4. 重症薬疹に対する日本での取り組み

Changらの報告を受けて，日本人においてもHLA-B*1502がカルバマゼピンによるSJSのリスク因子となるかどうか，横浜市立大学皮膚科および静岡てんかん・神経医療センターとともに共同研究を開始した．当初は参加施設も少なく，患者数が危ぶまれたので，収集対象をカルバマゼピン投与による重症薬疹（入院加療が必要だったケースと定義）に拡大した．その理由は，SJS，TEN以外にも薬疹により入院に至るほどの重篤な症状であれば，その発症が予知でき，回避されることは有益であると判断したためである．研究が開始された2005年～07年3月までの間に重症薬疹患者22例を収集した．これらすべてに対してHLAの解析を行ったが，2例のSJS症例を含む22例すべてにおいて，HLA-B*1502はみられなかった．そのかわりHLA-A*3101がリスクアレルとして観察された（オッズ比4.33，95% CI 2.02～9.06）（未発表）．

さらに大きな患者収集システムの必要性から2006年度より，厚労省研究班「重篤な皮膚有害事象の診断・治療と遺伝子マーカーに関する研究」が国立医薬品食品衛生研究所を中心に立ち上がった．本格的な多施設共同研究による新たな体制ではSJS/TENの症例に絞るものの，原因薬剤はカルバマゼピンに限定しないという方針に改められた．今後はより多くの患者の協力を得て，予定されているHLA解析はもとよりDNAチップを使った網羅的なゲノム解析が進展することが期待される．

本研究はどの先生方にも御参加いただけるようシステムを整備している．SJS/TENの症例を経験された先生は，ぜひ国立医薬品食品衛生研究所医薬安全科学部のホームページ（http://www.nihs.go.jp/mss/MSS%20folder/JSCAR/jscar_index.html）を参照して本研究に参加していただき，将来のSJS/TEN症例を救っていただきたいと思う．

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重症薬疹をはじめとする薬の有害事象をゲノム情報で予測するための研究は端緒についたばかりである．重症薬疹の場合，原因薬剤が多岐にわたること，発症例が少ないこと，発現する薬疹の種類も大きく異なることなどから，症例や精確な診療情報の収集が困難である．これまでも症例報告など小規模なものはあるものの，より深い検討を加えるためには新規発症例を全国規模で症例を収集する必要がある．多施設共同研究としての大規模な研究が始まったばかりであるが，多くの現場臨床医の協力を得て，臨床に還元できる成果が早急にもたらされることを祈っている．

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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*5801* 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.

Acknowledgement

We deeply appreciate the Federation of Pharmaceutical Manufacturers' Association of Japan for their assistance in recruiting patients. We also thank all patients and medical doctors for their cooperation with our study.

Financial & competing interests disclosure

This study was supported in part by the Health and Labor Sciences Research Grant (Research on Advanced Medical Technology) from the Ministry of Health, Labor and Welfare. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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.

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重篤副作用のバイオマーカー探索の最新の動向

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1　はじめに

命を脅かす重篤な副作用は様々な原因によって発症するが，その中でも，いわゆる特異体質によって発症する副作用の占める割合が大きい。特異体質による副作用はタイプBと分類され，薬物の標的臓器以外の部位で薬理作用とは全く無関係な症状を呈するのが特徴であり，前臨床試験や臨床試験からの予測は難しく，市販後に初めて経験されることが多い。また，タイプBの副作用は用量非依存性であることが多いために，服用を開始してしまうとコントロールも困難である。近年，このような特異体質による副作用の発症と関連が認められる遺伝子マーカーが報告されるようになったが，紙面が限られているので，本稿では重症薬疹の遺伝子マーカーを中心に紹介することにする。

2　重症薬疹の遺伝子マーカー

薬疹は広く見られる副作用であるが，スティーブン・ジョンソン症候群（SJS），中毒性表皮壊死融解症（TEN），薬剤過敏症（DIHS）は致死率が5～30%にも及び，重篤な副作用に数えられる。近年，これらの副作用の発症にHLA（Human leukocyte antigen, MHCとも略される）の特定のタイプが強く関与していることが明らかになってきた。

抗HIV薬であるアバカビルを服用すると，白人では5～10%の頻度でDIHSが発症する。主としてオーストラリアの白人を対象としたコホート研究では，248人のコホートのうち18名がアバカビルの服用開始後6週間以内にDIHSを発症したが，DIHS発症患者の94.4%（17名）は*HLA-B*5701*を保有しており，非発症者の保有率1.7%に比較して有意に高く（$p < 0.00001$），そのオッズ比は960であった[1]。アバカビル誘因性のDIHSと*HLA-B*5701*との強い関連は，その後，19カ国の患者と医師が参加した大規模な国際的コホート研究により確認され[2]，2008年の夏，FDAは，アバカビル製剤による治療の開始にあたっては，スクリーニング試験を行い，*HLA-B*5701*保有者には原則としてアバカビル製剤の投与を禁止する警告を発した。現在，黒人のアバカビルによるDIHS発症における*HLA-B*5701*のマーカーとしての有用性については，結論が出ていない。アバカビル誘因性のDIHSと関連する遺伝子マーカーに関する日本人を対象とした研究は報告されていないが，日本人におけるアバカビルによるDIHSの発症頻度は低く[3]，*HL-B*5701*の保有率自体も非常に稀で0.1%以下である[4]。

