by inflammatory cells was more common in EMM than in SJS/TEN. Thus, the intensity of the cellular infiltrate in the dermis appears to be a key histological feature, in addition to the presence/absence of epidermal necrosis. Moreover, these observations may explain the recent discovery that the widespread death of keratinocytes in SJS/TEN patients is caused by soluble mediators, such as granulysin, despite there being few infiltrating cells in the dermis [27] .

In conclusion, this study revealed statistically significant differences between EMM and SJS/TEN in terms of clinical features, such as fever, mucous membrane involvement, and lymph node swelling; the results of laboratory investigations, including measures of hepatic dysfunction, CRP levels, and the appearance of atypical lymphocytes; and histopathological findings, specifically lymphocyte infiltration in the skin. Although there are a few exceptions, such as the existence of unclassified EMM, these results suggest that the pathogenesis of EMM differs from that of SJS/TEN, and that these two disorders may be distinguishable. In this study, although the number of patients with SJS/TEN was limited, these findings may help in administering suitable treatments to EMM and SJS/TEN patients earlier. ■

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INVITED ARTICLE

Pharmacogenetics of cutaneous adverse drug reactions

Michiko AIHARA

Department of Dermatology, Yokohama City University School of Medicine, Yokohama, Japan

ABSTRACT

Drug-induced hypersensitivity reactions are of major medical concern because they are associated with high morbidity and high mortality. In addition, individual patients' reactions are impossible to predict in each patient. In the field of severe cutaneous adverse drug reactions (cutaneous ADR) such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-induced hypersensitivity syndrome (DHIS) or drug rash with eosinophilia and systemic symptoms (DRESS), major advances have recently been gained through studies of an association between HLA alleles and drug hypersensitivity induced by specific drugs. The results of these pharmacogenomic studies allow prediction of the risk of adverse reactions in patients treated with certain drugs, including carbamazepine and other aromatic antiepileptic drugs, allopurinol and abacavir. However, different ethnic populations show variations in the genetic associations. A strong association between carbamazepine-induced SJS/TEN and HLA-B*1502 has been found in Southeast Asian patients but not in Caucasian and Japanese patients. Moderate associations between aromatic amine anticonvulsants and other HLA alleles have been proposed in Japanese patients. In contrast, HLA-B*5801 was found to be associated with allopurinol-induced cutaneous ADR, including SJS/TEN and DIHS/DRESS, in Caucasian and Asian patients, including the Japanese. These differences may, at least in part, be due to the differences in allele frequency in different ethnic populations. This article reviews the progress in pharmacogenomics, associated mainly with carbamazepine and allopurinol in different ethnic populations. Pharmacogenetic screening based on associations between adverse reactions and specific HLA alleles helps to avoid serious conditions associated with drug hypersensitivity.

Key words: adverse drug reaction, drug-induced hypersensitivity syndrome, pharmacogenetics, Stevens–Johnson syndrome, toxic epidermal necrolysis.

INTRODUCTION

Drug hypersensitivities develop in susceptible patients as adverse drug reactions (ADR) following exposure to certain drugs. Many of the ADR are thought to be immunologically mediated and are of major concern to clinicians, because severe hypersensitivity is life-threatening and cannot be predicted.

Toxic epidermal necrolysis (TEN) and Stevens–Johnson syndrome (SJS) are major cutaneous ADR characterized by destruction of the epidermis and mucosal epithelium, often with organ involvement. They are considered variants of the same disorder differentiated by the presence of skin separation and extent of the body surface area involved.[1,2] Although they are rare disorders, the mortality is as high as 1–5% for SJS and 20–30% for TEN.[3,4] Common drugs that cause SJS/TEN include allopurinol, anticonvulsants, antimicrobials, non-steroidal anti-inflammatory agents and aromatic sulphonamide, although many other drugs can be implicated in SJS/TEN.

Correspondence: Michiko Aihara, M.D., Department of Dermatology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan. Email: maihara1@med.yokohama-cu.ac.jp
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Drug-induced hypersensitivity syndrome (DIHS) or drug rash with eosinophilia and systemic symptoms (DRESS) are other severe cutaneous ADR. In patients with DIHS/DRESS, skin rash usually occurs more than 2 weeks after the initial administration of the drug, associated with fever, hepatitis and/or other internal organ involvement, lymphadenopathy and hematological abnormalities (leukocytosis, hypereosinophilia and atypical lymphocytosis). Reactivation of human herpesvirus (HHV), mainly HHV-6, and less frequently cytomegalovirus, has been described during the course of DIHS/DRESS.[5–8] These viral reactivations have been reported in association with recurrence of symptoms more than 2 weeks after the drug was discontinued.[7,8] Common drugs associated with DIHS/DRESS include aromatic amine anticonvulsants (carbamazepine, phenytoin and phenobarbital), allopurinol, minocycline, sulfa antimicrobials and aromatic sulfonamides.

Not only immunological but also genetic factors have recently been suggested to contribute to the pathogenesis of cutaneous ADR. This notion is supported by studies associating human leukocyte antigen (HLA) class I alleles with SJS/TEN induced by anticonvulsants. In addition to playing a role as a genetic marker for cutaneous ADR, the particular HLA molecule is also functionally involved in the pathogenesis of cutaneous ADR. The drug antigen (e.g. drug–peptide complex) is presented by the specific HLA molecule on the antigen-presenting cells and recognized by effector T cells through the T-cell receptor for HLA-restricted T-cell activation. HLA class I restricted $CD8^+$ T cells and HLA class II restricted $CD4^+$ T cells are thought to induce an immune response, including cutaneous ADR. In SJS/TEN, $CD8^+$ cytotoxic T cells in the skin lesions may play an important role in eliciting keratinocyte death.[9]

In 2004, a very strong association of HLA-B*1502 with carbamazepine-induced SJS/TEN was reported in southeast Asian patients[10–12] and patients of Asian ancestry living in Europe.[13] This was an epoch-making finding in the field of pharmacogenetics of cutaneous ADR. However, the association has not shown across different populations or ethnicities. Moderate associations between aromatic amine anticonvulsants and other HLA alleles have been proposed in different ethnic populations. In contrast, some studies showed an association between the HLA class I allele and allopurinol-induced ADR, including TEN/SJS and DIHS/DRESS, across different populations.[14,15]

This review is focused on the recent pharmacogenetic studies of cutaneous ADR, including TEN/SJS and DIHS/DRESS, mainly induced by carbamazepine and allopurinol, and hypersensitivity induced by antiretroviral drugs, and discusses future perspectives of pharmacogenomics in cutaneous ADR.

ALLELE ASSOCIATIONS WITH CUTANEOUS ADR INDUCED BY AROMATIC AMINE ANTICONVULSANTS

Recently, many studies on allele associations with cutaneous ADR induced by aromatic amine anticonvulsants have been reported in Asian and European populations. Current studies indicate that HLA-B*1502 is a marker for carbamazepine-induced SJS/TEN in southeast Asian populations, where the prevalence of HLA-B*1502 is relatively high.

