et al. Signal-regulatory

proteinα (SIRPα) but not SIRPβ is involved in T-cell activation, binds to CD47 with high affinity, and is expressed on immature $CD34^+$ $CD38^-$ hematopoietic cells. Blood 2001 ; 97 : 2741-2749.

14. Oldenborg PA, Zheleznyak A, Fang YF, et al. Role of CD47 as a marker of self on red blood cells. Science 2000 ; 288 : 2051-2054.
15. Okazawa H, Motegi S, Ohyama N, et al. Negative regulation of phagocytosis in macrophages by the CD47-SHPS-1 system. J Immunol 2005 ; 174 : 2004-2011.
16. Ishikawa-Sekigami T, Kaneko Y, Okazawa H, et al. SHPS-1 promotes the survival of circulating erythrocytes through inhibition of phagocytosis by splenic macrophages. Blood 2006 ; 107 : 341-348.
17. Alblas J, Honing H, de Lavalette CR, et al. Signal regulatory protein α ligation induces macrophage nitric oxide production through JAK/STAT- and phosphatidylinositol 3-kinase/Rac1/NAPDH oxidase/H_2O_2-dependent pathways. Mol Cell Biol 2005 ; 25 : 7181-7192.
18. Inagaki K, Yamao T, Noguchi T, et al. SHPS-1 regulates integrin-mediated cytoskeletal reorganization and cell motility. EMBO J 2000 ; 19 : 6721-6731.
19. Tomizawa T, Kaneko Y, Saito Y, et al. Resistance to experimental autoimmune encephalomyelitis and impaired T cell priming by dendritic cells in Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 mutant mice. J Immunol 2007 ; 179 : 869-877.
20. Fang D, Elly C, Gao B, et al. Dysregulation of T lymphocyte function in itchy mice : a role for Itch in TH2 differentiation. Nat Immunol 2002 ; 3 : 281-287.
21. Kumanogoh A, Shikina T, Suzuki K, et al. Nonredundant roles of Sema4A in the immune system : defective T cell priming and Th1/Th2 regulation in Sema4A-deficient mice. Immunity 2005 ; 22 : 305-316.
22. Reddy M, Eirikis E, Davis C, et al. Comparative analysis of lymphocyte activation marker expression and cytokine secretion profile in stimulated human peripheral blood mononuclear cell cultures : an in vitro model to monitor cellular immune function. J Immunol Methods 2004 ; 293 : 127-142.
23. Iwakura Y, Ishigame H. The IL-23/IL-17 axis in inflammation. J Clin Invest 2006 ; 116 : 1218-1222.
24. Weaver CT, Harrington LE, Mangan PR, et al. Th17 : an effector CD4 T cell lineage with regulatory T cell ties. Immunity 2006 ; 24 : 677-688.
25. Gadina M, Stancato LM, Bacon CM, et al. Involvement of SHP-2 in multiple aspects of IL-2 signaling : evidence for a positive regulatory role. J Immunol 1998 ; 160 : 4657-4661.
26. Leonard WJ, Lin JX. Cytokine receptor signaling pathways. J Allergy Clin Immunol 2000 ; 105 : 877-888.
27. Fukao T, Frucht DM, Yap G, et al. Inducible expression of Stat4 in dendritic cells and macrophages and its critical role in innate and adaptive immune responses. J Immunol 2001 ; 166 : 4446-4455.
28. Watford WT, Hissong BD, Bream JH, et al. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. Immunol Rev 2004 ; 202 : 139-156.
29. Yu WM, Wang S, Keegan AD, et al. Abnormal Th1 cell differentiation and IFN-γ production in T lymphocytes from motheaten viable mice mutant for Src homology 2 domain-containing protein tyrosine phosphatase-1. J Immunol 2005 ; 174 : 1013-1) 0199.
30. Ravetch JV, Bolland S. IgG Fc receptors. Annu Rev Immunol 2001 ; 19 : 275-290.
31. Samelson LE. Signal transduction mediated by the T cell antigen receptor : the role of adapter proteins. Annu Rev Immunol 2002 ; 20 : 371-394.

HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens–Johnson syndrome and toxic epidermal necrolysis

Introduction: Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but life-threatening severe cutaneous adverse reactions. Recently, strong associations of *HLA-B*1502* and *HLA-B*5801* with carbamazepine- and allopurinol-induced severe cutaneous adverse reactions were found in Han Chinese patients, respectively, but ethnic differences in the associations have been reported. The objective of this study is to clarify the involvement of *HLA-B*1502* and *HLA-B*5801* in Japanese SJS/TEN patients. **Methods:** *HLA-B* genotyping was performed on 58 Japanese SJS/TEN patients between July 2006 and April 2008 from multicenters in Japan. **Results:** There were no *HLA-B*1502* carriers among 58 SJS/TEN patients. This patient group included seven carbamazepine-related and 11 aromatic anti-epileptic agent-related SJS/TEN patients. In addition, there were five *HLA-B*5801* carriers, which included four allopurinol-related SJS/TEN patients. **Conclusion:** While *HLA-B*1502* is unlikely to be associated with carbamazepine-related or aromatic anti-epileptic agent-related SJS/TEN, *HLA-B*5801* was significantly associated with allopurinol-related SJS/TEN in Japanese.

KEYWORDS: allopurinol, anti-epileptic drugs, carbamazepine, *HLA-B*1502*, *HLA-B*5801*, Japanese patient, Stevens–Johnson syndrome, toxic epidermal necrolysis

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Introduction

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening severe adverse drug reactions with mucosal and cutaneous disorders, and very often accompanied by high fever and systemic complications. Some investigators have proposed that SJS and TEN are variations of the same disease expressed with different severity [1,2], although this is controversial. Although SJS and TEN incidence is very low (0.4–6 per million per year) [3,4], more than 100 different causative drugs have been reported [5]. The diseases are probably T-cell-mediated delayed allergic reactions [4], and typically begin within 1–3 weeks after first exposure to a drug.

Recently, an extremely strong association (odds ratio: ~2504) between human leukocyte antigen (*HLA*)-*B*1502* and carbamazepine-induced SJS/TEN in Han Chinese patients in Taiwan was reported [6]. Another Taiwanese study showed that *HLA-B*5801* was detected in all Han Chinese patients with SJS/TEN or drug-induced hypersensitivity (DIHS) induced by allopurinol [7]. The involvement of *HLA-B* 1502* was also confirmed in SJS/TEN caused by other aromatic epileptic agents such as phenytoin in Han Chinese or Thai population [8,9]. However, such a strong association between *HLA-B*1502* and carbamazepine-induced SJS/TEN was not detected in Caucasian patients [5]. These reports suggested that HLA involvement in severe cutaneous adverse reactions may be drug-specific as well as ethnic group-specific. Thus, we started a retrospective case–control study to explore genetic biomarkers related to SJS and TEN in Japanese patients living in Japan.

Patients & methods

Patients

The ethics committees of each participating institute of the Japan Severe Adverse Reactions (JSAR) research group approved this study. Written informed consent was obtained from each patient. A total of 58 Japanese patients from unrelated families in Japan were recruited from JSAR research group hospitals or through a nationwide blood-sampling network system in Japan for SJS/TEN onset patients, operated by the National Institute of Health Sciences in cooperation with the Ministry of Health, Labour and Welfare in Japan and the Federation of Pharmaceutical Manufacturers' Association of Japan. All patients, two of whom were referred to in a previous report [10], were diagnosed as SJS or TEN by JSAR research group experts based on diagnostic criteria proposed by Bastuji-Garin *et al.* [1], which are currently used in Japan [11,12] using a standardized case report form including medicinal records,

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disease progress and involvement of systemic complication, as well as SJS/TEN treatment [1]. TEN and SJS are defined as mucocutaneous disorders characterized by extensive erythema, blisters, epidermal detachment, erosions, enanthema and high fever. SJS is defined as skin detachment of 10% or less of the body surface area, and TEN is defined as skin detachment of more than 10%, excluding staphylococcal scaled skin syndrome. The severity of ocular complication was scored as follows: 0: no involvement; 1: only hyperemia of bulbar and palpebral conjuctiva; 2: pseudomembrane formation; 3: defect of conjunctival or corneal epithelia.

HLA-B typing

High-resolution *HLA-B* typing was performed by a sequence-based method using SeCore™ B Locus Sequencing Kit (Invitrogen Corp., WI, USA) and an Applied Biosystems (ABI) 3730 DNA sequencer (ABI, CA, USA). Genomic DNA (250 ng) was used for PCR amplification and sequencing exons 2, 3 and 4. *HLA-B* haplotype was estimated with the Assign SBT software (version 3.2.7b, Conexio Genomics, Western Australia, Australia).