カルバマゼピン誘因性の台湾の漢民族SJS/TEN患者を対象としたケース・コントロール研究では，SJS/TEN発症群44名の全員が*HLA-B*1502*を保有していたが，カルバマゼピン服用SJS/TEN非発症群での保有率は3%であり，オッズ比は2504（$p = 3.13E\text{-}27$）であった[5]。カルバマゼピン誘因性のSJS/TENへの*HLA-B*1502*の強い関与は，ヨーロッパ在住のアジア系住民[6]，香港在住の漢民族[7]，タイ人[8]で追認されてきた。また，香港の漢民族やタイ人では，フェニトイン等のカルバマゼピン以外の芳香環を有する抗てんかん薬で発症するSJS/TENにおいても，*HLA-B*1502*の関与が確認されている[7,8]。しかし，これまで，白人のSJS/TEN患者（8

名）を対象にした研究[6]，日本人のSJS/TEN患者18名を対象とした著者らの研究[9]，及び，同じく日本人の重症薬疹患者を対象とした柏木らの研究[10]において，*HLA-B*1502*の保有者はひとりも検出されておらず，カルバマゼピンを含む抗てんかん薬由来のSJS/TENへの*HLA-B*1502*の関与は，白人及び日本人では確認されていない。なお，漢民族においても，SJS/TEN以外のカルバマゼピン由来の薬疹と*HLA-B*1502*との関連は認められていない。*HLA-B*1502*の保有率は漢民族及び東南アジア人の間では5%以上と高いが，白人では1%前後，日本人においては0.1%以下と非常に稀であることから，遺伝子マーカーの民族依存性と関連している可能性がある。このような結果を受けて，FDAは，2007年暮れに，漢民族を祖先にもつ患者がカルバマゼピンによる治療を開始する場合には，*HLA-B*1502*によるスクリーニング試験を義務付けるよう，カルバマゼピン製剤の添付文書の改訂を承認した。

同じく漢民族おいては，アロプリノール誘因性の重症薬疹と*HLA-B*5801*との強い関連性（オッズ比580）が認められた[11]。カルバマゼピンの場合と異なり，アロプリノールでは，白人[6]や日本人[9,12]の間でも，弱いながらも*HL-B*5801*の関与は確認されている。

以上のように，重症薬疹の発症と関連する遺伝子バイオマーカーは，原因薬物，副作用のタイプ，あるいは民族によって異なるので，さらに多くの薬物及び民族を対象にした研究が必要である。

3　その他の重篤副作用の遺伝子マーカー

薬剤性肝障害の遺伝子マーカーとしては，トログリタゾン[13]及びジクロフェナク[13]では薬物動態に関連する遺伝子多型が，また，アモキシシリン[13]やチクロピジン[14]ではHLAのクラスI及びIIの特定のタイプが報告されているが，いずれもオッズ比は10以下である。一方，スタチンによる筋障害の発症には，スタチンの肝取込に関与するトランスポーターOATP1B1をコードする*SLCO1B1*の遺伝子タイプが関与すると報告されている。すなわち，日本人では，ハプロタイプ*SLCO1B1*15*を1個以上有するとそのオッズ比は11.3と高くなり[15]，また，英国の研究では，ハプロタイプ*SLCO1B1*15*における機能低下責任SNP（single nucleotide polymorphism）とされるアミノ酸変異を伴う*521T＞C*と強い連鎖を示すイントロンSNP1個につきオッズ比は4.5ずつ高くなるという[16]。臨床応用もされ始めた重症薬疹における遺伝子マーカーに比較し，その他の重篤副作用においては，まだそれほど強い関連を示すマーカーは見つかっていないのが現状と言えよう。

4　重篤副作用のバイオマーカー探索研究のネットワーク

重篤副作用は発生頻度が非常に低く，一方，原因となる医薬品は多岐にわたるために，一製薬企業や一医療機関がバイオマーカー探索研究を行うことは困難である。このため，近年では，バイオマーカー探索研究のために，規制当局，アカデミア，製薬企業などが協力して，国家的あるいは国際的なバイオバンクやネットワークを立ち上げている。日本においては，文部科学省が主導するバイオバンクジャパンのように，がんや糖尿病，心臓疾患などの原疾患を対象としたバイオバンクが活動しているが，副作用を対象としたものはなかった。