ASSOCIATION BETWEEN HLA-B*1502 AND CARBAMAZEPINE-INDUCED SJS/TEN IN SOUTHEAST ASIAN AND EUROPEAN PATIENTS

In 2004, Chang *et al.*[10] reported a strong association between HLA-B*1502 and carbamazepine-induced SJS/TEN in Han-Chinese residing in Taiwan (Table 1). In this case–control study, 100% of 44 Han-Chinese SJS/TEN patients were HLA-B*1502 positive versus 3% of 101 tolerant patients and 8.6% in the general population ($P = 3.1 \times 10^{-27}$; odds ratio [OR] = 2505).[10] A follow-up study by Hung *et al.*[16] confirmed this association not only in Han-Chinese residing in Taiwan but also in those residing in Hong Kong and China and in Chinese descendents residing in the USA (98.3% of 60 patients, $P = 1.6 \times 10^{-41}$; OR = 1357). Further studies have confirmed the association between HLA-B*1502 and carbamazepine-induced SJS/TEN in Chinese, Thai and Indian populations.[11,12,17] Tassaneeyakul *et al.*[18] have performed a case–control study using 42 carbamazepine-induced SJS/TEN patients and 42 carbamazepine-tolerant controls in a Thai population. In their study, 37 SJS/TEN patients carried HLA-B*1502, thus suggesting a very strong association of HLA-B*1502 with

Table 1. Reported genetic biomarkers for anticonvulsants-induced cutaneous ADR

Causative drug	HLA-B	Race		Selectivity	References
Carbamazepine	*1502	Han Chinese (Taiwan)	SJS/TEN	59/60	16
		Han Chinese (Hong Kong)	SJS/TEN	4/4	11
		Asians in Europe	SJS/TEN	4/4	13
		Thai	SJS	37/42	18
		Indians	SJS	6/8	17
		Caucasians	SJS/TEN	0/8	13
		Japanese	SJS/TEN	0/15	22
		Han Chinese (Taiwan)	DIHS	0/13	16
		Caucasians	DIHS	0/56	29
	*1511	Japanese	SJS/TEN	4/15	22
Phenytoin	*1502	Han Chinese (Taiwan)	SJS/TEN	8/26	28
		Thai	SJS/TEN	4/4	18
Lamotrigine	*1502	Han Chinese (Taiwan)	SJS	2/6	28
Oxcarbazepine		Han Chinese (Taiwan)	SJS	3/3	28

ADR, adverse drug reactions; HLA, human leukocyte antigen; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

SJS/TEN ($P = 2.89 \times 10^{-12}$; OR = 54.76). In India, the same association was shown in six out of eight patients by Mehta *et al.*[17]

In contrast, this association has not been detected in Caucasian populations.[19] A European study performed by Lonjou *et al.*[19] included 12 carbamazepine-induced SJS/TEN patients, four HLA-B*1502-positive patients who had Asian ancestry and eight HLA-B*1502-negative Caucasian patients. These studies demonstrate that an association between HLA-B*1502 and carbamazepine-induced SJS/TEN is observed only in southeast Asian populations.

ALLELE ASSOCIATIONS WITH CARBAMAZEPINE-INDUCED SJS/TEN IN JAPANESE PATIENTS

In Japanese studies, none of the SJS/TEN patients receiving aromatic anti-epileptic drugs, including carbamazepine, carried HLA-B*1502 (Table 1).[20–23] Ueta *et al.* reported a case–control study on the relationships between HLA class I and II genetic polymorphisms with severe ocular complications using 71 Japanese drug-unspecified SJS/TEN patients and 101 Japanese controls. No HLA-B*1502 carriers were detected in cases or controls.[24] Instead, the investigators reported that HLA-A*0206 was associated with SJS/TEN with severe ocular complications ($P = 4 \times 10^{-5}$; OR = 4.1).[24]

Recently, our group detected four patients carrying HLA-B*1511 among 15 carbamazepine-induced SJS/TEN patients (26.7%). The allele frequency of HLA-B*1511 was significantly increased in the patients (13.3%) compared with that of the Japanese population (1%) ($P < 10^{-4}$; OR = 19.52).[22] These data suggest that HLA-B*1511, a member of the HLA-B75 group, as well as HLA-B*1502, are risk factors for carbamazepine-induced SJS/TEN in Japanese populations. Other major members of HLA-B75 are HLA-B*1508, HLA-B*1515 and HLA-B*1521. Interestingly, HLA-B*1508, HLA-B*1511 and HLA-B*1521 were detected in studies on SJS/TEN in Thailand and India.[12,17] These findings suggest that subfamilies belonging to the HLA-B75 serotype are involved in carbamazepine-induced SJS/TEN.

ALLELE FREQUENCIES OF INDIVIDUAL HLA-B75 (B*1502, B*1508, *1511, B*1515, B*1521) GENOTYPES

Allele frequencies of individual HLA genotypes in worldwide populations are shown at www.allelefrequencies.net (Table 2).[25] The prevalence of HLA-B*1502 is relatively high in southern Chinese and southeast Asian populations where HLA-B*1502 is in fact a marker for carbamazepine-induced SJS/TEN.[22] In contrast, the prevalence of HLA-B*1502 is very low in Caucasian and Japanese populations. This suggests that one reason for not detecting HLA-B*1502 in carbamazepine-induced SJS/TEN in Japanese and Caucasian patients is the low allele frequency. In addition, the extremely low allele frequencies of HLA-B75 subfamilies in Caucasians

Table 2. Population allele frequencies of major subfamilies of serotype B75

	Population allele frequencies reported in www.allelefrequencies.net website[†]				
Ethnic group	HLA-B*1502	HLA-B*1515	HLA-B*1521	HLA-B*1508	HLA-B*1511
Japanese	0.001				0.004–0.008[‡]
Koreans	0.002	0	0	0	0.02
Han Chinese	0.019–0.124	0.01	0.000–0.002	0.005–0.015	0.000–0.017[§]
Thai	0.061–0.085		0.007–0.010	0.01	0.01
Indians	0.000–0.060			0.005–0.033	
Caucasians	0	0	0	0.000–0.004	0.000–0.003

[†]New Allele Frequency Database: www.allelefrequencies.net/ (Middleton *et al.*).[25] [‡]The frequency of 0.1 was reported by Tanaka *et al.*[45] [§]Higher value than 0.038 in Han Chinese in Beijing was recently reported by Yang *et al.*[46] This table was partly quoted from Kaniwa *et al.*[22] HLA, human leukocyte antigen.

may be a reason for detecting no HLA-B75 subfamilies, including HLA-B*1511, in the Caucasian patients with carbamazepine-induced SJS/TEN.

HLA-B ASSOCIATION IN OTHER AROMATIC AMINE ANTICONVULSANT-INDUCED SJS/TEN

Aromatic amine anticonvulsants such as carbamazepine, phenytoin, phenobarbital, oxcarbazepine and lamotrigine are metabolized to arene oxide metabolites. Clinical cross-reactivity among aromatic amine anticonvulsants is observed with high frequency.[26,27] Small case studies in Thailand (four cases phenytoin induced) and Hong Kong (single cases of phenytoin and lamotrigine induced) showed the presence of HLA-B*1502 in all SJS patients.[11,12] In a current case–control association study in a Taiwanese population, the association between HLA-B*1502 and phenytoin-, lamotrigine- and oxcarbazepine-induced SJS/TEN was observed in 30.8% of 26 patients ($P = 4.1 \times 10^{-3}$; OR = 5.1), 33% of six patients ($P = 1.3 \times 10^{-1}$; odds ratio = 5.1) and 100% of three patients ($P = 8.4 \times 10^{-4}$; OR = 80.7), respectively.[28] These results indicate that aromatic anticonvulsants share a common risk allele, HLA-B*1502, presumably by similar antigen recognition, although the association is highest with carbamazepine. Other genetic factors may also contribute to the pathomechanism of the disease. Thus, HLA-B*1301, Cw*0801 and DRB1*1602 also showed an association with phenytoine-SJS/TEN in the same study ($P = 0.0128$–0.0281; OR = 3.0–4.3).[28]

In Europe, where the allele frequency of HLA-B*1502 is extremely low, a rare allele, HLA-B*38, showed a weaker association ($P < 2 \times 10^{-2}$; OR = 6.8) with SJS/TEN in a limited number of patients treated with lamotrigine.[15]

ALLELE ASSOCIATIONS WITH DIHS/DRESS AND MACULOPAPULAR ERUPTION INDUCED BY AROMATIC AMINE ANTICONVULSANTS

In addition to SJS/TEN, carbamazepine also induces other types of cutaneous ADR, including maculopapular eruption (MPE) and DIHS/DRESS. The association between HLA-B*1502 and carbamazepine-induced MPE was not detected in Han-Chinese populations in Taiwan and Hong Kong or in the Thai population.[11,12,16] Studies in 18 Han-Chinese patients residing in Taiwan and 56 Caucasian patients showed that carbamazepine-induced DIHS/DRESS was not associated with HLA-B*1502.[16,29] These data suggest that the association between HLA-B*1502 and carbamazepine-induced cutaneous ADR is specific to SJS/TEN.