Statistical analysis

*HLA-B*5801* allele frequency reported by Tanaka *et al.*, who performed typing of *HLA-A*, and *-B* for 493 Japanese healthy subjects living in Japan was used as the frequency in control subjects [13]. Fisher's exact test was conducted using Prism 4 (GraphPad Software, Inc., CA, USA) to calculate the odds ratio and the 95% confidence interval.

Results

Demographics of patients recruited in this study are summarized in TABLE 1. A total of 36 and 22 patients were diagnosed with SJS and TEN, respectively. Approximately 80% of SJS/TEN patients complained of ophthalmic disorders, and two patients were coadministered anti-epileptic agents and allopurinol.

*HLA-B*1502* & *HLA-B*0702* in carbamazepine-related SJS/TEN

In our study, carbamazepine was prescribed for seven patients, and other aromatic anti-epileptic agents, such as phenytoin, phenobarbital or zonisamide, were prescribed for 11 patients. By contrast to data on the Han Chinese [6,8] and Thai populations [9], *HLA-B*1502* was neither detected in patients administered carbamazepine, nor in patients administered other aromatic epileptic drugs (TABLE 2).

Alfirevic *et al.* reported a potential protecting effect of *HLA-B*0702* against carbamazepine-induced severe cutaneous adverse reactions in Caucasian patients [14]. In line with this, no SJS/TEN patients receiving carbamazepine or other aromatic anti-epileptic drugs carried *HLA-B*0702* in this study. However, we found *HLA-B*0702* in two patients who did not receive anti-epileptic drugs, and there was no significant difference in the carrier frequencies between patients (1.72%) and the Japanese population (5.17%) ($p = 0.1113$).

*HLA-B*5801* in allopurinol-related and -unrelated SJS/TEN patients

As shown in TABLE 3, we found five carriers of *HLA-B*5801*, and four patients (patients 23,

Table 1. Demographics of Japanese patients recruited in the current study.

Factor	Value
Disease (SJS, TEN)	36, 22
Sex (male, female)	35, 23
Age (mean [range])	55 (5–94)
Severity in ophthalmic disorders	
Score 0 (no ophthalmic involvement)	12
Score 1 (only hyperemia of bulbar and palpebral conjunciva)	21
Score 2 (pseudomembrane without epithelial defect)	1
Score 3 (conjunctival and/or corneal epithelial defect)	14
Severity unknown ocular disorders	9
No description on ophthalmic symptom	1
Administered drugs before development of SJS/TEN	
Carbamazepine	7
Other aromatic anti-epileptic drugs	11
Allopurinol	10*

*One patient was treated with both carbamazepine and allopurinol, and another patient was treated with phenytoin and allopurinol.
SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis.

Table 2. Characteristics of SJS/TEN patients administered aromatic anti-epileptic drugs.

ID number	Sex	Age (years)	Disease	Aromatic anti-epileptic drugs prescribed	Severity score in ophthalmic disorders	*HLA-B* diplotype
1	M	73	SJS	Carbamazepine	1	*1511/*4801
2	F	42	SJS	Carbamazepine	3	*4001/*5201
3	M	45	SJS	Carbamazepine	3	*4801/*5601
4	M	54	SJS	Carbamazepine	0	*1501/*3501
5*	F	6	SJS	Carbamazepine	Severity unknown	*4006/*5101
6*	F	52	SJS	Carbamazepine/zonisamide	Severity unknown	*4601/*5901
7	M	17	TEN	Carbamazepine/zonisamide	3	*4601/*5601
8	M	67	SJS	Phenytoin	Ocular involvement unknown	*4001/*4601
9	F	5	SJS	Phenytoin	0	*5504/*6701
10	F	64	TEN	Phenytoin	3	*1501/*5101
11	F	56	TEN	Phenytoin	0	*1501/*5401
12	M	6	SJS	Phenobarbital	Severity unknown	*1501/*5101
13	M	69	SJS	Phenobarbital	1	*1501/*5101
14	F	42	TEN	Phenobarbital	0	*5101/*5401
15	M	25	SJS	Zonisamide	2	*1301/*4601
16	F	71	SJS	Zonisamide	1	*4002/*5101
17	M	52	TEN	Zonisamide	Severity unknown	*3501/*4601
18	M	78	TEN	Zonisamide	Severity unknown	*3901/*6701

*These patients were reported in the previous report [10]. F: Female; M: Male; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis.

24, 27 and 28) received allopurinol. Since a total of ten patients received allopurinol, *HLA-B*5801* carrier frequency in allopurinol-related patients was 40.0%. Table 4 shows a significant increase in *HLA-B*58*01 allele frequency in allopurinol-administered patients when compared with the Japanese population (odds ratio: 40.83, $p < 0.0001$). *HLA-B*5801* was detected in one patient (patient 41) who did not receive allopurinol. This is the first report that *HLA-B*5801* was detected in a SJS/TEN patient unrelated to allopurinol.

Discussion

Recently, involvement of *HLA* loci have been detected in idiosyncratic adverse drug reactions, including cutaneous [6,7,15] or liver [16] injury. Regarding severe cutaneous reactions, some HLA class I antigen genotypes, such as *HLA-B*1502* [6], *HLA-B*5801* [7] and *HLA-B*5701* [15], have been reported to be very promising biomarkers for discriminating patients at high risk of SJS, TEN or DIHS induced by carbamazepine, allopurinol or abacavir, respectively. The very strong association of *HLA-B*1502* with

Table 3. Characteristics of SJS/TEN patients administered allopurinol and an allopurinol-unrelated patient carrying *HLA-B*5801*.

ID number	Sex	Age (years)	Disease	Allopurinol prescribed	Severity score in ophthalmic disorders	*HLA-B* diplotype[‡]
1	M	73	SJS	Yes	1	*1511/*4801
8	M	67	SJS	Yes	Severity unknown	*4001/*4601
23	F	53	SJS	Yes	1	*4002/**5801**
24	M	77	TEN	Yes	Severity unknown	*5201/**5801**
25	M	75	SJS	Yes	Severity unknown	*4002/*4006
26	M	67	SJS	Yes	1	*3901/*4001
27	F	81	SJS	Yes	1	*4601/**5801**
28	M	83	SJS	Yes	1	*3901/**5801**
29	M	58	TEN	Yes	1	*1501/*5601
30	M	75	TEN	Yes	0	*3501/*5201
41	F	55	TEN	No[†]	Severity unknown	*5401/**5801**

[†]Leflunomid was prescribed for this patient.
[‡]*5801 is indicated in bold.
F: Female; M: Male; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis.

Table 4. Associations of *HLA-B*5801* with Japanese SJS/TEN patients.

Patient group	Allele frequency (%)		p-value (Fisher's exact test)	Odds ratio	95% confidence interval for odds ratio
	SJS/TEN patients	*Japanese population**			
Allopurinol-related patients	20.0 (4/20)	0.61 (6/986)	<0.0001	40.83	10.50–158.9

**Data of 493 healthy Japanese reported in [13].*
SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis.

carbamazepine-induced SJS/TEN found in Han Chinese patients in Taiwan [6] was further confirmed by an extended study in Taiwan [17], and studies on Asian patients living in Europe [5], Han Chinese patients in Hong Kong [8] and the Thai population [9]. Man *et al.* and Locharernkul *et al.* reported that *HLA-B*1502* was also detected in patients who suffered from SJS/TEN caused by aromatic anti-epileptic agents such as phenytoin and lamotrigine [8,9]. By contrast, no SJS/TEN patients receiving aromatic anti-epileptic drugs including carbamzepine carried *HLA-B*1502* in our study using Japanese patients. Thus, we could not confirm the association of *HLA-B*1502* with SJS/TEN in Japanese patients. This is reminiscent of the lack of the association in Caucasian carbamazepine-induced SJS/TEN patients [5]. *HLA-B*1502* was not detected in 486 healthy Japanese [13], while its allele frequency in Han Chinese was reported to be 8.6% [6]. The very low allele frequency of *HLA-B*1502* in the Japanese may account for why no association between *HLA-B*1502* and SJS/TEN was detected in our study. To date, useful genetic biomarkers have not been found for carbamazepine-induced SJS/TEN in ethnic groups other than some Asian ethnic groups, including Han Chinese.

Alfirevic *et al.* reported a significant low carrier frequency of *HLA-B*0702* in Caucasian patients with carbamazepine-induced severe cutaneous adverse reactions, and its potential protecting effect against severe cutaneous adverse reactions [14]. Since we detected *HLA-B*0702* in two SJS/TEN patients unrelated to carbamazepine administration, further studies are necessary to clarify the relationship between *HLA-B*0702* and SJS/TEN.

