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[V]

班会議プログラム

厚生労働省科学研究費補助金

難治性疾患克服研究事業

「重症多形滲出性紅斑に関する調査研究（H19－難治－一般－004）」

平成１９年度班会議プログラム

主任研究者：愛媛大学医学部皮膚科、橋本公二

日時：　平成２０年１月２６日（土）
　　９：３０から１７：００まで

場所：　東京駅前：マルビルコンファレンススクエア　**ルーム３**

住所〒100-6307 東京都千代田区丸の内 2-4-1 丸ビル 7・8 階
TEL　03-3217-7111　（平日　10：00～19：00）　FAX 03-3217-7501
E-mail　welcome@marubiru.info
URL　http://www.marubiru.jp/hc/

●JR ご利用の場合／東京駅丸の内南口より徒歩１分
●地下鉄をご利用の場合／丸ノ内線東京駅より直結
千代田線二重橋前駅７番出口より徒歩２分

開会の挨拶　　主任研究者　橋本公二　　9:30-9:45

分担研究発表　09:45-10:45　（発表７分、質疑応答３分）

「DIHSにおけるサイトメガロウイルス再活性化の意義」
浅野　祐介、狩野　葉子、塩原　哲夫（杏林大学）

「長期にわたり再発を繰り返すDIHS症例の臨床的およびウイルス学的特徴について」
繁平　有希、山根　裕美子、相原　道子、池澤　善郎（横浜市立大学）

「テグレトールによるDIHSのHLA解析」
新原　寛之、森田　栄伸（島根大学）

「ＳＪＳ眼後遺症患者の解析」
外園　千恵、木下　茂（京都府立医科大学）

「重症薬疹と可溶性Fasリガンド」
藤山　幹子、橋本　公二（愛媛大学）

特別講演 10:45-12:00　　座長　橋本公二
①「トリクロロエチレンの代謝と有害性」
那須　民江　教授（名古屋大学大学院医学系研究科　環境労働衛生学）
②「中国のトリクロロエチレン使用職場に発生する重症薬疹様皮膚肝障害」
上島　通浩　准教授（名古屋大学大学院医学系研究科　環境労働衛生学）

12:00-13:00　　事務局連絡、昼食

13:00-13:15
分担研究発表
「SJS/TEN登録票の作成」
北見　周、渡辺　秀晃、飯島　正文（昭和大学）

13:15-17:00
議題　「DIHSの治療ガイドライン案の検討」
「症例検討」

[VI]

研究成果印刷物

 DOI 10.1111/j.1365-2133.2007.08167.x

Association of human herpesvirus 6 reactivation with the flaring and severity of drug-induced hypersensitivity syndrome

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Accepted for publication
29 April 2007

Key words
DRESS, drug-induced hypersensitivity syndrome, HHV-6, reactivation

Conflicts of interest
None declared.

Summary

Background Drug-induced hypersensitivity syndrome (DIHS) is an adverse reaction with clinical signs of fever, rash and internal organ involvement. In the vast majority of patients in Japan, the causative drugs for DIHS are limited to the following eight: carbamazepine, phenytoin, phenobarbital, zonisamide, mexiletine, dapsone, salazosulfapyridine and allopurinol. The association of human herpesvirus (HHV)-6 reactivation with DIHS has been reported by various groups.

Objectives To confirm the relationship between the flaring and severity of DIHS and HHV-6 reactivation.

Methods We evaluated 100 patients with drug rash and systemic symptom(s) caused by the drugs associated with DIHS. HHV-6 reactivation was examined by serological antibody assay and quantitative real-time polymerase chain reaction assay of serial serum samples.

Results Anti-HHV-6 IgG titres increased in 62 of 100 patients, 14–28 days after the onset of symptoms. These patients suffered from severe organ involvement and a prolonged course compared with 38 patients showing no reactivation of HHV-6. Significant amounts of HHV-6 DNA were detected in serum samples from 18 of the 62 patients. Flaring of symptoms such as fever and hepatitis was closely related to HHV-6 reactivation in these 18 patients. It should be emphasized that all five patients with fatal outcome and 10 patients with renal failure were in the HHV-6 reactivation group.