著者は，皮膚科，精神科，眼科の臨床医及び薬理ゲノム学の専門家と共に，2006年度よりSJS/TENを対象としたバイオマーカーの探索研究のための研究班を立ち上げ，その基盤整備として，症例集積システムを構築した。本システムは製薬企業による副作用報告制度を利用したもので，厚労省医薬食品局安全対策課及び日本製薬団体連合会の協力を得て，国立医薬品食品衛生研究所で運営されている。この症例集積システムは，SJS/TENに限らず，日本における重篤な副作用の症例集積ネットワークとして発展させることが可能であると考えている。本症例集積システムを基にして，産官学が共同して，薬剤性肝障害などを初めとする重篤な副作用の日本人患者と関連するバイオマーカーの探索研究が発展し，重篤な副作用の回避へ貢献することを希望して止まない。

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Topics
トピックス

重症薬疹の発症に関連する遺伝子マーカーの探索研究

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はじめに

薬疹は頻繁にみられる副作用であるが，そのなかでスティーブン・ジョンソン症候群（SJS），中毒性表皮壊死融解症（TEN）および薬剤過敏症（DIHS）は，発生頻度こそ低いが致死率が5～30%にも及ぶ重篤な副作用である。いずれも主症状は皮膚障害であるが，高熱と肝臓などの内臓障害を伴う。SJS/TENでは目，口腔内などの粘膜の障害も著しいが（両者の区別は主として表皮剥離面積の大きさで行う），DIHSでは粘膜障害の関与は低くヘルペスウイルスの関与がある。原因薬物は，DIHSではアロプリノール，メキシレチン，抗てんかん薬など極めて限られているが，SJS/TENでは100以上に及ぶ。いずれの副作用もT細胞が関与する遅延型のアレルギーであり，通常，薬物の服用から発症までに数日から数週間を要する。現在は，薬物を投与する前に重症薬疹の発症を予測することは困難であるため，バイオマーカーを用いて高リスク患者をあらかじめ識別できるようになることが望ましい。本稿では，重症薬疹の発症と関連する遺伝子マーカーに関する最新の研究，規制当局の対応，およびわが国における取り組みについて紹介することにする。

遺伝子マーカーの薬物・副作用・民族特異性

近年，重症薬疹の発症には，HLA（human leukocyte antigen）の特定のタイプが強く関与していることが明らかになってきた。しかし表1に示すように，重症薬疹と遺伝子マーカーとの関係は，原因となる薬物，重症薬疹のタイプや民族・人種に依存しており，かなり複雑であることもわかってきた。以下に，少し詳しく説明しよう。

1．カルバマゼピン誘因性のSJS/TENの遺伝子マーカー

台湾の漢民族のカルバマゼピン誘因性SJS/TEN患者を対象にしたケース・コントロール研究では，44名の患者全員が*HLA-B*1502*を保有していたのに対し，カルバマゼピンを服用してもSJS/TENを発症しなかったコントロール群での保有率は3%であった（p=3.13E-27）[1]。この研究はその後も続けられ，表1に示したように，症例数が60例となってもその陽性率は依然高い値を保持している[2]。カルバマゼピン誘因性のSJS/TENの発症への*HLA-B*1502*の関与は，その後，欧州在住のアジア系住民[3]，香港在住漢民族[4]，タイ人[5]などで確認されてきた（表1）。しかし，アジア系民族ではカルバマゼピン誘発性SJS/TENと強い関連がみられた*HLA-B*1502*であるが，こ

表1　重症薬疹発症と関連する遺伝子マーカーの薬物・民族依存性

薬物名	遺伝子マーカー HLA	民族	疾病	症例陽性率	オッズ比	文献
カルバマゼピン	*B**1502*	漢民族（台湾）	SJS/TEN	59/60	1,357	2
		漢民族（台湾）	DIHS	0/13		2
		アジア系欧州在住者	SJS/TEN	4/4		3
		タイ人	SJS	6/6	26	5
		白人	SJS/TEN	0/8		3
		日本人	SJS/TEN	0/7		12
		漢民族（香港）	SJS/TEN	4/4		4
芳香族抗てんかん薬		漢民族（香港）	SJS/TEN	2/2	72	4
フェニトイン		タイ人	SJS	4/4	19	5
芳香族抗てんかん薬		日本人	SJS/TEN	0/11		12
アロプリノール	*B**5801*	漢民族（台湾）	SJS/TEN/DIHS	51/51	580	6
		白人	SJS/TEN	19/31	80	3
		日本人	SJS/TEN/DIHS	3/3		7
		日本人	SJS/TEN	4/10	40	12
アバカビル	*B**5701*	白人	DIHS	57/130	19	10
		黒人	DIHS	10/69	17	10
		白人(PT positive)*	DIHS	42/42	1,945	10
		黒人(PT positive)*	DIHS	5/5	900	10

＊：アバカビルに対するパッチテストが陽性の患者

れまでのところ白人のSJS/TEN患者においては検出されておらず[3]，また，漢民族においてもカルバマゼピン誘発性のDIHS患者では検出されていない（表1）[2]。