Kano *et al.*[30] showed that four out of 13 Japanese patients (30.8%) with DIHS/DRESS – all associated with HHV-6 reactivation – induced by aromatic amine anticonvulsants (carbamazepine, eight; phenobarbital, two; phenytoin, one) had HLA-B*1301 (allele frequency 15.4%). This allele frequency of HLA-B*1301 was much higher than that reported for the Japanese population (1.3%),[31] although the difference was not statistically significant after correction for multiple comparisons. They supposed that the effect of certain HLA-B alleles on the virus reactivation contributed, in part, to the HLA-B allele association with DIHS/DRESS.

Recently, we found a significant association between carbamazepine-induced cutaneous ADR

and HLA-A*3101 in 22 Japanese patients, including MPE, erythema multiforme, erythroderma, DIHS, SJS and other types. Eleven patients (50%), including two SJS patients and others, carried HLA-A*3101, and the allele frequency was much higher in the patients (25%) than that reported for the Japanese population (7.1%) ($P = 4 \times 10^{-4}$; OR = 4.33).[23] Another study involving carbamazepine-induced MPE in 18 Han-Chinese also suggested the association with HLA-A*3101 ($P = 2.2 \times 10$; OR = 17.5).[16] The sample sizes of these studies were small, so further study on a large sample size is needed to clarify whether or not HLA-A*3101 is a risk allele.

ASSOCIATION BETWEEN HLA-B*5801 AND ALLOPURINOL-INDUCED CUTANEOUS ADR

Allopurinol is a xanthine oxidase inhibitor used to treat gout and hyperuricemia (Table 3). A case–control study in a Han-Chinese population showed an extremely strong association between HLA-B*5801 and allopurinol-induced SJS/TEN or DIHS/DRESS.[14] In this study, all 51 patients (100%) with allopurinol-induced SJS/TEN or DIHS/DRESS carried HLA-B*5801, compared with only 20 out of 135 (15%) allopurinol-tolerant patients and 19 out of 93 (20%) population controls ($P < 10^{-6}$; OR = 580). Regarding the association in other southeast Asian populations, a similar strong association between HLA-B*5801 and allopurinol-induced SJS/TEN was shown in a case–control study in a Thai population.[32]

The association of HLA-B*5801 with allopurinol-induced SJS/TEN was observed in a European study as well ($P < 10^{-8}$; OR = 80).[15] The carrier frequency was 55% in 27 European patients. One of the reasons for the lower carrier frequency seems to be the lower allele frequency of HLA-B*5801 (1–6%) in the European population than in southeast Asian populations, although HLA-B*5801 is more broadly distributed than HLA-B*1502.

In the Japanese population, the allele frequency of HLA-B*5801 is less than 1%.[20] We have reported earlier that four out of 10 Japanese patients (40%) with allopurinol-induced SJS/TEN carried HLA-B*5801.[20] A moderate but statistically significant association ($P < 10^{-4}$, OR = ~40) between HLA-B*5801 and allopurinol-induced SJS/TEN was detected in that study. Our recent data have shown that 10 out of 18 Japanese patients (55.6%) with allopurinol-induced SJS/TEN carried HLA-B*5801 (M. Tohkin, unpubl. data, 2010). Dainichi *et al.*[33] also detected three HLA-B*5801 carriers in all three allopurinol-treated Japanese patients diagnosed with SJS, DIHS and TEN, respectively. Although the sample size in our study was not sufficient to estimate the accurate carrier frequency in Japanese patients, it showed a possible association between HLA-B*5801 and allopurinol-induced SJS/TEN in Japan.

These studies lead to the conclusion that HLA-B*5801 is a potential genetic biomarker for allopurinol-associated SJS/TEN across different populations or ethnicities, although there is less information regarding the association in other populations than the Japanese.

ALLELE ASSOCIATION WITH CUTANEOUS ADR INDUCED BY ANTIRETROVIRAL DRUGS

HIV patients treated with antiretroviral drugs show a high frequency of cutaneous ADR, including SJS/TEN and hypersensitivity syndrome (Table 4). The hypersensitivity syndrome is associated with fever,

Table 3. Reported genetic biomarkers for allopurinol-induced cutaneous ADR

HLA-B	Race	ADR	Selectivity	References
*5801	Han Chinese (Taiwan)	SJS/TEN or DIHS/DRESS	51/51	14
	Thai	SJS/TEN	27/27	32
	Caucasians	SJS/TEN	15/27	15
	Japanese	SJS/TEN/DIHS	3/3	33
	Japanese	SJS/TEN	4/10	20

ADR, adverse drug reactions; DIHS, drug-induced hypersensitivity syndrome; DRESS, drug rash with eosinophilia and systemic symptoms; HLA, human leukocyte antigen; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

Table 4. Reported genetic biomarkers for cutaneous ADR induced by antiretroviral drugs

Causative drug	HLA	Race	ADR	Selectivity	References
Abacavir	B*5701	Caucasians	Hypersensitivity	57/130	40
			Hypersensitivity (patch test+)	42/42	40
		Black	Hypersensitivity	10/69	40
			Hypersensitivity (patch test+)	5/5	40
		Japanese	Hypersensitivity	0/7	38
Nevirapine	B*3505	Thai	Hypersensitivity	25/143	42
	Cw8	Japanese	Hypersensitivity	5/12	44
	Cw8	Sardinian	Hypersensitivity	6/3	43
	B14	Sardinian	Hypersensitivity	6/13	43

ADR, adverse drug reactions; HLA, human leukocyte antigen.

rash and internal organ involvement (gastrointestinal symptoms in abacavir-treated patients and hepatitis in nevirapine-treated patients).[34]

ABACAVIR

Abacavir is a guanosine analog that belongs to the family of nucleoside reverse transcriptase inhibitors used for treatment of HIV infection. Hypersensitivity to abacavir occurs in approximately 5–8% of patients within 1–6 weeks of the initial dose.[35] The initial association between abacavir-induced hypersensitivity and HLA-B*5701 was reported in Australian and British populations.[36,37] However, abacavir-induced hypersensitivity is present at a high frequency only in Caucasians and at a very low frequency in Asian and black populations.[38,39] In fact, the allele frequency of HLA-B*5701 is approximately 8% in Caucasians, but lower in Asian and African populations.[36,37] To examine the universality of the sensitivity and specificity of HLA-B*5701 association with abacavir hypersensitivity across ethnicities, the Study of Hypersensitivity to Abacavir and Pharmacogenetic Evaluation (SHAPE) was performed. It was a case–control study that enrolled both white and black patients in the USA.[40] This study showed that 100% of both white and black patch test-positive patients carried HLA-B*5701, suggesting a predictive value of HLA-B*5701 for abacavir-induced hypersensitivity across ethnicities. This study demonstrated the clinical utility of testing for HLA-B*5701 prior to prescription of abacavir.

Although all current studies show the requirement for HLA-B*5701 presence for development of abacavir-induced hypersensitivity syndrome, 45% of patients who carry HLA-B*5701 do not develop the hypersensitivity syndrome.[41] Therefore, it is likely that HLA-B*5701 is necessary but not sufficient for development of abacavir-induced hypersensitivity syndrome.