Conclusions A combination of immunological reaction to a drug and HHV-6 reactivation results in the severe course of DIHS. The demonstration of HHV-6 reactivation is a useful marker of diagnosis as well as prognosis in DIHS.

For more than 50 years, a specific syndrome caused by drug administration has been reported under various names such as allopurinol hypersensitivity syndrome,[1] dapsone hypersensitivity[2] and anticonvulsant hypersensitivity syndrome.[3] In 1994, Roujeau and Stern proposed 'hypersensitivity syndrome', regardless of causative drugs.[4] Later, they discarded this term and used the acronym DRESS (drug rush with eosinophilia and systemic symptoms).[5] However, the lack of a specific and sensitive diagnostic test sometimes resulted in confusion when diagnosing this syndrome. Interestingly, about 10 years ago, the involvement of human herpesvirus (HHV)-6 reactivation in this syndrome was demonstrated independently by a French group and two Japanese groups.[6–8] Based on this observation, a Japanese consensus group proposed a newly defined name

for this syndrome, drug-induced hypersensitivity syndrome (DIHS), and established a set of criteria for diagnosis of DIHS.[9]

DIHS is distinguished from other drug eruptions by certain characteristics: a limited number of causative drugs, late onset, clinical similarity to mononucleosis-like syndrome and prolonged course.[4,5] Anticonvulsants are the most common cause of DIHS.[3,10] Allopurinol, dapsone, salazosulfapyridine, minocycline and mexiletine can also cause DIHS.[10,11] The syndrome typically develops 2–6 weeks after the initiation of drug administration. The initial signs are fever and maculopapular eruptions that can progress to exfoliative dermatitis. Lymphadenopathy, hepatitis, renal dysfunction and haematological abnormalities, such as leucocytosis, eosinophilia and atypical lymphocytosis, are observed to various degrees.

It is noteworthy that the flaring of clinical signs such as fever, eruption and hepatitis, often occurs several weeks after the withdrawal of the causative drug in DIHS patients.[4] DIHS is attributed to an immunological reaction to a drug or to metabolites of that drug,[12,13] because the signs, such as eruption and fever, reappear upon re-administration of the drug. However, the mechanism underlying the flaring of symptoms of DIHS cannot be explained solely by an immunological reaction to a drug.

We established a link between DIHS and HHV-6 reactivation.[6] Over recent years, there have been numerous case reports of DIHS associated with HHV-6 reactivation,[14] in which encephalitis[15,16] and the development of fulminant type 1 diabetes,[17] possibly caused by HHV-6 infection, have been involved. HHV-6 generally infects people in early childhood and results in a latent infection of the peripheral blood mononuclear cells.[18,19] Reactivated HHV-6 is pathogenic for both normal and immunocompromised patients.

To confirm the relationship between the flaring and severity of this syndrome and HHV-6 reactivation, we examined the replication of HHV-6 by serological antibody assay and quantitative real-time polymerase chain reaction (PCR) testing, and assessed the relevance of this virus to the clinical signs of the syndrome. We found an association between the appearance of HHV-6 DNA and the flaring and severity of signs and symptoms in DIHS.

Materials and methods

Patients and samples

Between 1998 and 2004, we collected serial serum samples from 100 patients with drug rash and systemic symptom(s). These patients fulfilled the following criteria: (i) at least one systemic symptom such as fever, haematological abnormality (leucocytosis, eosinophilia and/or appearance of atypical lymphocytes), or liver dysfunction was observed; (ii) culprit drugs were anticonvulsants (carbamazepine, phenytoin, phenobarbital and zonisamide), allopurinol, dapsone, salazosulfapyridine and mexiletine, which are the major causative drugs of DIHS in Japan; (iii) Stevens–Johnson syndrome and toxic epidermal necrolysis were excluded.