この遺伝子マーカーの民族依存性は，*HLA-B*1502*の保有率が漢民族および東南アジア人の間では高く（5％以上），白人では低いこと（1％前後）が一因と考えられる。なお，表1に示すように，漢民族[4]およびタイ人[5]では，カルバマゼピン以外の芳香族抗てんかん薬が原因のSJS/TENにおいても，*HLA-B*1502*との関連が認められた。

2．アロプリノール誘因性の重症薬疹

アロプリノール由来の重症薬疹の発症と関連する遺伝子マーカーとしては，*HLA-B*5801*が検出されている。台湾の研究チームの発表では，漢民族のアロプリノール誘因性のSJS/TEN/DIHS患者51人全員がこの遺伝子マーカーを保有していたのに対し，コントロール群の*HLA-B*5801*保有率は15％にすぎなかった（表1）[6]。

興味深いことには，アロプリノールを原因とする重症薬疹と*HLA-B*5801*との関連は，漢民族ほどには強くはないが白人においても確認されており[3]，また，日本人においても3人のSJS，TENあるいはDIHSの患者全員から*HLA-B*5801*が検出されたという報告もある（表1）[7]。ただし，後で述べるように，日本人の場合には，必ずしも重症薬疹の患者のすべてが*HLA-B*5801*を保有しているわけではない。

3．アバカビル誘因性のDIHS

抗HIV薬であるアバカビルは，白人では服用開始後6週間以内に5～10％の頻度でDIHSを発症するが，アバカビル誘因性DIHS発症と関連するマーカーとしては*HLA-B*5701*が報告されている。

主としてオーストラリアの白人248人を対象と

したコホート研究の結果では，アバカビル服用後にDIHSを発症した患者18名のうち17名が*HLA-B*5701*を保有していた。発症者における保有率（94.4%）は，非発症者の保有率1.7%に比較して有意に高く（$p<0.00001$），オッズ比は960であった[8]。アバカビルによるDIHS発症率は黒人においては白人よりも低く，また，*HLA-B*5701*が黒人においてもマーカーとなりうるか否かについては疑問視されていた[9),10)]。しかし，表1に示したように，アバカビルに対するパッチテスト陽性患者に絞ると，白人と同様に*HLA-B*5701*の保有率は100%となり，黒人においても*HLA-B*5701*はアバカビルによるDIHS発症のマーカーとして有用であることが示唆された[10)]。

規制当局の対応

遺伝子マーカーを利用したテーラーメイド医療が提唱されて久しいが，臨床応用された例は意外に少ない。バイオマーカーの予測性能は，副作用発症者中のバイオマーカー陽性者の割合を表す感度（sensitivity）や，副作用非発症者中のバイオマーカー陰性者の割合を表す特異度（specificity）で表される。台湾の漢民族におけるカルバマゼピン由来のSJS/TEN発症を予測するうえで，*HLA-B*1502*は感度と特異度はいずれも1に近く，非常に優れた遺伝子マーカーである。FDAは，2007年暮れにカルバマゼピン製剤の添付文書の改訂を承認し，漢民族を祖先にもつ患者がカルバマゼピンによる治療を開始する場合には，*HLA-B*1502*によるスクリーニング試験を義務づけ，保有者にはカルバマゼピンによる治療を開始してはならないとさせた。

一方，19カ国の患者と医師が参加した大規模なコホート研究により，*HLA-B*5701*に基づくスクリーニング試験を実施すると，アバカビルによるDIHS発症を半分以下に下げられることが判明し[11)]，2008年に入り，米国および欧州におけるアバカビル製剤の添付文書は治療の開始時のスクリーニング試験を義務づけ，*HLA-B*5701*保有者には原則としてアバカビル製剤の投与をしてはならないという警告を記載するようになった。

日本人の*HLA-B*5701*の頻度は非常に低く，またアバカビルによるDIHS発症頻度自体も欧米に比較して低いことから，日本におけるアバカビル製剤の添付文書においては，海外におけるアバカビル由来のDIHSと*HLA-B*5701*との関連性に関する情報提供を行うにとどめている。

わが国における重篤副作用のバイオマーカー探索研究

このように，重症薬疹の発症と関連する遺伝子バイオマーカーは，原因薬物，副作用のタイプ，あるいは民族によって異なることが示されてきた。このことは，重症薬疹を発症しやすい高リスクな日本人患者を識別できる遺伝子マーカーを見出すためには，日本人を対象にした研究が必要であることを意味している。筆者は2006年に，皮膚科，眼科，精神科の臨床医，薬理ゲノム学の専門家らとともに，SJS/TENの発症と関連する遺伝子マーカーを探索する研究班を立ち上げた。