NEVIRAPINE

Nevirapine is another antiretroviral agent that is a potent non-nucleoside reverse transcriptase inhibitor. Nevirapine often causes cutaneous ADR with a frequency of approximately 5% for hypersensitivity syndrome and 0.3% or less for SJS/TEN.[34] A recent case–control study in Thailand showed a high frequency of HLA-B*3505 (17.5%) in patients with nevirapine-induced hypersensitivity syndrome.[42] Because HLA-B*3505 is carried by less than 1% of the Thai population, a strong association between HLA-B*3505 and nevirapine-induced hypersensitivity syndrome is suggested. HLA-Cw8 and HLA-B*1402 associations with nevirapine-induced hypersensitivity were also reported in a Sardinian population,[43] and a HLA-Cw8 association was noted in a Japanese population.[44] To date, no specific HLA association has been described in nevirapine-induced SJS/TEN.

FUTURE PERSPECTIVE OF PHARMACOGENOMICS IN CUTANEOUS ADR

Elucidation of associations between HLA alleles and drug hypersensitivity will make it possible to predict immunologically mediated drug reactions and prevent them in the future. As a result of current studies, the strong associations between HLA-B*1502 and

carbamazepine-induced SJS/TEN in patients of Asian ancestry, and between HLA-B*5701 and abacavir hypersensitivity, have been included in the labels on these drugs, and screening for these alleles is recommended by the US Food and Drug Administration (FDA) prior to initiating therapy with these drugs. The FDA has also updated the genetic information on other drug labels and now recommends genetic testing for more than 10 drugs. In order to perform a widespread genetic screening for these drugs, technology advancement is needed to decrease the cost of the screening. It is to be hoped that pharmacogenetic testing kits will be available in the near future for prevention of severe reactions such as SJS/TEN and hypersensitivity syndrome. In addition, the ability to predict the propensity of drugs to cause ADR will make pharmacogenomic screening an important tool in new drug development in the future.

Although strong associations have been shown between HLA alleles and some types of cutaneous ADR, there has been no definitive proof or data published concerning the functions of the implicated HLA alleles. HLA-restricted T-cell activation is needed for induction of immunological reactions and, in addition, there is a possibility that some HLA proteins have higher binding affinity than others toward a drug or drug metabolite through covalent or non-covalent mechanisms. On the other hand, a protecting effect of HLA has been suggested. Alfirevic *et al.*[29] reported a potential protecting effect of HLA-B*0702 against carbamazepine-induced severe cutaneous adverse reactions in Caucasian patients. Functional studies together with genomic approaches are required for further progress in understanding the pathogenesis of ADR.

Many questions are still unresolved. For instance, it is still unclear what the genetic difference is between the patients who develop severe reactions such as SJS/TEN and milder skin reactions, and between the patients who develop severe skin reactions and those who have only internal organ involvement with the same drug. In order to elucidate the pathogenesis of these diseases, definite case–control studies will be needed.

For further development of pharmacogenomics, collaboration between different research groups is needed to collect larger numbers of biological samples from ADR patients, particularly from those with rare ADR such as SJS/TEN. This association is also needed across ethnicities, based on consistent definitions of diseases.

CONCLUSIONS

Studies presented in this review show the tremendous progress in the area of pharmacogenetics of cutaneous ADR in recent years. It is likely that further progress will be made in this field by the continuous development of genetic technologies and the international well-defined sampling of the patients. This could result in the reduction of serious cutaneous ADR by screening prior to initiating drug therapies. Further studies, such as confirmatory haplotype-mapping, are required to definitively identify the susceptibility region responsible for the hypersensitivity.

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ORIGINAL ARTICLE

Statistical Analysis of Stevens-Johnson Syndrome Caused by *Mycoplasma pneumonia* Infection in Japan

Yuko Kunimi[1,2], Yuko Hirata[3], Michiko Aihara[1], Yumiko Yamane[4] and Zenro Ikezawa[1]

ABSTRACT

Background: Stevens-Johnson syndrome (SJS) associated with *Mycoplasma pneumoniae* (*M. pneumoniae*) infection is mainly observed in children. In adults, drugs are a major cause of SJS, but some adult patients with SJS are infected with *M. pneumoniae*. We analyzed patients with SJS associated with *M. pneumoniae* infection to elucidate the differences between drug-induced SJS and *M. pneumoniae*-associated SJS and also to study differences between *M. pneumoniae*-associated SJS in children and adults.

Methods: This is a retrospective review of Japanese patients who have been reported as *M. pneumoniae*-associated SJS in medical Journals published from 1981 to 2009, compared with data of Japanese patients with drug-induced SJS reported from 2000 to 2009.

Results: Thirty-eight cases of *M. pneumoniae*-associated SJS and 78 cases of drug-induced SJS were analyzed in this study. Ocular lesions were observed more frequently in *M. pneumoniae*-associated SJS than in drug-induced SJS ($p < 0.01$), and adult patients showed a higher ratio of sequelae in their eyes than did patients under 20 years of age ($p < 0.01$). Sixty-six percent of adult patients with *M. pneumoniae*-associated SJS developed fever/respiratory symptoms and mucocutaneous lesions on the same day. In contrast, most of the patients under 20 years of age developed fever/respiratory symptoms before mucocutaneous involvement. This means that these adult patients were infected and immunized previously and developed allergic reactions to *M. pneumoniae* soon after the later infection.

Conclusions: In order to prevent ocular sequelae in adult patients when *M. pneumoniae* infection is suspected, more intensive treatment may be needed in adult patients than in younger patients.

KEY WORDS

Mycoplasma pneumoniae, ocular involvement, sequelae, Stevens-Johnson symdrome, treatment

INTRODUCTION

Stevens-Johnson syndrome (SJS) is a potentially fatal disorder characterized by high fever, widespread blistering exanthema of macules and atypical target-like lesions accompanied by mucosal involvement. It often advances to toxic epidermal necrolysis (TEN) characterized by widespread epidermal detachment.[1] SJS and TEN are usually related to medications, but some cases of SJS have been reported to be associated with infection by microorganisms such as *Mycoplasma pneumoniae* (*M. pneumoniae*).[2,3]

M. pneumonia is a common respiratory pathogen that produces disease of varied severity, ranging from mild respiratory tract infection to severe atypical pneumonia.[4] This organism is also responsible for producing a wide spectrum of non-pulmonary manifestations, including neurological, hepatic, and cardiac diseases and erythema multiforme.[5-8] It may occur before, during or after pulmonary infection, as

[1]Department of Immuno-dermatology, Yokohama City University Graduate School of Medicine, [2]Present address: Department of Dermatology, Yokohama City University Medical Center, [3]Department of Dermatology, Yokohama Seamen's Insurance Hospital and [4]Department of Dermatology, Yokosuka Kyosai Hospital, Kanagawa, Japan.
Correspondence: Yuko Kunimi, Department of Dermatology, Yokohama City University Medical Center, 4-57 Urafunecho, Minami-ku, Yokohama, Kanagawa 232-0024, Japan.
Email: yukowata@yokohama-cu.ac.jp
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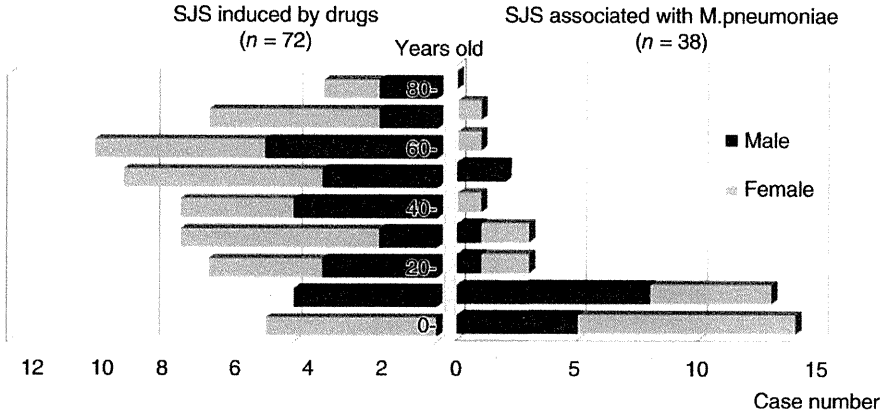

Fig. 1 Age and sex. The patients with *M. pneumoniae*-associated SJS were much younger than those with drug-induced SJS.

well as in the absence of any pulmonary symptoms. *M. pneumoniae* is also known as a cause of SJS especially in childhood and adolescence. However only a few articles have reported on the clinical characteristics of SJS associated with *M. pneumoniae*.[9-13]

To elucidate the clinical characteristics of SJS associated with *M. pneumoniae* infection (*M. pneumoniae*-associated SJS), we retrospectively analyzed reports on Japanese patients with *M. pneumoniae*-associated SJS and compared them with reports on patients with drug-induced SJS. In addition, we studied the clinical differences between children and adolescent patients and adults.