Detection of antihuman herpesvirus (HHV)-6 antibodies and HHV-6 DNA

An indirect immunofluorescence test was carried out to determine anti-HHV-6 IgG antibody titres in serum samples, as described previously.[17] A fourfold or greater increase in IgG antibody titre was interpreted as significant.

DNA was extracted from serum using a QIAamp Viral RNA Kit (Qiagen, Chatsworth, CA, U.S.A.) and HHV-6 DNA in the samples was quantified by real-time PCR.[20]

Statistical analysis

The characteristics of the two groups of patients were compared by using the Student's t-test or Fisher's two-tailed exact test, as appropriate.

Results

Antihuman herpesvirus 6 IgG titres and characteristics of patients

The preliminary study of paired serum samples indicated a significant increase in the anti-HHV-6 IgG titre between days 14 and 28. We analysed paired serum samples from 100 patients, which were normally obtained before day 14 and after day 28. Seroconversion or a significant increase in anti-HHV-6 IgG antibody titre was detected in 62 of the 100 patients. Note that in many of these patients, a marked increase in anti-HHV-6 IgG titre occurred before day 28 (Fig. 1).

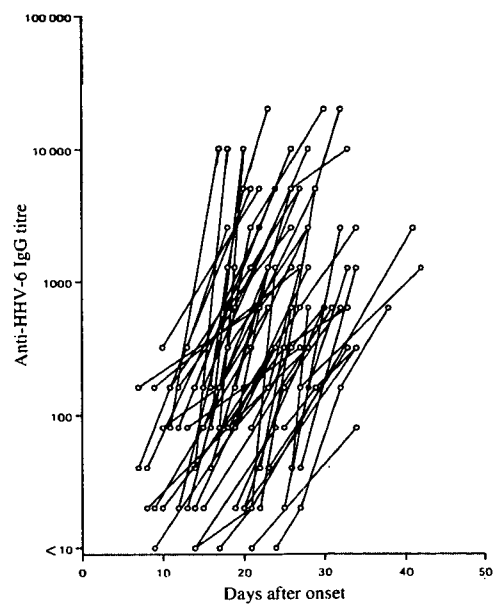

Fig 1. Rise of antihuman herpesvirus (HHV)-6 IgG titres in patients with drug-induced hypersensitivity syndrome. The results are limited to the duration of increase of anti-HHV-6 IgG titre.

Table 1 Clinical characteristics of 100 patients, according to whether or not the human herpesvirus (HHV)-6 IgG titre increased

	Increase of HHV-6 IgG titres (n = 62)	No increase of HHV-6 IgG titres (n = 38)	P-value
M/F	37/25	19/19	0·34
Age (years)			
Mean	48·1	57·8	< 0·001
Median	49	61	
Range	5–88	21–90	
Causative drug[a]			
Anticonvulsants	42	26	
Allopurinol	9	4	
Mexiletine	9	1	
Dapsone	3	2	
Salazosulfapyridine	3	5	
Days after administration of the drug (range, median)	41·7 (8–700, 29·5)	66·6 (5–650, 29·5)	0·29

[a]Multiple drugs caused drug rash in three patients whose HHV-6 titre increased.

We compared 62 patients whose anti-HHV-6 IgG titre increased with 38 patients whose anti-HHV-6 IgG titres did not change. The characteristics of these two groups were similar, except for the average age ($P < 0·001$) (Table 1): the sex ratio, causative drugs and the elapsed time between the initiation of drug administration and the appearance of clinical signs were not significantly different between the two groups.

However, severity of the clinical course was clearly different between these two groups. The 62 patients whose anti-HHV-6 IgG titre increased showed severe symptoms including long-lasting fever, lymphadenopathy, leucocytosis, appearance of atypical lymphocytes and hepatitis, compared with the 38 patients whose anti-HHV-6 IgG titre did not increase (Table 2). This resulted in a prolonged course; the duration of illness is clearly longer in the patients with an increase of HHV-6 IgG titre (Table 2). It should be emphasized that all five patients with fatal outcome and 10 patients with renal failure were in the HHV-6 reactivation group (Table 2).