○研究班の症例集積システム

SJS/TENの年間発症数は500例足らずである。研究班においては，まず全国で散発する症例を把握し集積する必要があった。そこで，厚生労働省（厚労省）医薬食品局安全対策課および日本製薬団体連合会の協力を得て，日本全国をカバーする症例集積システムを構築した（図1）。製薬企業および医療従事者は，重篤な副作用が発生した場合には，速やかに厚労省に報告する義務を負っているが，

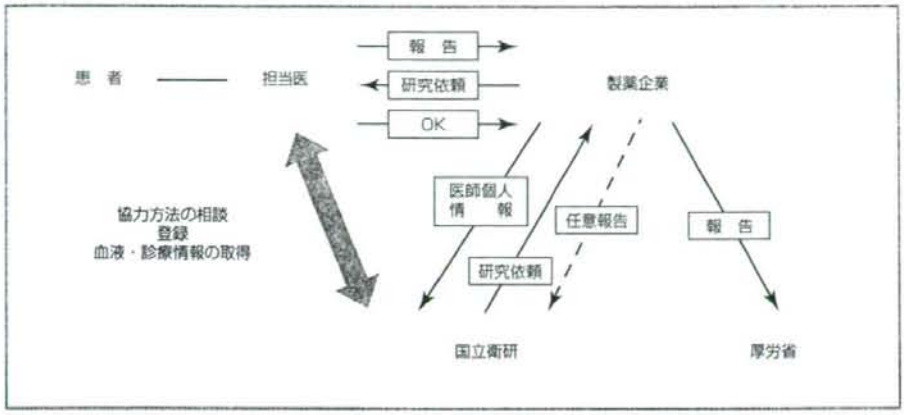

図1 SJS/TEN発症と関連する遺伝子マーカーの探索研究のための症例集積システム
〔協力：厚生労働省医薬食品局安全対策課，日本製薬団体連合会〕

本システムはこの制度を利用したものである。

すなわち，製薬企業が厚労省に重篤副作用を報告する際に，副作用がSJS/TENの場合には，国立医薬品食品衛生研究所（国立衛研）に任意に副作用発生の事実を報告してもらい，国立衛研からその製薬企業を通して主治医へ研究班への協力を依頼し，主治医が協力に応諾した場合には主治医の個人情報が国立衛研に連絡され，国立衛研と主治医とが直接に協力について協議を行うのである。

研究班では，研究班の臨床医が所属する医療機関および本システムを通じて，これまでに約100例の症例を集積した。読者のなかには病院の薬剤部にお勤めの方も多いと思われるが，SJS/TENの発症を把握された場合には，主治医の先生とご相談のうえで，被疑薬と考えられるメーカーのMRを通じて研究にご協力いただければ幸いである。本研究に関するウェブサイトは次のとおりである。

http://www.nihs.go.jp/mss/MSS%20folder/JSCAR/jscar_index.html

研究班のこれまでの成果を表1に記載した[12]。本研究においても，アロプリノール服用SJS/TEN患者では，台湾の漢民族ほどの強い関連は認められないが，一部の患者では*HLA-B*5801*が関与していることが確認された。一方，カルバマゼピンを含む芳香族系抗てんかん薬誘因性のSJS/TENについては，日本人では*HLA-B*1502*の関与は確認されていない。この結果を受けて，わが国でもカルバマゼピンの添付文書が改訂されたが，そのなかでは，海外で行われた研究の紹介にとどめ，日本人患者に対する*HLA-B*1502*によるスクリーニング試験は勧めていない。

今後も可能なかぎり症例を集積し，SJS/TENの発症と関わる日本人に有用な遺伝子マーカーの探索に努め，テーラーメイド医療に貢献したいと考えている。

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Epitope of Autoantibodies to *N*-methyl-D-aspartate Receptor Heteromers in Paraneoplastic Limbic Encephalitis

Yukitoshi Takahashi, MD, PhD[1]

Dalmau and colleagues[1] have presented interesting data about paraneoplastic anti–*N*-methyl-D-aspartate receptor (NMDAR) encephalitis associated with ovarian teratoma. They found antibodies to NR2B- and NR2A-containing heteromers of the NMDAR by ingenious technique using functional heteromeric NMDAR and its antagonists. All 12 patients' cerebrospinal fluid and serum reacted with heteromeric NMDAR composed of NMDAR1 (NR1) and NMDAR2B (NR2B), and sera and CSF from eight patients also recognized heteromeric NMDAR composed of NR1 and NMDAR2A (NR2A). Patients' sera did not react with cells transfected with individual subunits (NR1, NR2A, or NR2B), and they could not confirm the definitive epitope of antibodies to NR2B- and NR2A-containing heteromers of the NMDARs.