The association of *HLA-B*5801* with allopurinol-induced severe cutaneous adverse reactions detected in Han Chinese in Taiwan [7] has been confirmed in Caucasians [5]. Although the association observed in Han Chinese in Taiwan was extremely strong (odds ratio: ~580), only a moderate association of *HLA-B*5801* with allopurinol-induced SJS/TEN was observed in a European study by Lonjou *et al.* ($p < 10^{-18}$, odds ratio: 80) [5]. In their study, the carrier frequency in European patients was 55.6%, while that in a European population was 1.5%. A moderate but statistically significant association ($p < 0.0001$, odds ratio: ~40) between *HLA-B*5801* with allopurinol-administered SJS/TEN was also detected in the current study using Japanese patients. Although the carrier frequency of *HLA-B*5801* in the Japanese population (1.2%) [13] is comparable to that in the European population (1.5%), the carrier frequency of *HLA-B*5801* in allopurinol-administered Japanese patients (40.0%) was lower than that observed in European patients. The sample size of our study was not sufficient to estimate the accurate carrier frequency in patients. Recently, Ueta *et al.* reported a case–control study on relationships between HLA class I and II genetic polymorphisms with severe ocular

Executive summary

Backgrounds of genetic biomarkers for severe cutaneous adverse reactions

- Recently, strong drug-specific associations of human leukocyte antigen (*HLA*)-*B*1502* and *HLA-B*5801* with carbamazepine- and allopurinol-induced severe cutaneous adverse drug reactions were found in Han Chinese patients, respectively.
- However, a European study suggested that HLA involvement in severe cutaneous adverse reactions may be ethnic-group-specific, as well as drug-specific.

Objective of this study

- We began a retrospective case–control study to explore genetic biomarkers related to Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in Japanese patients living in Japan.

Conclusion

- We could not find any association between *HLA-B*1502* and carbamazepine-aromatic anti-epileptic agent-associated SJS/TEN in Japanese patients.
- We detected a moderate association of *HLA-B*5801* with Japanese allopurinol-related SJS/TEN patients.

complications using 71 Japanese drug-unspecified SJS/TEN patients and 111 Japanese controls, and they did not detect any *HLA-B*5801* carriers both in cases and controls [18]. However, no allopurinol-induced patients were included in their sample [UETA M, TOKUNAGA K, SOTOZONO C *ET AL.*: PREFECTURAL UNIVERSITY OF MEDICINE, KYOTO, JAPAN. PERS. COMMUN.]. On the other hand, Dainichi *et al.* detected three *HLA-B*5801* carriers in all three allopurinol-associated patients diagnosed with SJS, DIHS and TEN, respectively [19]. Their data and the current study lead to a conclusion that *HLA-B*5801* is one of the (surrogate) genetic biomarkers for allopurinol-associated SJS/TEN also in Japanese patients.

Conclusion

While *HLA-B*1502* is unlikely to be associated with carbamazepine-related or aromatic anti-epileptic agent-related SJS/TEN, *HLA-B*5801* was significantly associated with allopurinol-related SJS/TEN in Japanese.

Future perspective

Recently, the US FDA approved the revision of the label of products containing carbamazepine. In the updated label, it is clearly stated that patients with Chinese ancestry should be screened for the *HLA-B*1502* allele before starting treatment with carbamazepine, and that *HLA-B*1502*-positive patients should basically not be given the drug. On July the 24th, 2008, FDA published an 'alert' [101] based on several studies [15,20–22] informing healthcare professionals that the screening for *HLA-B*5701* is necessary before initiating treatment with abacavir ,and that abacavir should not be administered to *HLA-B*5701* carriers. The Committee for Medicinal Products for Human Use (CHMP) is also considering the revision of the Summary of Product Characteristics (SPC) of abacavir-containing products. Thus, personalized medicine based on pharmacogenomics using biomarkers with excellent performance characteristics has started to identify patients at high risk of idiosyncratic adverse reactions. However, biomarkers only for restricted drugs such as carbamazepine (*HLA-B*1502* for some Asian ethnic groups excluding Japanese), abacavir (*HLA-B*5701* for people living in the USA and Europe) or allopurinol (*HLA-B*5801*) among more than 100 causative ones have been detected to date. Therefore, more intensive, nationwide or even international case–control studies are necessary to find corresponding biomarkers identifying patients at high risk for individual ethnic populations or individual causative drugs. The accumulation of such data may uncover pathogenic mechanisms of SJS/TEN, which will be useful for the identification of new molecules that cause severe cutaneous adverse reactions at an early stage of the drug-development process.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Bibliography

Papers of special note have been highlighted as:
* of interest
** of considerable interest

1 Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC: Clinical classification of cases of toxic epidermal necrolysis, Stevens–Johnson syndrome, and erythema multiforme. *Arch. Dermatol.* 129(1), 92–96 (1993).

2 Roujeau JC: Stevens-Johnson syndrome and toxic epidermal necrolysis are severity variants of the same disease which differs from erythema multiforme. *J. Dermatol.* 24(11), 726–729 (1997).

3 Roujeau JC, Stern RS: Severe adverse cutaneous reactions to drugs. *New Eng. J. Med.* 331(19), 1272–1285 (1994).

4 Chave TA, Mortimer NJ, Sladden AP, Hall AP, Hutchinson PE: Toxic epidermal necrolysis: current evidence, practical management and future directions. *Br. J. Dermatol.* 153(2), 241–253 (2005).

5 Lonjou C, Borot N, Sekula P *et al.*: A European study of *HLA-B* in Stevens–Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet. Genomics* 18(2), 99–107 (2008).

* **The associations between *HLA-B* genotypes and Steven–Johnson syndrome (SJS) were confirmed to be less significant in Europeans than in Han Chinese in Taiwan.**

6 Chung WH, Hung SI, Hong HS *et al.*: Medical genetics: A marker for Stevens–Johnson syndrome. *Nature* 428(6982), 486 (2004).

- **A promising genetic biomarker for carbamazepine-induced SJS in Han Chinese patients in Taiwan was detected.**

7 Hung SI, Chung WH, Liou LB *et al.*: *HLA-B*5801* allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc. Natl Acad. Sci. USA* 102(11), 4134–4139 (2005).

- **A promising genetic biomarker for allopurinol-induced SJS in Han Chinese patients in Taiwan was detected.**

8 Man CB, Kwan P, Baum L *et al.*: Association between *HLA-B*1502* allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 48(5), 1015–1018 (2007).

9 Locharernkul C, Loplumlert J, Limotai C *et al.*: Carbamazepine and phenytoin induced Stevens–Johnson syndrome is associated with *HLA-B*1502* allele in Thai population. *Epilepsia* (2008) (Epub ahead of print).

10 Kashiwagi M, Aihara M, Takahashi Y, Muramatsu M, Ikezawa Z: *HLA* genotypes in carbamazepine induced severe cutaneous adverse drug response in Japanese patients. *J. Dermatol.* (2008) (In Press).

11 Hashimoto K, Iijima M, Shiobara T *et al.*: Stevens–Johnson syndrome. In: *Treatment Manuals for Individual Severe Adverse Reactions (Volume 1)*. Shudo K (Ed.). Japan Pharmaceutical Information Center, Tokyo, Japan, 5–19 (2007).

12 Hashimoto K, Iijima M, Shiobara T *et al.*: Toxic epidermal necrolysis (TEN). In: *Treatment Manuals for Individual Severe Adverse Drug Reactions (Volume 1)*. Shudo K (Ed.). Japan Pharmaceutical Information Center, Tokyo, Japan, 21–35 (2007).

13 Tanaka H, Akaza T, Juji T: Report of the Japanese Central Bone Marrow Data Center. *Clin. Transpl.* 139–144 (1996).

14 Alfirevic A, Jorgensen AL, Williamson PR, Chadwick DW, Park BK, Pirmohamed M: *HLA-B* locus in Caucasian patients with carbamazepine hypersensitivity. *Pharmacogenomics* 7(6), 813–818 (2006).

15 Martin AM, Nolan D, Gaudieri S *et al.*: Predisposition to abacavir hypersensitivity conferred by *HLA-B*5701* and a haplotypic *Hsp70-Hom* variant. *Proc. Natl Acad. Sci. USA* 101(12), 4180–4185 (2004).

16 O'Donohue J, Oien KA, Donaldson P *et al.*: Co-amoxiclav jaundice: clinical and histological features and HLA class II association. *Gut* 47(5), 717–720 (2000).

17 Hung SI, Chung WH, Jee SH *et al.*: Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet. Genomics* 16(4), 297–306 (2006).

18 Ueta M, Tokunaga K, Sotozono C *et al.*: HLA class I and II gene polymorphisms in Stevens–Johnson syndrome with ocular complications in Japanese. *Mol. Vis.* 14, 550–555 (2008).

19 Dainichi T, Uchi H, Moroi Y, Furue M: Stevens–Johnson syndrome, drug-induced hypersensitivity syndrome and toxic epidermal necrolysis caused by allopurinol in patients with a common *HLA* allele: what causes the diversity? *Dermatology* 215(1), 86–88 (2007).