METHODS

Reports on Japanese patients with SJS published in medical journals were analyzed. Data on patients who had been diagnosed with *M. pneumoniae*-associated SJS were collected from 1981 to 2009 and those with drug-induced SJS were collected from 2000 to 2009 (published Yamane *et al.*[14] and additional reports from 2007 to 2009). Clinical reports were selected when enough information was available to make a credible diagnosis. Symptoms of SJS should include severe mucous membrane lesions (eyes, oral cavity and lips, genital area) and skin lesions (macules, atypical target-like lesions, bullae and erosion) with maximum skin detachment on less than 10% of the skin surface area. In order to exclude patients with erythema multiforme, patients were not included if they had slight or very mild mucous membrane lesions.

Diagnostic procedures associated with *M. pneumoniae* infection included serological findings, such as an increase of mycoplasma IgG antibody with or without positive mycoplasma IgM antibody, or clinical features and roentgenogram findings compatible with *M. pneumoniae* infection.

The collected and analyzed data were as follows: Demographic information (age, sex), skin and mucous lesions, internal organ involvement, time interval between appearance of fever/respiratory symptoms and onset of skin/mucosal lesions, treatments, terms of hospitalization, sequelae and mortality. The data on patients with *M. pneumoniae*-associated SJS were divided into two groups according to age (less than 20 years old, and older) in order to clarify differences between the groups.

Data were compared using the chi-square test. A P-value less than 0.05 indicated a significant difference.

RESULTS

AGE AND SEX (Fig. 1)

Thirty-eight patients (17 males and 21 females, 1 : 1.2) with *M. pneumoniae*-associated SJS and 72 patients (30 males and 42 females, 1 : 1.4) with drug-induced SJS were analyzed in this study. The ages of patients with *M. pneumoniae*-associated SJS were between 1 and 74 years (mean, 15.3 years). Twenty-seven patients (71.1%) were less than 20 years old. In drug-induced SJS, the ages were between 2 and 89 years (mean, 46.6 years). The reason why patients with *M. pneumoniae*-associated SJS were much younger than patients with drug-induced SJS is thought to be that *M. pneumoniae* infections were more frequent in childhood and adolescence. There was no significant difference in the male to female ratio between *M. pneumoniae*-associated and drug-induced SJS.

SKIN AND MUCOUS MEMBRANE INVOLVEMENT (Fig. 2)

Skin lesions were ranged from mild to severe in both *M. pneumoniae*-associated and drug-induced SJS. There was no remarkable difference between these groups, though the extent of the lesions was not always fully described in the reports.

The ocular lesions were observed in 94.4% of *M.*

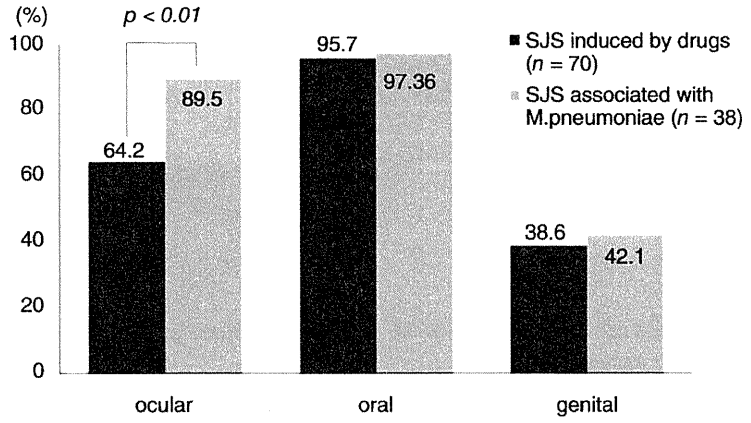

Fig. 2 Symptoms of mucous lesions. Ocular lesions were observed in most of the *M. pneumoniae*-associated SJS patient but only in two-thirds of patients with drug-induced SJS.

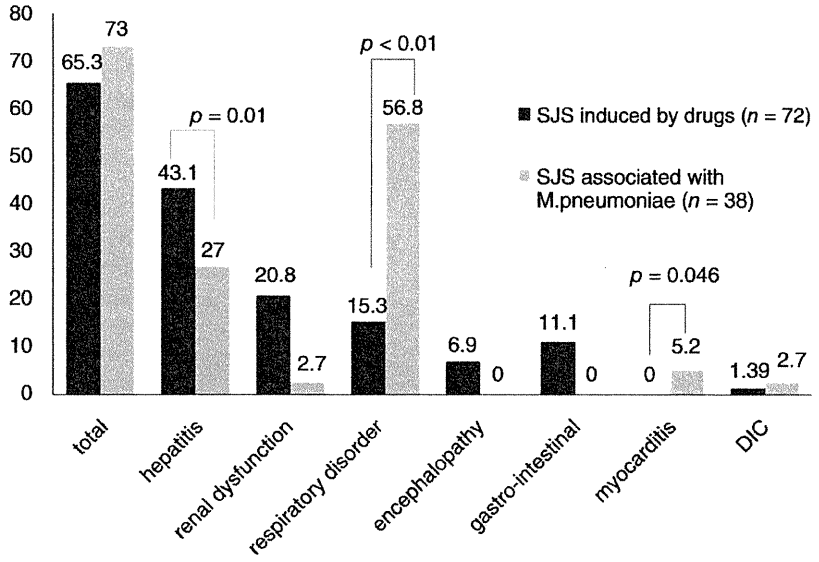

Fig. 3 Internal organ involvement. Hepatitis was the most common complication in patients with drug-induced SJS, while respiratory disorders were more common in patients with *M. pneumoniae*-associated SJS ($p < 0.01$).

pneumoniae-associated SJS and in 64.2% of drug-induced SJS, thus, a significantly higher percentage of these lesions was seen in *M. pneumoniae*-associated SJS ($p < 0.01$). The oral lesions were observed in most of the patients in both groups. The genital lesions were observed in 38.6% and 42.1% of drug-induced SJS and *M. pneumoniae*-associated SJS, respectively ($p = 0.72$). These results showed that in regard to mucous membrane involvement, ocular lesions appeared more frequently in *M. pneumoniae*-associated SJS than in drug-induced SJS.