NMDARs are heterotetrameric cation channels composed of NR1 and NR2/3 subunits.[2] NMDARs are assembled early in the endoplasmic reticulum, and both NR1 and NR2 subunits are necessary for their association and their successful cell-surface targeting.[2] NR2B of rat is homologous to GluRε2 in mice. We established stable NIH3T3 transformant cell lines expressing full-length glutamate receptor ε2 (GluRε2) (NR2B) for screening of autoantibodies to GluRε2.[3] Our cell lines (transfected only with GluRε2) did not react with rabbit antibodies to GluRε2 in cell-sorter analyses. These data confirm that individual subunit of NMDARs cannot be targeted on cell surface, and that Dalmau's methods cannot determine which subunit of heterotetrameric NMDAR is autoantigen of antibodies in patients with anti-NMDAR encephalitis.

We have examined autoantibodies to full-length GluRε2 molecules by immunoblot, and N-terminal epitope of the antibodies by immunoblot using bacterial fusion proteins containing peptides from the GluRε2 (amino acid residues 1–48) (NT1).[3] We reported that autoantibodies to GluRε2 in patients with nonparaneoplastic acute limbic encephalitis

appeared usually in the early acute stages, and the autoantibodies had epitope to NT1.[4,5] Recently, we examined autoantibodies to GluRε2 with full-length GluRε2 molecules in five Japanese patients who had been proved in Dalmau's laboratory to have antibodies to NR2B- and NR2A-containing heteromers of the NMDARs. Three patients had ovarian teratoma, one had no findings of teratoma in ovary, and one had no examination of ovary. All five patients had autoantibodies to GluRε2. These data suggest that some part of autoantibodies to NMDARs in acute limbic encephalitis with and without teratoma have epitope to N terminal of GluRε2 (NR2B), and some part of antibodies detected by Dalmau's method recognize N terminal of GluRε2 (NR2B) of the heterotetrameric NMDARs.

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DOI: 10.1002/ana.21362

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	Journal Name	Manuscript No.		Author Received:	No. of pages: 13	PE: Saravanan

Epilepsia, **(*):1–13, 2008
doi: 10.1111/j.1528-1167.2008.01977.x

FULL-LENGTH ORIGINAL RESEARCH

A substantial number of Rasmussen syndrome patients have increased IgG, $CD4^+$ T cells, TNFα, and Granzyme B in CSF

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SUMMARY

Purpose: We studied the immunologic molecules in cerebrospinal fluid (CSF) and discussed their evolutional changes in pediatric patients with Rasmussen syndrome (RS).

Methods: CSF samples collected from 27 patients with RS (average onset age, 7.5 ± 5.6 years) were studied. Cell count, protein, glucose, albumin, chloride, and immunoglobulin G (IgG) levels were measured by conventional methods. Surface markers of lymphocytes in CSF were examined by a cell sorter. Granzyme B, interferon γ (IFNγ), interleukin 4 (IL-4), tumor necrosis factor α (TNFα), and IL-12 in CSF were quantitated by enzyme-linked immunosorbent assay (ELISA). Autoantibodies against GluR ε2 (NR2B) were examined by immunoblot.

Results: The data of the first CSF examination showed that IgG levels (Mann-Whitney *U* test, $p < 0.01$), $CD4^+$ T cells ($p = 0.02$), TNFα levels ($p < 0.01$), and Granzyme B levels ($p < 0.01$) were elevated compared with disease controls. White blood cell count, IFNγ level, IL-12 level, and Granzyme B level were elevated, especially in the early stage of disease. $CD4^+$ T cells, $CD8^+$ cells, $CD3^+$ T cells, IgG levels, and TNFα levels were elevated at all stages of disease evolution. Protein levels and albumin levels were elevated in the progressed stage. Autoantibodies against GluR ε2 (NR2B) (IgG) were found in 50% of patients in the early stage, and the positive rate was low at the progressed stage.

Discussion: The present findings suggest that complex pathophysiologic mechanisms involving $CD4^+$ T cells and $CD8^+$ T cells change evolutionally during the progression of RS. A crucial cytotoxic process occurs in the early stage, and declines in the progressed stage.

KEY WORDS: Rasmussen syndrome, Granzyme B, Interferon γ, Tumor necrosis factor α, GluR ε2 (NR2B).

Rasmussen syndrome (RS) is considered to be an autoimmune disease, and is usually diagnosed by comprehensive consideration of characteristic clinical symptoms, electroencephalography (EEG), magnetic resonance imaging (MRI), and histopathologic findings (Rasmussen et al., 1958, 1978; Anderman & Rasmussen, 1991; Oguni et al., 1991; Bien et al., 2005; Takahashi, 2006). Although a correct early diagnosis is essential to the achievement of a good outcome, patients with RS are usually diagnosed with localization-related epilepsy at about onset, because they show few characteristic features of RS (including epilepsia partialis continua, hemiparesis, and focal slowing of EEG) at the onset stage.