20 Mallal S, Phillips E, Carosi G *et al.*: *HLA-B*5701* screening for hypersensitivity to abacavir. *N. Engl. J. Med.* 358(6) 568–579 (2008).

21 Hughes CA, Foisy MM, Dewhurst N *et al.*: Abacavir hypersensitivity reaction: an update. *Ann. Pharmacother.* 42(3), 387–396 (2008).

22 Saag M, Balu R, Phillips E *et al.*: High sensitivity of human leukocyte antigen-*B*5701* as a marker for immunologically confirmed abacavir hypersensitivity in white and black patients. *Clin. Infect. Dis.* 46(7), 1111–1118 (2008).

Website

101 FDA ALERT (7/24/2008): Information for healthcare professionals regarding abacavir (marketed as Ziagen™) and abacavir-containing medications www.fda.gov/cder/drug/InfoSheets/HCP/abacavirHCP.htm

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Clinical Features of Opticospinal Multiple Sclerosis with Anti-Aquaporin 4 Antibody

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Key Words
Anti-aquaporin 4 antibody · Neuromyelitis optica · Opticospinal multiple sclerosis · Somatosensory-evoked potential · Syrinx formation

Abstract
Background: We have followed 9 Japanese patients with opticospinal multiple sclerosis (OSMS), some of whom showed longitudinally extensive spinal cord lesions, deep sensory disturbances and resistance to treatment. We investigated the patients for anti-aquaporin 4 (AQP4) antibodies and related this to their neuroimaging, clinical and laboratory features. ***Methods:*** We studied the clinical course, neurological findings, cerebrospinal fluid (CSF), and electrophysiological findings, and determined the presence of anti-AQP4 antibody and human leukocyte antigen DPB1 and DRB1 alleles. ***Results:*** Five patients (56.6%) had anti-AQP4 antibody. Antibody-positive patients displayed female predominance, longitudinally extensive spinal cord lesions, higher frequency of exacerbations, severe disability, and higher cell counts and total protein content without IgG oligoclonal bands in the CSF. They also showed poor steroid responsiveness and poor therapeutic response to interferon β_{1b}. ***Conclusions:*** The presence of anti-AQP4 antibodies correlates with clinical severity and poor prognosis in OSMS.

Introduction

The reported prevalence of multiple sclerosis (MS) ranges from 0.7 to 8.6 per 100,000 in Japan [1]. Some recent reports have indicated rates of 8.57 per 100,000 in the Tokachi area [2] and 10.2 per 100,000 in Asahikawa city [3]; these higher rates are possibly attributable to the geographical location of these areas, which are in northern Japan. Fifteen to 40% of Japanese MS patients have the opticospinal form (opticospinal MS; OSMS) [1], characterized by the selective involvement of the optic nerve and spinal cord. The proportion of OSMS cases is higher in Japan than in Western countries, where conventional MS is more common [1, 4, 5]. OSMS has similar features to relapsing neuromyelitis optica (NMO) and it has been suggested that they are the same disease [6–8]. Recently, NMO-IgG, which interacts selectively with the aquapo-

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rin 4 (AQP4) water channel, was found in the serum of both NMO and Japanese OSMS patients [9, 10]. Additionally, several reports showed a clinicopathological relationship between NMO, MS and AQP4 [11–14]. However, there are few reports describing a correlation between Japanese OSMS patients and anti-AQP4 antibodies [15, 16]. On the other hand, we have been treating OSMS patients for a long time. Some of the patients have long lesions in the spinal cord, deep sensory disturbances and are refractive to corticosteroid and interferon β_{1b} (IFN-β_{1b}) treatment. We investigated the relationship between the presence of anti-AQP4 antibodies and the neuroimaging, neurophysiological and clinical-laboratory features in these patients.

Materials and Methods

Patients

We recruited 9 consecutive Japanese patients with MS (2 men and 7 women; mean age 49.9 years, SD 10.4 years), whose main lesions were in the spinal cord and optic nerve, and who had been treated for more than 10 years in our hospital. The patients fulfilled the criteria for clinically definite MS developed by Poser et al. [17]. Clinical course (age of onset, symptoms at onset, disease duration, frequency of exacerbations, annual relapse rate and therapy), the presence of anti-AQP4 antibody, neurological findings, cerebrospinal fluid (CSF), electrophysiological findings in the acute phase, and human leukocyte antigen (HLA) DRB1 and DPB1 alleles were evaluated. Optic nerve involvement was confirmed using visual evoked potentials and flicker tests. Disability was scored using the Expanded Disability Status Scale (EDSS) [18]. The Research Ethics Committee of the National Defense Medical College approved the study and informed consent was obtained from each patient.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) was performed using a 1.5-tesla Vision system (Siemens, Germany). For brain MRI, T_2-weighted, spin-echo images [repetition time (TR), 3,520–3,800 ms; echo time (TE), 95.2–102 ms] and T_1-weighted images (TR, 400 ms; TE, 13 ms) were collected as axial and coronal images. T_1-weighted images were used in enhancement studies with gadolinium diethylene triamine pentaacetic acid (Gd-DTPA). T_2-weighted images (TR, 3,000–4,100 ms; TE, 102–120 ms) and T_1-weighted images (TR, 500–751 ms; TE, 9.4–19.1 ms) of the spinal cord were obtained in the sagittal and axial planes. A Gd-DTPA enhancement study of the spinal cord was also performed using T_1-weighted images.

Electrophysiological Study

Three kinds of electrophysiological examination were performed. Firstly, motor (right median, ulnar and tibial nerves) and sensory (right median, ulnar and sural nerves) nerve conduction studies were performed in all patients before therapy. Secondly, median nerve somatosensory-evoked potentials (SEPs) were studied, with analysis of N9 (recorded at Erb's point ipsilateral to the stimulated nerve – contralateral Erb's point cEP), N11 (sixth cervical spinous process – Fz of the international 10–20 system), P13/14 (contralateral hand sensory area HS – cEP), and N20 (HS–Fz). Thirdly, tibial nerve SEPs were studied, with analysis of P15 (contralateral iliac crest – ipsilateral greater trochanter iGT), N21 (first lumbar spinous process – iGT), and P38 (C3–C4 or Fz–Cz). A stimulus was delivered at the wrist or ankle and the strength was adjusted to produce minimal contraction of the abductor pollicis brevis or abductor hallucis muscle. Potentials were amplified by filters set at 1 and 1,500 Hz, and at least 500 responses were averaged. To ensure reproducibility of results, SEPs were recorded at least twice. Normal limits were established in 14 healthy volunteers of a mean ± SD age of 42 ± 6 years, and a mean ± SD height of 167 ± 6 cm.

Anti-AQP4 Antibody

For the anti-AQP4 antibody detection, we constructed AQP4 antigen-presenting cells as follows [15]: total RNA was extracted from an adult human cerebellum and cDNA-encoding human AQP4 (AQP4 M23 isoform; GenBank accession No. U63623) was cloned by the reverse transcription polymerase chain reaction technique. Full-length cDNA was inserted into the *XbaI* site of a pEF-BOS expression vector and transfected to HEK 293 cells. The HEK 293 cells were then fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.4). Nonspecific binding was blocked with 10% goat serum/phosphate-buffered saline, the cells were incubated with the patient serum for 60 min at room temperature, then incubated with fluorescein-isothiocyanate-conjugated rabbit anti-human IgG (BD Biosciences, San Jose, Calif., USA). Then, a SlowFade Gold anti-fade reagent (Molecular Probes, Carlsbad, Calif., USA) was applied to the slide.

Statistical Analyses

Statistical analyses were performed with SPSS version 12.0J for Windows (SPSS Inc., Chicago, Ill., USA). Two-tailed p values of <0.05 were considered significant. One-factor factorial analysis of variance was used to compare ages or years. The Kruskal-Wallis test was used for nonparametric comparisons of EDSS scores. Differences in proportions were examined using contingency tables and the χ^2 test or Fisher's exact test.