INTERNAL ORGAN INVOLVEMENTS (Fig. 3)

Internal organ involvement was shown in many SJS patients. Hepatitis was the most frequent complications in drug-induced SJS (43.1%). In contrast, respiratory disorders were most frequent in *M. pneumoniae*-associated SJS (56.8%), with a frequency that is significantly higher than the frequency of respiratory disorders in drug-induced SJS (15.3%) ($p < 0.01$). This difference is probably due to an association of *M. pneumoniae* with respiratory tract infections. Respitatory disorders included pneumonia, hydrothorax, progressive respiratory failure and hypoxemia. However, abnormal shadows in roent-

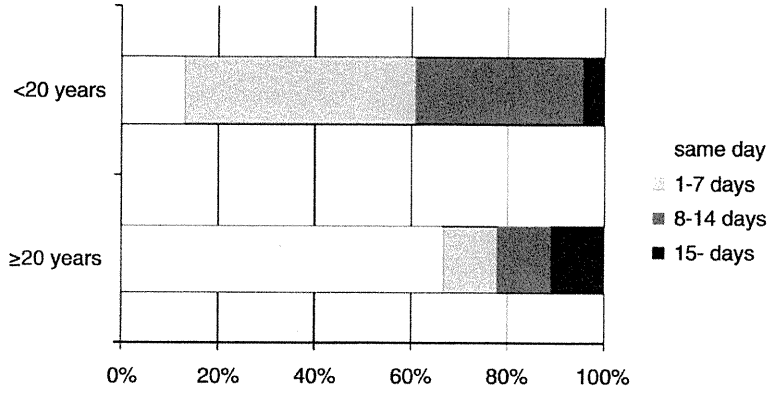

Fig. 4 Time interval between appearance of fever/respiratory symptoms and onset of skin/mucosal lesions. The time interval between appearance of fever/respiratory symptoms and onset of skin/mucosal lesions was more than 1 week in 64% of patients under 20 years old. In contrast, 66% of older patients developed fever/respiratory symptoms and skin/mucosal lesions on the same day.

genograms were observed only in 21 patients (55.2%) with *M. pneumoniae*-associated SJS. Renal dysfunction was significantly higher in drug-induced SJS (20.8%) than in *M. pneumoniae*-associated SJS (2.7%) ($p = 0.01$). Myocarditis was observed in a small number of patients with *M. pneumoniae*-associated SJS.

DURATION OF HOSPITALIZATION AND SEQUELAE

We compared the duration of hospitalization in the two groups, except in a deceased case. A total of 57% of *M. pneumoniae*-associated SJS and 51.8% of drug-induced SJS were discharged within 30 days. There were no significant differences between the two groups.

A total of 18% of patients in each group had chronic sequelae. Ocular complications, including corneal disorders, dry eyes, and loss of eyelashes, were observed in 13.5% and 12.5% of patients with *M. pneumoniae*-associated SJS and drug-induced SJS, respectively. Although *M. pneumoniae* infection itself presented with respiratory disorders, bronchitis as a sequela was only observed in 4.2% of patients with drug-induced SJS. Two patients died of drug-induced SJS. One 18-year-old male patient died of SJS that developed after *M. pneumoniae* infection, but he was administered acetaminophen before appearance of SJS symptoms. Therefore the cause of his SJS was not determined. He died of severe diffuse alveolar damage.

COMPARISON OF SYMPTOMS OF *M. pneumoniae*-ASSOCIATED SJS BETWEEN 2 GROUPS OF PATIENTS LESS THAN 20 YEARS OLD AND OLDER THAN 20 YEARS.

Time Interval between Appearance of Fever/Respiratory Symptoms and Onset of Skin/Mucosal Lesions (Fig. 4)

In *M. pneumoniae*-associated SJS, the time interval between appearance of fever/respiratory symptoms and onset of skin/mucosal lesions was compared between patients who were more than 20 years old and those who were less than 20 years old. The interval was less than 24 hours (the same day) in 13.0% and more than 1 week in 39.1% of younger patients. In contrast, 66.7% of older patients developed fever/respiratory symptoms and skin/mucosal lesions on the same day. According to these results, it seems likely that these adult patients were infected and immunized previously and then developed an allergic reaction to *M. pneumoniae* soon after the later infection.

Mucous Membrane Involvement and Internal Organ Involvement (Fig. 5)

The frequency of each type of mucous membrane involvement did not differ with age. Respiratory disorders and hepatitis were observed in 59.3% and 25.9%, respectively, of patients under 20 years old, and in 50.0% and 30.0%, respectively, of older patients. There was no significant difference between the frequencies of these disorders in the 2 groups. Renal dysfunction, myocarditis and DIC were observed only in the patients who were under 20 years old.

Treatments, Length of Hospitalization and Sequelae

Systemic treatment with antibiotics and corticosteroids was mainly used in *M. pneumoniae*-associated

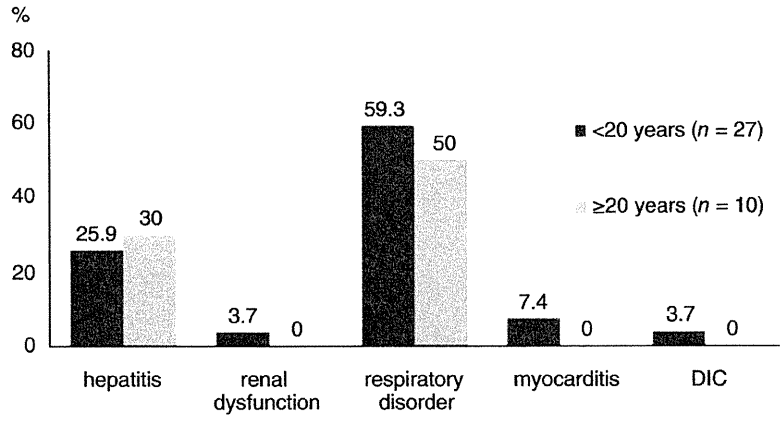

Fig. 5 Internal organ involvement in *M. pneumoniae*-associated SJS. Hepatitis and respiratory disorders were more frequent in patients under 20 years old, although there were no significant differences between the two groups.

Table 1

	Number of cases (%)	
	<20 years (n = 26)	≥20 years (n = 11)
No steroid and antibiotics therapy	2 (7.7)	0
Steroid therapy	9 (34.6)	0
Steroid pulse therapy	1 (3.8)	1 (9.0)
Steroid pulse therapy + Antibiotics	1 (3.8)	0
Steroid pulse therapy + High dose immunoglobulin + Antibiotics	0	2 (18.1)
High dose immunoglobulin + Antibiotics	1 (3.8)	0
Antibiotics	5 (19.2)	0
Antibiotics + Corticosteroids	6 (23.0)	8 (72.7)
Semipulse therapy + Plasma exchange + Antibiotics	1 (3.8)	0

SJS. Although all patients more than 20 years old were treated with corticosteroids, 8 patients (30.8%) younger than 20 years were treated without corticosteroids (Table 1). Steroid pulse therapy and high-dose immunoglobulin therapy were performed in some patients of both groups. Plasma-exchange was performed only in one patient (a 9-year-old boy) in combination with steroid pulse therapy.

Antibiotics were administered to 10 patients (90.9%) in the younger group and 14 patients (53.4%) in the older group. In the younger group, 5 patients were treated only with antibiotics.

Most of the younger patients (70.6%) could be discharged within 30 days. In contrast, 77.8% of adult patients were treated longer in hospitals (Fig. 6). The frequency of chronic sequelae was significantly higher in adult patients than in younger patients, especially chronic ocular complications ($p < 0.01$) (Fig. 7). Severe ocular lesions might be relevant to the longer hospitalization in adult patients.

DISCUSSION

M. pneumoniae is the most common organism to cause SJS, especially in children and young adults.[15,16] Although the association of SJS with *M. pneumoniae* infection has been widely accepted, the clinical characteristics of *M. pneumoniae*-associated SJS have not been examined. In order to reveal the differences in clinical characteristics between *M. pneumoniae*-associated SJS and drug-induced SJS, we reviewed the reports published in the medical journals in Japan.