Lymphocytic infiltration containing predominantly T cells and sparse B cells is found in surgically resected tissues from patients with RS (Farrell et al., 1995), and local central nervous system (CNS) immune responses in RS include local clonal expansion of T cells responding to discrete antigen epitopes (Li et al., 1997). Apoptosis of astrocytes by Granzyme B produced by cytotoxic T cells (CTLs) has been demonstrated in resected tissues from patients (Bien et al., 2002; Bauer et al., 2007). Peripheral blood lymphocytes in patients are sensitized to glutamate

Accepted October 23, 2008; Early View publication xxxx xx, 200x
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receptor (GluR) ε2 = *N*-methyl-D-aspartate (NMDA) type GluR 2B (NR2B) (Takahashi et al., 2005). Heterogeneous autoantibodies against neuronal molecules (including GluR3, GluR ε2 (NR2B), neuronal acetylcholine receptor alpha7, and munc-18) and glial cells are detected in RS (Rogers et al., 1994; Takahashi et al., 2003, 2005; Watson et al., 2005; Yang et al., 2000; Roubertie et al., 2005). Autoantibodies against GluR ε2 (NR2B) have epitopes predominantly in intracellular domains, and show epitope spreading evolutionally (Takahashi et al., 2003). In animal models of RS, anti-GluR3B T cells are found (Levite & Hermelin, 1999). From these data, we suggested that cellular autoimmunity mediated by CTLs plays a primary role in the development of RS, and that subsequent humoral autoimmunity mediated by autoantibodies also contributes to the immunopathogenesis (Takahashi et al., 2003).

Autoantibodies against GluR3 were found at first in RS (Rogers et al., 1994). Recent studies have revealed non-RS epileptic patients with the autoantibodies and RS patients without the autoantibodies against GluR3 (Wiendl et al., 2001; Watson et al., 2004; Ganor et al., ~~2005a,~~ 2005b). Autoantibodies against GluR ε2 (NR2B) are also detected in patients with RS and other epilepsies, and are rarely negative in patients with RS (Takahashi et al., 2003, 2006). These data suggest that RS is heterogeneous not only in clinical characteristics, but also in immunopathogenesis. Autoantibodies against GluR3 have been shown to induce currents through GluR (Rogers et al., 1994; Twyman et al., 1995) and promote death of cortical neurons by complement-dependent (He et al., 1998) and complement-independent mechanisms (Levite & Hermelin, 1999; Ganor et al., 2004a, ~~2004b~~) as well as neuronal excitotoxicity. Animals immunized with the self AMPA GluR3B peptide, to obtain a possible animal model for RS developed excitotoxic anti-GluR3B antibodies, anti-GluR3B T-cells, and brain damage (Levite & Hermelin, 1999; Ganor et al., 2005a, ~~2005b~~). Production of autoantibodies against GluR3 may depend on cleavage of GluR3B-containing fragments from the TCR-activated T cells by Granzyme B (Gahring et al., 2001; Ganor et al., 2007).

Although reports about the pathogenic role of cytokines in RS are few (Tekgul et al., 2005; Ganor et al., ~~2005a,~~ 2005b), cytokines are highlighted as inflammatory mediators that alter neuronal excitability and affect cell survival (Vezzani et al., 2008). We studied surface markers of T cells, cytokines, and Granzyme B in CSF samples from 27 patients with RS, and evaluated their evolutional changes to reveal their roles in immunopathogenesis, and examine their possible contribution to the diagnosis.

Patients and Methods

Patients

We studied CSF samples from 27 patients (male, 12; female, 15), who were diagnosed with RS based on clinical characteristics at the National Epilepsy Center from 2002 to 2006. Clinical criteria include (1) intractable partial seizures, and (2) interictal symptoms and EEG suggesting progressive involvement of unilateral hemisphere at the early stage. In nine patients, histologic findings at surgical intervention also confirmed the diagnosis. Histologic criteria include microglia nodules and perivascular cuffing. In Japan, it is difficult to obtain a patient's consent to undergo brain biopsy but, for cultural and traditional reasons, it is easy to obtain consent for CSF examination. Of the 27 patients, 16 attended the National Epilepsy Center. They fulfilled the clinical criteria for RS and were included in this study after giving written informed consent (Table 1). The diagnosis was proven histologically in 5 of 16 patients. The remaining 11 patients attended other hospitals and were suspected of having RS. Their clinical data were sent to the National Epilepsy Center for evaluation, which confirmed a clinical diagnosis of RS. The diagnosis was proven by histologic findings in 4 of 11 patients. CSF samples were collected after obtaining written informed consent. CSF samples collected at other hospitals were sent to the National Epilepsy Center for analysis of autoantibodies against GluR ε2 (NR2B).

Clinical characteristics, treatment at sampling points, and surgical outcome are shown in Table 1. Sixteen patients had epilepsia partialis continua (EPC). The average onset age was 7.5 ± 5.6 years. The sampling time ranged from 1–288 months after onset. Epileptic patients without infectious etiology or progressive clinical course served as disease controls (n = 16). In disease controls, the average age at examination was 5.8 ± 4.9 years, and the average duration of epilepsy at examination was 4.5 ± 4.0 years. They had seizures at different frequencies ranging from daily to yearly.