Results

Clinical features of all patients are summarized in table 1. Five of the 9 patients (56.6%) had anti-AQP4 antibodies; all of them had frequent relapses of myelitis with or without optic neuritis and were females. Optic neuritis was severe in the anti-AQP4 antibody-positive patients, and 3 patients in the antibody-positive group but none in the negative group showed complete blindness. The antibody-positive group exhibited a later age of onset, but this was not significant (table 1). The positive group showed significantly more frequent exacerbations ($p < 0.05$; one-factor analysis of variance), and significantly higher EDSS scores during remission ($p < 0.01$; Kruskal-Wallis

Table 1. Clinical features of OSMS with anti-AQP4 antibody

	Anti-AQP4 Ab-positive (n = 5)	Anti-AQP4 Ab-negative (n = 4)
Male/female	0/5	2/2
Age of onset, years	40.4 ± 12.9	26.8 ± 7.0
Symptoms at onset		
Visual impairment	3 (60)	2 (50)
Numbness of lower limbs	2 (40)	2 (50)
Disease duration, years	14.4 ± 4.6	17.0 ± 5.8
Frequency of exacerbations	9.6 ± 1.8*	6.8 ± 0.9
Annual relapse rate per year	0.74 ± 0.3	0.49 ± 0.3
EDSS score		
At relapse	7.8 ± 1.7	5.3 ± 1.0
During remission	7.1 ± 2.0**	3.3 ± 0.3
Neurological findings in the lower extremities		
Positive Babinski sign	4 (80)	4 (100)
Decreased tendon reflexes with Babinski sign	3/4	1/4
Deep sensory disturbance	5 (100)	3 (75)
CSF analysis		
Increased MBP (≥0.8 ng/ml)	5 (100)	0
IgG oligoclonal bands	1 (20)	0
Pleocytosis (≥20 cells/μl)	4 (80)	1 (25)
Increased protein	4 (80)	1 (25)
HLA class II alleles		
DPB1*0501	4 (80)	2#
DPB1*02012	2 (40)	0#
DPB1*0901	1 (20)	1#
Administration of immunotherapies		
IVMP	5 (100)	4 (100)
CPA	2 (40)	1 (25)
Plasmapheresis	3 (60)	1 (25)
AZP	4 (80)	2 (50)
Administration of IFN-β_{1b}	3 (60)	3 (75)
Good response	0/3	2/3
Poor response	3/3	1/3

Anti-AQP4 Ab = Anti-AQP4 antibody; IVMP = intravenous methylprednisolone; CPA = cyclophosphamide; AZP = azathioprine. * $p < 0.05$; ** $p < 0.01$. #One patient in the anti-AQP4 antibody negative group declined the examination. Figures in parentheses indicate percentages.

Table 2. MRI findings

	Anti-AQP4 Ab-positive (n = 5)	Anti-AQP4 Ab-negative (n = 4)
Spinal MRI		
Lesions in the spinal cord	5	4
Lesions in the thoracic cord	5 (100)	3 (75)
Lesions in the cervical cord	3 (60)	3 (75)
Both thoracic and cervical lesions	2 (40)	1 (25)
Longitudinally extensive spinal cord lesions		
≥3 vertebral segments	5 (100)	0
<3 vertebral segments	0	4 (100)
Syrinx formation	4 (80)	1 (25)
Brain MRI		
Lesions in the cerebrum or cerebellum	5 (100)	3 (75)
Periventricular lesions	3 (60)	3 (75)

Anti-AQP4 Ab = Anti-AQP4 antibody. Figures in parentheses indicate percentages.

test) than the negative group. The number of patients who displayed decreased tendon reflexes in the lower extremities despite a positive Babinski sign was larger in the antibody-positive group. In CSF analyses, pleocytosis (more than 20 cells/μl), a slight increase in protein, and increased myelin basic protein (MBP) were more common in the positive group than in the negative group, while IgG oligoclonal bands were not detected in either group. The patients in both groups responded well to corticosteroid therapy in the initial relapses using high-dose intravenous methylprednisolone pulse, which was then tapered, and their symptoms mostly subsided. However, the antibody-positive group showed poor steroid responsiveness in subsequent relapses, and anti-immunosuppressants such as cyclophosphamide, or azathioprine and plasmapheresis were more commonly used. On the other hand, the negative group responded well to corticosteroids even in subsequent relapses. Three patients in the positive group administered IFN-β_{1b} subsequently had marked exacerbations and 2 of them had new white matter lesions in the cerebrum on MRI. In the negative group, 2 of 3 patients given IFN-β_{1b} experienced a reduction in frequency and severity of relapse, but in 1 patient IFN-β_{1b} was discontinued because of severe adverse effects. One patient in each group had the autoantibody to SS-A/Ro, however, no patient was diagnosed as having Sjögren's syndrome according to the results of lip biopsy. All patients in the antibody-positive group and 2 in the negative group (1 in the negative group had not been examined) had HLA-DPB1*0501 alleles.

MRI Findings

Spinal MRI showed at least two hyperintense spinal cord lesions on T_2-weighted images, with Gd-DTPA enhancement in the thoracic or cervical cord of all cases (table 2). In the positive cases, all patients had longitudinally extensive spinal cord lesions, in at least 3 vertebral segments (fig. 1a). On the other hand, in all the antibody-negative patients, MRI showed no longitudinally exten-

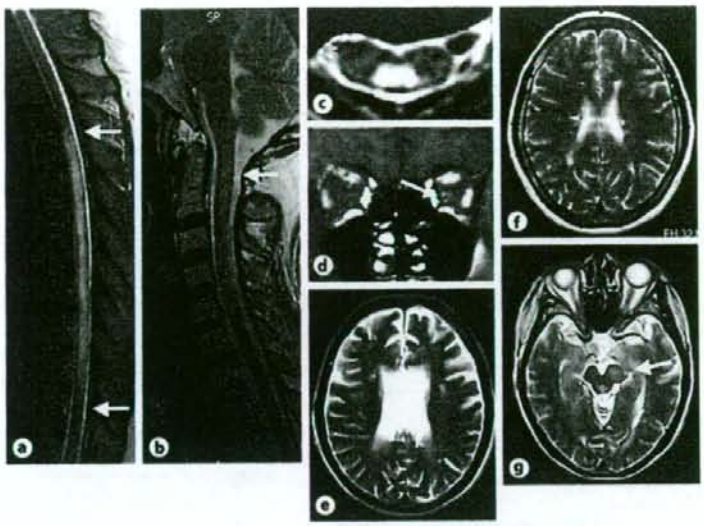

Fig. 1. Representative MR images of anti-AQP4 antibody-positive and antibody-negative patients with OSMS. **a** Spinal cord T_2-weighted MRI of a 60-year-old patient with anti-AQP4 antibody, showing longitudinal lesions of high signal intensity extending over several segments at relapse. **b** Spinal cord MRI of a 49-year-old female patient who has no anti-AQP4 antibody, showing longitudinal lesions of high signal intensity but less than 3 vertebral segments at relapse. **c** T_2-weighted axial image of a 52-year-old patient with anti-AQP4 antibody, showing syrinx formation mainly located in the center of the spinal cord as signal hyperintensity with a similar signal to that of the CSF in spite of remission. **d** Brain fat saturation pulse MRI of a 58-year-old patient with anti-AQP4 antibody, showing the left optic nerve with abnormal intensity in remission. **e** T_2-weighted axial MRI of a 60-year-old patient with anti-AQP4 antibody, showing periventricular hyperintense lesions at relapse. **f** T_2-weighted image of a 49-year-old female patient who has no anti-AQP4 antibody, showing periventricular hyperintense lesions at relapse. **g** T_2-weighted image of a 58-year-old patient with anti-AQP4 antibody, showing a new high-intensity lesion in the left middle cerebellar penduncle after IFN-β_{1b} treatment.

sive spinal cord lesions (fig. 1b). The spinal cord was severely atrophied in 3 of the antibody-positive cases. In addition, 4 of the 5 positive cases showed syrinx formation as signal hypointensity on T_1-weighted images and hyperintensity on T_2-weighted images. These cavity-like lesions showed a similar signal to CSF and were not enhanced by Gd-DTPA (fig. 1c). Several months after initiation of corticosteroid therapy, the cavity sizes had reduced only slightly. Brain MRI was performed in each case. In the antibody-positive cases, some scans revealed an increased signal in the optic nerve on fat saturation images (fig. 1d). All patients in the antibody-positive group and 3 in the negative group had cerebral lesions (table 2). Three patients in each group had a few periventricular lesions (table 2; fig. 1e, f). Two of the positive cases administered IFN-β_{1b} had new lesions of the pyramidal tracts in the brainstem (fig. 1g).

Electrophysiological Findings

Nerve conduction studies were normal in all patients of each group. One patient in the antibody-negative group declined the SEP study. The right median SEPs showed the following abnormalities: in the antibody-positive group, SEPs showed delayed latencies between the N9 and N20 onsets in 2 cases and reduced amplitudes at P13/14 in 2 cases. The right tibial nerve SEPs showed no abnormalities in all the antibody-positive patients but 2 patients in the negative group showed delayed latencies between the N21 and N28 peaks.

Discussion

The present study confirmed that patients with anti-AQP4 antibody had these previously reported features: female predominance, longitudinally extensive spinal

cord lesions, higher frequency of exacerbations, higher EDSS scores, higher cell counts and total protein content without IgG oligoclonal bands in the CSF [1, 16]. All patients in the antibody-positive group showed increased MBP in the CSF and had HLA-DPB1*0501 alleles which have been thought to be associated with OSMS [19]. In the antibody-positive group, 3 of 4 patients with a positive Babinski sign had decreased tendon reflexes in the lower extremities indicating mixed upper and lower motor neuron signs. Although abnormalities of tendon reflexes and vibration sense of the lower extremities may suggest lesions in dorsal roots and dorsal columns, we were unable to correlate electrophysiological data with these signs. This poor correlation between clinical and electrophysiological changes in MS has also been reported previously [20].