Patients with *M. pneumoniae*-associated SJS were much younger than those with drug-induced SJS. *M. pneumoniae* infected children in early childhood,[17] and the infection was repeated in young adults. This seems to be a major reason for the difference in the mean age of patients. Ocular involvement was more frequently observed in *M. pneumoniae*-associated SJS than in drug-induced SJS, but the difference was not significant. As *M. pneumoniae* infects the respiratory mucous membrane, respiratory disorders were observed more frequently in *M. pneumoniae*-associated SJS. However, bronchitis as a sequela was shown only in drug-induced SJS. This might suggest that serious lesions of epithelium in the respiratory tract are mainly induced by drug-induced SJS. Regarding other internal organ involvement, patients with drug-induced SJS showed hepatitis, renal dysfunction, encephalopathy and gastro-intestinal symptoms more frequently (significant difference in renal dysfunction, $p = 0.01$). These results were compatible with results of a study reported by Wetter DA *et al.* They analyzed 27 SJS patients and concluded that *M. pneumoniae*-

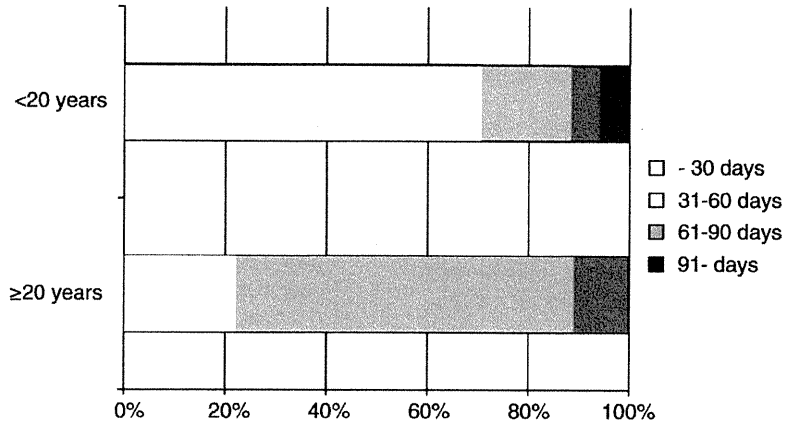

Fig. 6 Length of hospitalization for patients with *M. pneumoniae*-associated SJS. Most patients under 20 years old could leave the hospital within 30 days. In contrast, many of the older patients remained longer in hospital.

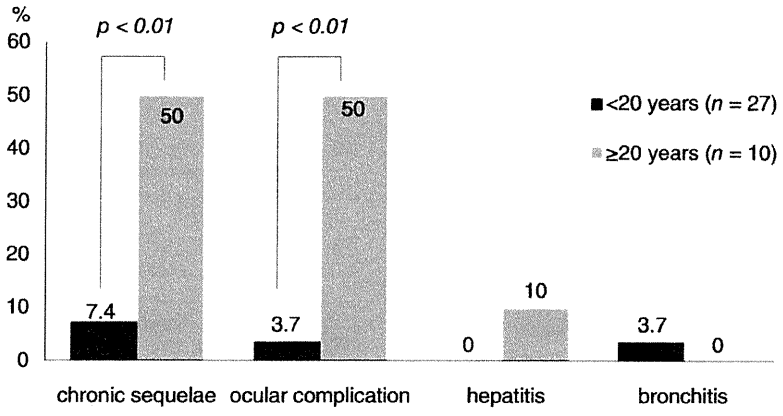

Fig. 7 Sequelae in patients with *M. pneumoniae*-associated SJS. The incidence of chronic sequelae was significantly higher in patients under 20 years old than in older patients ($p < 0.01$), especially chronic ocular complications ($p < 0.01$) .

associated SJS manifested less severely than drug-induced SJS, although only 6 patients with *M. pneumoniae*-associated SJS were included the study.[9]

Next, we examined the differences in clinical manifestations between children and adult patients with *M. pneumoniae*-associated SJS. We divided the patients into two groups according to age: under 20 years old and older than 20 years. Respiratory disorders were present in 50-60% of both groups. Despite a relatively high frequency of internal organ involvement—except hepatitis—in younger patients, ocular sequelae appeared more frequently in the adult patients. This means that the adults patients developed more severe ocular lesions compared with the younger patients. Thus it might be one of the reasons why the duration of hospitalization was longer in the adult group.

The pathomechanisms of skin manifestations due to *M. pneumoniae* infection remain unclear. Narita classified the extrapulmonary manifestation due to *M. pneumoniae* into three types: the first is a direct type in which inflammatory cytokines locally induced by lipoproteins contained in the bacterial cell membrane must play a role, the second is an indirect type in which immune modulation, such as autoimmunity, through cross-reaction between the bacterial cell components and human cells must play a role, and the third is a vascular occlusion type in which vasculitis and/or thrombosis with or without a systemic hypercoagulable state induced by the bacterium must play a role.[18] The author suggested that *M. pneumoniae*-associated SJS can be called a direct type because of *M. pneumoniae* isolation from blisters.[19,20] However, the isolation rarely succeeded. In this study, there was only one case report of *M. pneumoniae* isolation from a skin lesion of an SJS patient.

Thus the pathomechanism of *M. pneumoniae*-associated SJS is likely to involve immunological mechanisms, including cytotoxic T cells, similar to those shown in drug-induced SJS.[21-23]

Many adult patients with *M. pneumoniae* infection developed fever, respiratory disorders, and mucocutaneous lesions simultaneously, while many younger patients developed fever and respiratory disorders more than 1 week before the first manifestations of SJS appeared. This time difference may indicate the involvement of allergic mechanisms in *M. pneumoniae*-associated SJS. It seems likely that the adult patients were infected and immunized previously, and then developed an allergic reaction to *M. pneumoniae* soon after the later infection. In contrast, younger patients might need time for the immunization to take effect and therefore experienced an allergic reaction after the first infection.

In the treatment of *M. pneumoniae*-associated SJS, corticosteroids were used with or without antibiotics. Although the use of corticosteroids in *M. pneumoniae*-associated SJS is controversial, it was effective in the patients included in this study. No patient developed sepsis or severe organ involvement except one patient who died of severe diffuse alveolar damage after *M. pneumoniae* infection and acetaminophen administration.

In conclusion, patients with *M. pneumoniae*-associated SJS seem to show ocular manifestations more frequently than did those with drug-induced SJS, especially in adult patients. More intensive systemic treatment may be needed in adult patients than in younger patients with *M. pneumoniae*-associated SJS. The limitation of this study is that it was a retrospective review of cases from the literature and so a prospective study comparing the drug-induced SJS and *M. pneumoniae*-associated SJS is needed for further comfirmation of the results.

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CONFLICT OF INTEREST

No potential conflict of interest was disclosed.

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LETTERS TO THE EDITOR

Evaluation of serum cytokine levels in toxic epidermal necrolysis and Stevens–Johnson syndrome compared with other delayed-type adverse drug reactions

Dear Editor,

Toxic epidermal necrolysis (TEN) and Stevens–Johnson syndrome (SJS) are life-threatening disorders characterized by destruction of the epidermis and mucosal epithelium. They are mainly observed as consequences of adverse drug reactions (ADR) and are considered variants of the same disorder differentiated by the extent of the body surface area involved.[1]

It has been suggested that several pathways are implicated in the widespread apoptosis of keratinocytes in SJS/TEN.[2–5] Involvement of cytotoxic T cells and the molecular cytotoxicity of Fas and cytotoxic enzymes including granzyme B, perforine and granulysin have been shown in SJS/TEN.[3,5] Cytokines including tumor necrosis factor (TNF)-α and interferon (IFN)-γ were found to be overexpressed in the lesional skin.[2,3,6,7] It has also been shown that IFN-γ can activate production of TNF-α by keratinocytes in the skin.[4,7]

Plasma exchange has been reported as a useful method of TEN treatment.[8] In TEN patients, plasma exchange can effectively remove from patients' serum not only drugs and drug metabolites, but also increased amounts of soluble Fas ligand and cytokines. However, in patients with SJS/TEN, production of these cytokines were shown mainly in the skin lesions and blister fluids[2,6,7] and only a few reports have shown an increase of these cytokines in the patient serum.[6,9,10] In addition, there are few reports published about cytokine production other than IFN-γ and TNF-α in SJS/TEN patients.[7,9,10]

The aim of the present study was to evaluate a possible role of serum cytokines in SJS/TEN by comparing their concentration levels among delayed-type ADR.