Methods

All data analyzed except for those of patients 5, 14, and 25 were obtained before surgical interventions. Cell count, protein level, glucose level, albumin level, chloride level, and IgG in CSF were determined by conventional methods. Cell counts were examined in 29 samples from 18 patients, protein levels in 30 samples from 19 patients, glucose levels in 25 samples from 15 patients, chloride level in 22 samples from 12 patients, albumin level in 18 samples from 11 patients, and IgG levels in 20 samples from 13 patients. Surface markers of lymphocytes in CSF were examined in 17 samples from 10 patients using a cell sorter. Granzyme B (32 samples from 23 patients) were examined by Granzyme B enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. KT-078, KAMIYA BIOMEDICAL COMPANY, Seattle, WA, USA), using monoclonal antibody to human Granzyme B and Streptavidin-HRP. Interferon γ (IFNγ) (30 samples from 22 patients), interleukin 4 (IL-4) (29 samples from 22 patients), tumor necrosis factor α (TNFα) (28 samples

Epilepsia, **(*):1–13, 2008
doi: 10.1111/j.1528-1167.2008.01977.x

Table 1. Clinical characteristics and treatment at sampling points and surgical outcome

Pt	Sex	Age at onset (year)	Age at diagnosis (year)	Sampling stage (months)	Seizure	Motor dysfunction	Mental retardation	Immunologic treatment	Age at surgical intervention and method/surgical outcome	Histologic findings	Elevated molecules in CSF	GluR ε2 (NR2B) in CSF (months)
1	F	0.2	0.9	31	D	l-HP	++	–	2Y10M: r-FH/ seizure free	MGN+VBS+EP +SD+PVC	CD4, CD8, TNFα, GrB	8+, 17–, 31–
2	F	1.5	23.0	260	D	r-HP	–	–	–		CD4, IFN γ, TNFα, GrB	260–
3	F	2.4	2.5	288	Y	QP	+++	–	–			288–
4	M	3.6	3.9	4	D	l-HP	–	–	4.8Y: r-FH/ seizure free, MR+	MGN+VBS+EP +SD+PVC	GrB	4+
5	M	3.7	9.0	96	D	–	–	–	8Y: left temporal resection / daily EPC	FCD+MGN+VBS +EP+SD+PVC		96+
6	M	3.9	10.4	36	D	r-HP	++	–			IFNγ, TNF α, GrB	36–
				65	W	QP	+++	–			TNFα, GrB	65+
				68	D	QP	+++	-	-		CD4, CD8, TNFα, GrB	68–
7	M	4.0	5.3	18	D	–	–	–	5Y9M: r-FH/ seizure free	MGN+VBS+EP +SD+PVC	TNFα, GrB	18+
8	M	4.1	10.0	172	D	ru-MP	+	–				172+
				184	D	ru-MP	+	Tacrolimus			CD4, CD8, TNFα,	
				186	D	ru-MP	+	Tacrolimus			CD4, CD8, TNFα,	186+
				201	D	ru-MP	+	Tacrolimus			CD4, CD8, IFNγ, TNF α, GrB	201–
				204	D	ru-MP	+	Tacrolimus	–		CD4, CD8, IFNγ, TNF α, GrB	204–
9	F	4.7	6.0	45	D	rl-MP	–	–			CD8, TNF α,	45–
				51	D	rl-MP	–	–	–		CD4, CD8,	
10	F	5.3	6.3	12	D	ll-MP	–	–			GrB	
				15	D	l-HP	–	Pulse	6Y10M: r-FH/ weekly SPS	MGN+PVC		15–
11	F	5.6	22.0	192	D	l-HP	+	–	–		CD4, CD8, TNFα, GrB	192–
12	F	5.8	18.0	31	D	r-HP	++	–			TNFα,	31+
13	F	5.9	6.0	2	D	r-HP	+	–	–		IFNγ, TNF α, GrB	2+
14	M	5.9	16.0	132	W	–	–, r-EFD	–	15Y:left frontal resection/daily SPS	FCD+PVC+gliosis, phagocyte infiltration	TNFα,	132–
15	F	6.0	10.1	36	D	QP	++	–	–		GrB	36+
16	M	6.1	7.1	12	D	–	–	–	15Y:right frontal disconnection/ seizure free	not examined	IFNγ, TNF α, GrB	12+
17	F	6.5	12.7	168	D	r-HP	+	–	–			
18	F	6.6	6.9	4	D	l-HP	–, l-EFD	–	–		IFNγ, TNF α, GrB	4+, 5–
19	F	7.1	9.2	30	W	l-HP	+	Pulse	9Y8M: r-FH/ seizure free	MGN+VBS+EP +SD+PVC		31–

Continued

Epilepsia, **(*):1–13, 2008
doi: 10.1111/j.1528-1167.2008.01977.x