All patients in this study also met the revised diagnostic criteria for NMO [21]. NMO lesions are distributed in the hypothalamus and the grey matter of the spinal cord and correlate with the distribution of AQP4-rich areas [11, 22]. However, in the present study, brain lesions were also found in the long tracts such as the pyramidal tract or the middle cerebellar peduncle of the antibody-positive patients, which differed from previous reports. Almost all the patients in both groups had small periventricular lesions, but these findings did not fulfill the criteria of McDonald et al. [23] for MS on brain MRI. In the antibody-positive cases, MRIs demonstrated that syrinx formation was predominantly located in the central part of the spinal cord (fig. 1c). MS associated with a spinal cord cavity was initially reported radiographically in 1985 and it was suggested that a syrinx developed in MS secondary to spinal cord necrosis [24]. Syringomyelia has been associated with diseases such as spinal cord tumors, trauma, infection, arachnoiditis, and posterior fossa abnormalities [25, 26]. Misu et al. [11] have recently suggested that the expression of AQP4 was richer in the grey matter than the white matter of the normal human spinal cord and also showed that NMO lesions were characterized by absent or impaired AQP4, especially in the grey matter. Moreover, Matsuoka et al. [16] suggest that the spinal cord lesions in antibody-positive OSMS involve the upper to middle thoracic cord. The present study confirms these findings (table 2) and also suggests that the existence of anti-AQP4 antibody may cause NMO-like impairments.

Regarding treatment, plasmapheresis is effective in anti-AQP4 antibody-positive patients with poor steroid responsiveness, but these patients had a poor therapeutic response to IFN-β_{1b} therapy. On the other hand, the patients in the antibody-negative group responded well to IFN-β_{1b}. This indicates that anti-AQP4 antibody may correlate inversely with the response to IFN-β_{1b}. Hence IFN-β_{1b} should be used with caution in the antibody-positive patients. Our findings are in concordance with previous reports describing the correlation between Japanese OSMS and anti-AQP4 antibodies. The presence of anti-AQP4 antibodies is thus an indicator of severe OSMS with poor prognosis.

References

1 Kira J: Multiple sclerosis in the Japanese population. Lancet Neurol 2003;2:117–127.
2 Houzen H, Niino M, Kikuchi S, et al: The prevalence and clinical characteristics of MS in northern Japan. J Neurol Sci 2003;211:49–53.
3 Itoh T, Aizawa H, Hashimoto K, et al: Prevalence of multiple sclerosis in Asahikawa, a city in northern Japan. J Neurol Sci 2003;214:7–9.
4 Kira J, Yamasaki K, Horiuchi I, et al: Changes in the clinical phenotypes of multiple sclerosis during the past 50 years in Japan. J Neurol Sci 1999;166:53–57.
5 Kira J, Kanai T, Nishimura Y, et al: Western versus Asian types of multiple sclerosis: immunogenetically and clinically distinct disorders. Ann Neurol 1996;40:569–574.
6 Misu T, Fujihara K, Nakashima I, et al: Pure optic-spinal form of multiple sclerosis in Japan. Brain 2002;125:2460–2468.
7 Lucchinetti CF, Mandler RN, McGavern D, et al: A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain 2002;125:1450–1461.
8 Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG: The clinical course of neuromyelitis optica (Devic's syndrome). Neurology 1999;53:1107–1114.
9 Lennon VA, Wingerchuk DM, Kryzer TJ, et al: A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 2004;364:2106–2112.
10 Lennon VA, Kryzer TJ, Pittock SJ, et al: IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 2005;202:473–477.
11 Misu T, Fujihara K, Kakita A, et al: Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. Brain 2007;130:1224–1234.
12 Roemer SF, Parisi JE, Lennon VA, et al: Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. Brain 2007;130:1194–1205.
13 Sinclair C, Kirk J, Herron B, Fitzgerald U, McQuaid S: Absence of aquaporin-4 expression in lesions of neuromyelitis optica but increased expression in multiple sclerosis lesions and normal-appearing white matter. Acta Neuropathol 2007;113:187–194.
14 Takahashi T, Fujihara K, Nakashima I, et al: Anti-aquaporin-4 antibody is involved in the pathogenesis of NMO: a study on antibody titre. Brain 2007;130:1235–1243.

15 Tanaka K, Tani T, Tanaka M, et al: Anti-aquaporin 4 antibody in Japanese multiple sclerosis with long spinal cord lesions. Mult Scler 2007;13:850–855.
16 Matsuoka T, Matsushita T, Kawano Y, et al: Heterogeneity of aquaporin-4 autoimmunity and spinal cord lesions in multiple sclerosis in Japanese. Brain 2007;130:1206–1223.
17 Poser CM, Paty DW, Scheinberg L, et al: New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227–231.
18 Kurtzke JF: Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444–1452.
19 Yamasaki K, Horiuchi I, Minohara M, et al: HLA-DPB1*0501-associated opticospinal multiple sclerosis: clinical, neuroimaging and immunogenetic studies. Brain 1999;122:1689–1696.
20 Aminoff MJ, Davis SL, Panitch HS: Serial evoked potential studies in patients with definite multiple sclerosis. Clinical relevance. Arch Neurol 1984;41:1197–1202.
21 Wingerchuk DM, Lennon VA, Pittock SJ, et al: Revised diagnostic criteria for neuromyelitis optica. Neurology 2006;66:1485–1489.
22 Pittock SJ, Lennon VA, Krecke K, Wingerchuk DM, Lucchinetti CF, Weinshenker BG: Brain abnormalities in neuromyelitis optica. Arch Neurol 2006;63:390–396.
23 McDonald WI, Compston A, Edan G, et al: Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50:121–127.
24 Kwee IL, Nakada T: Syrinx formation in multiple sclerosis. Br J Radiol 1985;58:1206–1208.
25 Li KC, Chui MC: Conventional and CT metrizamide myelography in Arnold-Chiari I malformation and syringomyelia. Am J Neuroradiol 1987;8:11–17.
26 Sherman JL, Barkovich AJ, Citrin CM: The MR appearance of syringomyelia: new observations. Am J Roentgenol 1987;148:381–391.

INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Longitudinal Analysis of Cytokines and Chemokines in the Cerebrospinal Fluid of a Patient with Neuro-Sweet Disease Presenting with Recurrent Encephalomeningitis

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Abstract

Background Neuro-Sweet disease (NSD) has recently been identified as Sweet disease with central nervous system (CNS) involvement characterized by multisystem neutrophilic infiltration. However, the pathogenesis of this disease remains unknown. Neutrophil and other inflammatory cell activities are influenced by many cytokines and chemokines, but to date, no studies have examined the levels of these factors in patients with NSD.

Patient and Methods The patient presented with encephalomeningitis twice in one year and was diagnosed with NSD. We measured the levels of cytokines (i.e., IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α) and chemokines (i.e., CCL2, CCL3, CCL5, CXCL8, CXCL10 and GM-CSF) in 10 CSF samples from the patient longitudinally for one year including those during two episodes of encephalomeningitis.

Results The elevations of IL-6, IFN-γ, CXCL8 (IL8) and CXCL10 (IP10) were markedly higher than the levels in uninfected control subjects with neurological disorders. The levels of these cytokines and chemokines were statistically correlated with total CSF cell counts ($p < 0.01$).

Conclusion CD4+ helper T (Th) cells can be divided into the Th1 and Th2 subtypes according to their cytokine secretion patterns, and IFN-γ and IP10 are the Th1-type cytokine and chemokine indicating the involvement of Th1 cells in NSD. In addition, the level of IL8, a specific neutrophil chemoattractant, correlated well with the neutrophil cell counts in CSF. Our data suggest the important roles of Th1 cells and IL8 in the pathogenesis of NSD.

Key words: CXCL8 (IL-8), CXCL10 (IP-10), IL-6, IFN-γ, neutrophil cell, Th1 cell

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Introduction

Neuro-Sweet disease (NSD) has recently been identified as Sweet disease with central nervous system (CNS) involvement characterized by multisystem neutrophilic infiltration (1, 2). Patients present with painful erythematous plaques on their skin and histological examination of the plaques shows dense dermal infiltration of neutrophils with no signs of vasculitis. This characteristic finding, together with HLA B51 negativity, is important in distinguishing NSD from neuro-Behçet disease (NBD) (2, 3). Japanese patients with NSD also typically show high levels of HLAs B 54 and CW1 (2).