Eight SJS (male : female ratio, 4:4; mean age, 52.9 years; age range, 23–74 years) and three TEN (three males; mean age, 51.0 years; age range, 4–75 years) patients were included in this study. These patients were admitted to either one of our two hospitals and treated with corticosteroid therapy with or without plasma exchange. Maximum epidermal detachment was 30–70% of body surface area in the TEN patients. All of these patients recovered. Sera were obtained from these patients at the onset (within 3 days of hospitalization) before starting treatment with high-dose systemic corticosteroids and plasma exchange. One SJS patient with systemic lupus erythematodes had been treated with daily 20 mg prednisolone for 1 year before initiating SJS treatment. In order to compare the cytokine levels among ADR types, 34 patients with other types of generalized delayed-type ADR were also included in this study. Their final diagnoses were as follows; macropapular type (MP), 15 patients (male : female ratio, 7:8; mean age, 58.4 years), erythema multiforme type (EM), 12 patients (male : female ratio, 6:6; mean age, 60.7 years), and drug-induced hypersensitivity syndrome (DIHS) or drug rash with eosinophilia and systemic symptoms (DRESS), which is another systemic life-threatening ADR that is mostly associated with reactivation of human herpesvirus 6, six patients (male : female ratio, 3:3; mean age, 48.7 years). Obtained sera were stored at −80°C until cytokine measurement. The cytokine levels were measured using the BioPlex suspension array system (Bio-Rad, San Francisco, CA, USA). Results were expressed as the mean ± standard error of the mean. Statistical analysis was performed using Mann–Whitney's *U*-test, with $P < 0.05$ considered significant. Serum cytokine levels in normal volunteers ($n = 13$) were as follows: IFN-γ, 54.9 ± 16.8 pg/mL; TNF-α, 13.6 ± 10.0 pg/mL; interleukin (IL)-10, 3.8 ± 4.4 pg/mL; IL-1

Correspondence: Michiko Aihara, M.D., Department of Environmental Immuno-Dermatology, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan. Email: maihara1@med.yokohama-cu.ac.jp

receptor antagonist (IL-1ra), 157.3 ± 92.3 pg/mL; IL-5, 2.1 ± 1.2 pg/mL; IL-6, 7.4 ± 3.2 pg/mL; IL-13, 7.9 ± 4.9 pg/mL; and granulocyte macrophage colony-stimulating factor (GM-CSF), 14.2 ± 13.0 pg/mL.

Levels of cytokines at the onset are shown in Figure 1. Mean IFN-γ levels were higher in patients with SJS (185.0 ± 82.0 pg/mL) and TEN (217.8 ± 37.0 pg/mL) than in those with MP (59.6 ± 13.9 pg/mL), EM (56.0 ± 18.4 pg/mL) and DIHS/DRESS (127.1 ± 24.8 pg/mL), although not significantly. Mean TNF-α (360.3 ± 189.5 pg/mL), IL-6 (346.9 ± 272.9 pg/mL), IL-10 (48.0 ± 12.8 pg/mL), GM-CSF (232.5 ± 72.9 pg/mL) and IL-1ra (1491.7 ± 700 pg/mL) levels were significantly higher in patients with TEN than in those with the other types of ADR. Mean IL-5 levels in patients with SJS/TEN did not show a significant increase compared with those in patients with the other types of ADR. In contrast,

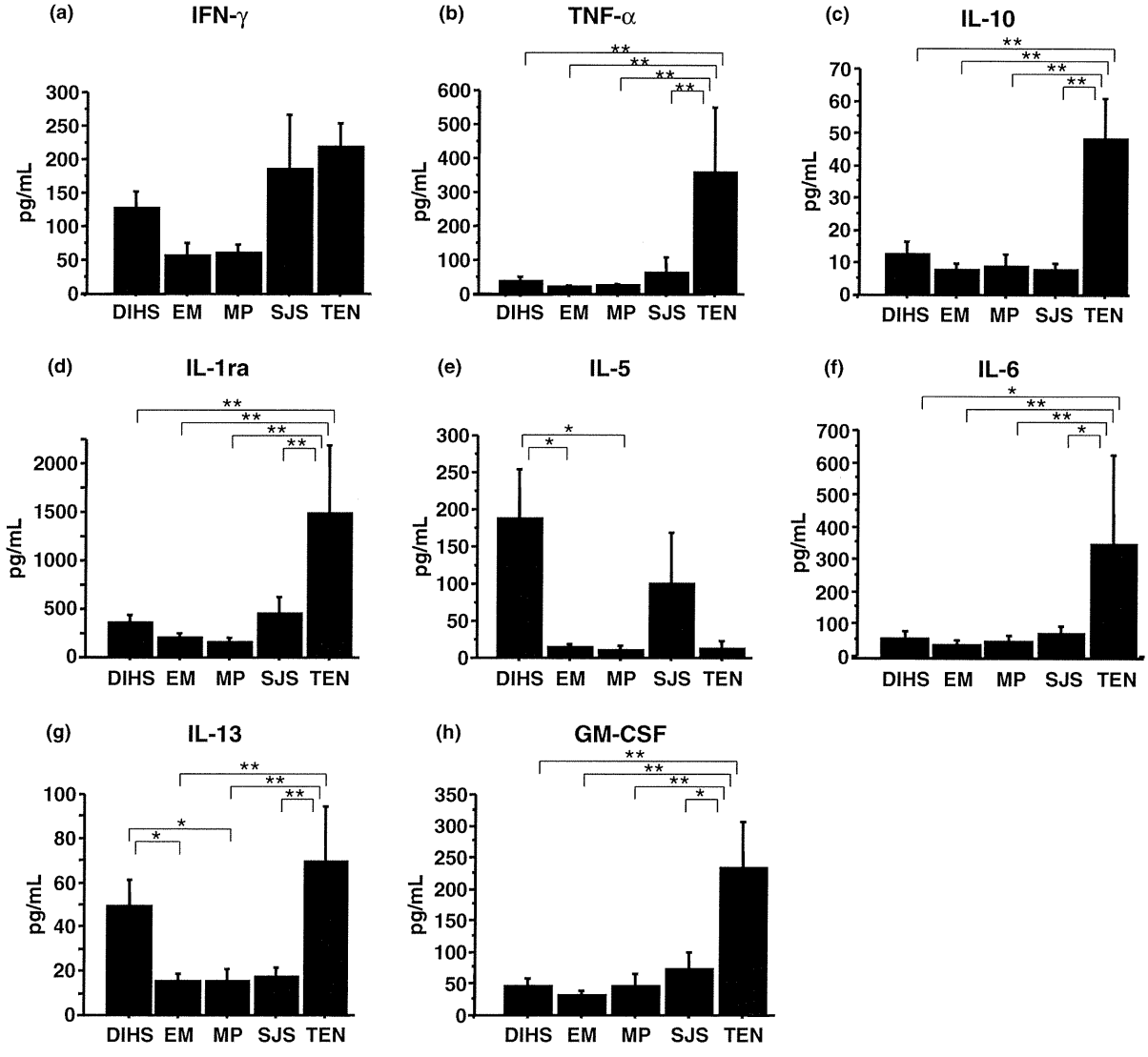

Figure 1. Comparison of serum cytokine levels at onset among patients with delayed-type adverse drug reactions including toxic epidermal necrolysis (TEN) (n = 3), Stevens–Johnson syndrome (SJS) (n = 8), macropapular type (MP) (n = 15), drug-induced hypersensitivity syndrome (DIHS) (n = 6) and erythema multiforme type (EM) (n = 12). (a) IFN-γ, (b) TNF-α, (c) IL-10, (d) IL-1ra, (e) IL-5, (f) IL-6, (g) IL-13, and (h) GM-CSF. *P < 0.05, **P < 0.001. GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.