Encephalitis and meningitis are common neurological manifestations of NSD (1). Systemic corticosteroid therapy is highly effective and most patients recover from their neurological deficits without sequelae (1, 2, 4, 5). Despite effective treatment, however, some patients have recurrent episodes indicating that more effective therapies are still needed. A clearly defined pathogenesis for NSD and reliable laboratory markers reflecting disease activity remain elusive. Here, we report the first longitudinal analysis of the levels of cytokines and chemokines in the cerebrospinal fluid

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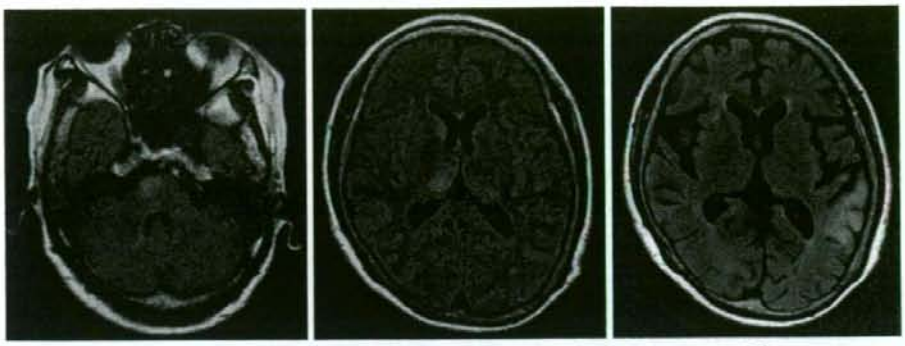

1-1 1-2 1-3

Figure 1. **Magnetic resonance images on admission. FLAIR images show high-intensity lesion in the brainstem (1-1), right thalamus and caudate nucleus (1-2) at first hospitalization, and high-intensity lesion in the cortex and subcortical white matter of the left temporal lobe at second hospitalization (1-3).**

(CSF) of a patient with NSD. Our results provide important clues to the pathogenesis of NSD and may contribute to the formulation of more effective preventative NSD therapies.

Patient and Methods

Patient

At first hospitalization

A 59-year-old woman had a sore throat and a fever in late August 2005. Four days later, she visited a local hospital. She was diagnosed with acute tonsilitis, admitted to a hospital and treated with antibiotic therapy in early September 2005. The day after admission, she became drowsy and she was transferred to our hospital. She had a history of acute hepatitis B viral infection. She had a temperature of 36.6℃, a pulse of 78/min, and a blood pressure of 148/65 mm Hg. She had erythematous plaques on both legs. On neurological examination, her consciousness level was semicoma and she presented with right pupillary dilatation and delayed light reflex. The deep tendon reflexes of all four limbs were hyperactive except for the bilateral Achilles tendon reflexes. Laboratory evaluation revealed increased numbers of peripheral blood leukocytes and neutrophils: white blood cell (WBC) count, $15.1\times10^3/\mu l$ (normal range: $3.3\times10^3\sim7.9\times10^3/\mu l$) and neutrophil cell count, $13.9\times10^3/\mu l$ (normal range $1.5\times10^3\sim5.9\times10^3/\mu l$). Her serum C reactive protein (CRP) level was 18.8 mg/dl (normal <0.20 mg/dl). CSF examination showed 341 cells/mm^3 (mononuclear cells, 298; neutrophilic cells, 43) and a total protein concentration of 172 mg/dl. A culture of a CSF sample was negative for bacteria, tuberculosis and fungi. Antibodies against herpes simplex virus were absent and PCR analysis also showed no herpes simplex virus. A brain MRI scan showed increased signal intensities on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images in the brainstem (Fig. 1-1), right thalamus and caudate nucleus (Fig. 1-2). The electroencephalogram showed slow basic rhythm and diffuse θ activity. After admission she was treated with an intravenous infusion of antibiotics and acyclovir. Subsequently, the disturbance of consciousness became progressively worse and mechanical respiratory management was required two days after admission. She suffered a generalized tonic seizure and was treated with phenytoin. The seizures were difficult to control, however, and required treatment with the anesthetic agent propofol. Because a brain MRI scan showed increased signal intensities on T2-weighted and FLAIR images in various subcortical brain structures, a diagnosis of acute disseminated encephalomyelitis (ADEM) was suspected. Thus, four days after admission intravenous dexamethasone (12 mg/day for 5 days) was administered for 4 days and then, ten days later, methylprednisolone (1,000 mg/day for 3 days) was administered for three days. Her condition gradually improved and she did not require respiratory management. However four weeks after admission a brain MRI scan showed an abnormal signal intensity lesion in the periventricular white matter of the left parietal lobe and expansion of the brainstem lesion. Then her symptoms and abnormal brain MRI findings gradually improved and she was discharged from the hospital without any sequelae in early November 2005.

At second hospitalization

The patient had a sore throat and a fever in mid-January 2006. Five days later, she consulted an otolaryngologist and was diagnosed with acute tonsilitis. She was treated with an intravenous infusion of antibiotics. Five days later she suffered a sudden, generalized tonic seizure during infusion and was referred to our department. She had a temperature of 37.8℃, a pulse of 95/min, and a blood pressure of 153/83 mm Hg. Her throat was reddish and the palatal tonsil was swelling with velaque. Erythematous plaques were apparent on her cheek, forearms and legs. On neurological examination, she was disoriented and could not remember her name

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and birthday correctly. The deep tendon reflexes of all four limbs were hyperactive predominantly in left upper and lower limbs. She presented with bilateral Hoffman reflexes and spasticity of the lower limbs. Laboratory tests revealed increased numbers of peripheral blood leukocytes and neutrophils: WBC count, $13.7\times10^3/\mu l$ and neutrophil cell count, $11.5\times10^3/\mu l$. Her serum CRP level was elevated at 11.3 mg/dl (normal <0.20 mg/dl). The serum rheumatoid factor and antibodies including antinuclear, anti-SS-A, anti-SS-B, anti-DNA, anti-Sm, and anti-RNP antibodies, and the perinuclear anti neutrophil cytoplasmic antibody (P-ANCA), and the cytoplasmic anti-neutrophil cytoplasmic antibody (C-ANCA) were all absent. Human leukocyte antigen (HLA) typing showed B-54 and CW1. CSF examination showed 108 cells/mm^3 (mononuclear cells, 91; neutrophilic cells, 17), a total protein concentration of 41 mg/dl. A culture of the CSF sample was negative for bacteria, tuberculosis and fungi. Antibodies against herpes simplex virus, varicella zoster virus and toxoplasma were negative. PCR analysis also showed no herpes simplex virus. A brain MRI revealed increased signal intensity on T2-weighted and FLAIR images in the cortex and subcortical white matter of the left temporal lobe (Fig. 1-3). ^{99m}Tc-HMPAO SPECT performed on the third day of hospitalization revealed hyperperfusion in the left temporal lobe. An electroencephalogram showed diffuse slow activity with small spikes and sharp waves in the left temporal region. There were no ocular lesions such as uveitis, episcleritis and conjunctivitis. Neither oral aphthae nor genital ulcers were obserbed. We performed a malignancy survey including a whole-body CT, an examination by gastrointestinal endoscopy, a bone marrow aspiration study, and a gynecological consultation, all of which showed negative results. After admission she was treated with an intravenous infusion of antibiotics and acyclovir. Her consciousness was progressively disturbed and she suffered frequent generalized tonic seizures; therefore, at ten days after admission she required propofol treatment and mechanical respiratory management. A skin biopsy of the erythema on her right forearm was performed. Histological examination showed dense dermal infiltration of neutrophils with no signs of vasculitis, and as a result she was diagnosed with Sweet's disease. Corticosteroid therapy was initiated with an intravenous administration of methylprednisolone (1,000 mg/day for 3 days) from the tenth day of admission, followed by 50 mg of prednisolone administrated orally. Her symptoms gradually improved by the end of January 2006 she no longer required mechanical ventilation. However, she continued suffering from a slight fever, and elevated levels of CRP and WBCs without signs of infection and presented with aphasia. As a result, she was treated with a second intravenous administration of methylprednisolone (1,000 mg/day for 3 days) in early Feburary 2006. Subsequently, her symptoms and laboratory data improved, and she was discharged from the hospital without any sequelae about three weeks later.

Methods

Analysis of levels of cytokines and chemokines

We measured the levels of cytokines (i.e., IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α) and chemokines (i.e., CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, CXCL8/IL-8, CXCL10/IP-10 and GM-CSF) in 10 CSF samples from the patient throughout the clinical course. We also measured the levels of those cytokines and chemokines in CSF samples from the control subjects. The control subjects for cytokines were 21 noninfected patients with neurological disorders (epilepsy, 8; psychomotor delay, 5; psychogenic response, 5; functional headache, 1; myopathy, 1; agenesis of corpus callosum, 1) and the control subjects for chemokines were 10 noninfected subjects with neurological disorders (functional headache, 3; Parkinson disease, 1; normal pressure hydrocephalus, 2; spinocerebellar degenetration, 2; amyotrophic lateral sclerosis, 2). CSF samples were obtained from them on routine analysis and they all had normal CSF cell counts. All upper values of control subjects are expressed as mean + 3SD.

Determination of cytokine levels

The levels of IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 in CSF were measured with a cytometric bead array (CBA) kit (BD PharMingen, San Diego, CA) as previously described (6-8), with the exception that data analysis was performed using GraphPad Prism software (GraphPad Prism Software, San Diego, CA). The lower detection limits for IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were 7.1 pg/mL, 2.8 pg/mL, 2.6 pg/mL, 2.6 pg/mL, 2.5 pg/mL, and 2.8 pg/mL, respectively.

Determination of chemokine levels

The levels of CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, CXCL8/IL-8, and GM-CSF were measured using ELISA kits (Endogen, Woburn, MA, USA), and the concentration of CXCL10/IP-10 was measured using an ELISA kit (R&D Systems, Minneapolis, MN, USA) on the basis of the quantitative sandwich enzyme immunoassay technique, as previously described (9). The sensitivity of these assays was 10 pg/mL.

Statistical analysis

The Spearman rank correlation was calculated to assess the correlation between the levels of cytokines and total CSF cell counts, and the levels of chemokines and total CSF cell counts.

Results

Clinical course (Fig. 2)

Clinical manifestations and brain MRI findings correlated

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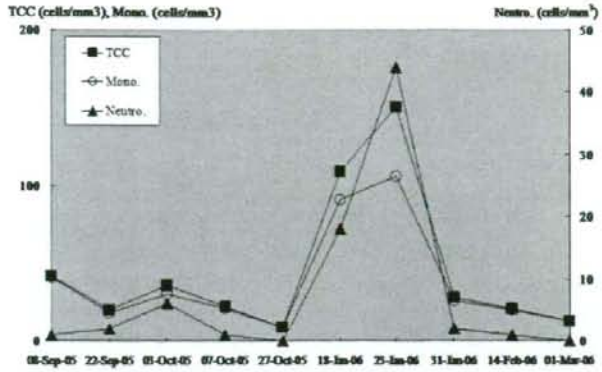

Figure 2. Cell counts [total cell count (TCC), mononuclear cell count (Mono.), neutrophilic cell count (Neutro.), cells/mm³] in CSF.

Table 1. The Levels (pg/mL) of Cytokines (1-1), Chemokines (1-2) and Total Cell Count [(TCC), Cells/mm³] in CSF

(1-1)

Date	8-Sep 2005	22-Sep 2005	3-Oct 2005	7-Oct 2005	27-Oct 2005	18-Jan 2006	23-Jan 2006	31-Jan 2006	14-Feb 2006	1-Mar 2006	*P value*
IL-6 (<12.1)*	209	14.5	255.8	12.3	9.3	2417.2	1329.4	182.7	13.6	12.6	<0.01
IL-4 (<14.3)*	7	<2.5	6	5	<2.5	17.6	13.2	4.4	<2.5	<2.5	<0.01
IL-2 (<5.5)*	2.7	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	ns
IFN-γ (<60.3)*	22.6	12.5	29.1	<7.1	<7.1	134.6	463.7	58	<7.1	<7.1	<0.01
TNF-α (<7.2)*	2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	ns
IL-10 (<7.2)*	4	3.3	4.6	<2.8	<2.8	5.9	5.3	2.9	<2.8	<2.8	<0.01
TCC	42	20	36	22	9	109	150	28	21	13	

(1-2)

Date	8-Sep 2005	22-Sep 2005	3-Oct 2005	7-Oct 2005	27-Oct 2005	18-Jan 2006	23-Jan 2006	31-Jan 2006	14-Feb 2006	1-Mar 2006	*P value*
MCP-1 (<1380)*	348.5	740.8	1344.6	730.6	962.5	1403.2	1217.2	689.9	1097.5	1235.1	ns
IL-8 (<55.23)*	198.5	146.2	283.6	96	86.5	449.4	441.4	264	50.9	80.7	<0.01
MIP-1α (<10.05)*	24.4	18.5	25.2	17.5	17.5	23.7	49.6	26.7	14.5	25.9	ns
RANTES (<7.22)*	40	56	48	26.7	21.3	13.3	50.7	37.3	42.7	50.7	ns
IP-10 (<579.2)*	1880.5	632.1	2417.8	1511.8	605.8	3076.2	3176.3	2639	358.2	932.2	<0.01
TCC	42	20	36	22	9	109	150	28	21	13	

* the levels of CSF cytokines and chemokines of the control subjects (< mean + 3SD)

well with CSF cell counts. The disease activity was divided into active and inactive phases. September 8, 2005, October 3, 2005, January 18, 2006, and January 23, 2006 correspond to the active phases.

Cytokine levels (Table 1-1, Fig. 3)

The levels of IL-6 and IFN-γ in CSF were statistically correlated with total CSF cell counts ($p < 0.01$). The elevations of these cytokines were markedly higher than the lev-

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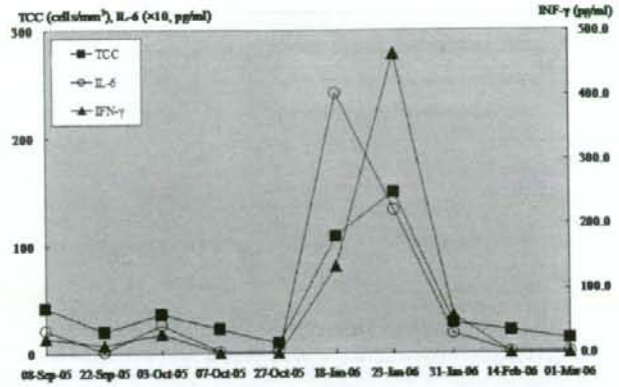

Figure 3. Levels of IL-6, IFN-γ (pg/mL) and total cell count [(TCC), cells/mm^3] in CSF.

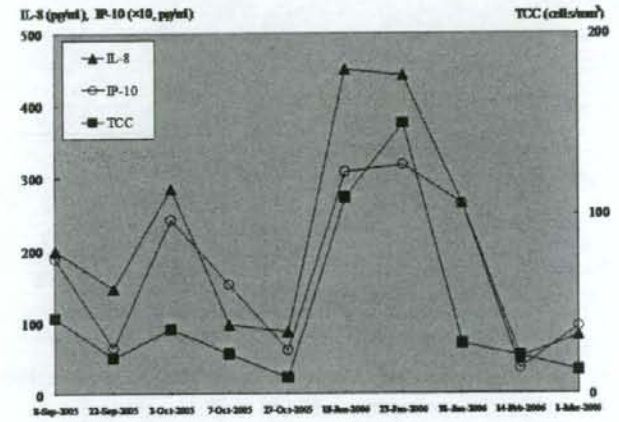

Figure 4. Levels of IL-8, IP 10 (pg/mL) and total cell count [(TCC), cells/mm^3] in CSF.

els in 21 uninfected subjects with neurological disorders. The levels of IL-4 and IL-10 in CSF were also statistically correlated with total CSF cell counts. However, the elevations of these cytokines were almost within normal ranges of control subjects. The levels of IL-2 and TNF-α in CSF were equal to or below the detection limits. The levels of CSF cytokines of the control subjects are shown in Table 1-1.

Chemokine levels (Table 1-2, Fig. 4)

The levels of IL-8 and IP-10 in CSF were statistically correlated with total CSF cell counts ($p < 0.01$). The elevations of these chemokines were markedly higher than the levels in 10 uninfected subjects with neurological disorders. The levels of other chemokines in CSF also showed various changes during the follow-up period; however, there was no significant correlation between these levels and total CSF cell counts. The levels of GM-CSF in all of the CSF samples were below the detection limits. The levels of CSF chemokines of the control subjects are shown in Table 1-2.

Correlations between level of IL-8 in CSF and neutrophilic cell counts in peripheral blood and CSF

The level of IL-8 in CSF correlated with the neutrophilic cell count in CSF (Fig. 5-1). The level of IL-8 in CSF also correlated with the peripheral neutrophilic cell count except for during the active phase (October 3, 2005) at the first time hospitalization (Fig. 5-2).

Discussion

The patient's symptoms are compatible with probable NSD consistent with the criteria advocated by Hisanaga et al and the Neuro-Sweet Disease Study Group (2). The present patient's clinical features are summarized according to the following findings: 1. She presented with recurrent encephalomeningitis with subsequent acute pharyngitis and tonsilitis. 2. She had erythematous plaques on her cheek, forearms and legs. A histological examination of the skin biopsy revealed predominant neutrophilic infiltration of the dermis, spared epidermis, and the absence of leukocytoclastic vasculitis. 3. On HLA typing, B-54 and CW1 were positive, but B-51