Results of human herpesvirus 6 DNA detection

To confirm the time of HHV-6 reactivation, we examined HHV-6 DNA in serum samples. We serially monitored the HHV-6 DNA load in 352 serum samples from 62 patients using real-time PCR and detected HHV-6 DNA in 33 serum samples of 18 patients (Table 3). The HHV-6 copy number was between 120 and 2 400 000 copies mL^{-1} of serum. Fourteen patients had markedly higher viral loads compared with the three patients with exanthem subitum in the febrile phase, who had 610–1000 HHV-6 genome copies mL^{-1} of serum. Moreover, it is clarified that viraemia is observed from day 10 to day 27 after the onset, and that an increase of anti-HHV-6

Table 2 Clinical course of 100 patients, according to whether or not the human herpesvirus (HHV)-6 IgG titre increased

	Increase of HHV-6 IgG titres (n = 62)	No increase of HHV-6 IgG titres (n = 38)	P-value
Symptoms and signs, n (%)			
Fever	61 (98)	29 (76)	< 0·001
Duration of fever (days)	12·4 ± 7·1	4·8 ± 2·9	< 0·001
Lymphadenopathy	44 (71)	10 (26)	< 0·001
Leucocytosis[a]	38 (61)	8 (21)	< 0·001
Eosinophilia[b]	38 (61)	19 (50)	0·27
Appearance of atypical lymphocytes	55 (89)	20 (53)	< 0·001
Flaring, n (%)	32 (52)	7 (18)	< 0·001
Fever	22 (35)	2 (5)	< 0·001
Hepatitis[c]	32 (52)	2 (5)	< 0·001
Skin rash	12 (19)	5 (13)	0·42
Use of systemic corticosteroid, n (%)	50 (81)	27 (71)	0·17
Prognosis			
Duration of illness (weeks)	5·3 ± 2·6	2·8 ± 1·5	< 0·001
Death, n (%)	5 (8)	0 (0)	0·07

[a]Leucocytosis was evaluated based on white blood count (WBC), i.e. leucocytosis-positive patients were defined as those who had more than $1·1 \times 10^{10}$ leucocytes L^{-1} if they had not received corticosteroids, or more than 5×10^{10} leucocytes L^{-1} if they had been treated with systemic corticosteroids. [b]Eosinophilia was scored as positive when the peripheral blood eosinophil count was higher than $1·5 \times 10^{9}$ L^{-1} or more than 10% of the WBC. [c]Hepatitis was evaluated by measuring alanine aminotransferase (ALT) levels. Severe hepatitis indicated that ALT levels revealed more than 10 times the normal value.

Table 3 Human herpesvirus (HHV)-6 DNA levels in serial serum samples, and correlation with clinical signs at relapse

Patient no.	Age/sex	Causative drug	Days after onset	HHV-6 DNA copy number (copy mL^{-1})	HHV-6 IgG titre	Flaring of symptoms
1	44/M	Carbamazepine	12	0	80	
			13	3300	80	
			16	2 400 000	80	Fever (day 16–18)
			17	1 600 000	80	
			18	4300	320	Hepatitis (day 18, ALT 404)
			19	0	10 240	
2	22/M	Phenobarbital	15	0	20	
		Zonisamide	22	310 000	20	Hepatitis (day 24, ALT 1200)
			29	0	5120	
3	66/M	Mexiletine	20	0	< 20	
			24	1200	< 20	
			27	73 000	20	Hepatitis (day 27, ALT 505)
			32	0	640	
4	72/M	Phenytoin	12	6700	40	
			14	2800	40	Fever (days 14–17)
			15	57 000	80	
			18	0	10 240	
5	55/F	Carbamazepine	12	60 000	20	
			14	51 000	80	Hepatitis (day 15, ALT 519)
			18	0	640	
6	88/F	Carbamazepine	14	16 000	80	Fever (days 13–18)
			16	40 000	80	
			20	270	10 240	
			23	0	10 240	
7	59/F	Carbamazepine	13	0	40	
			15	1100	40	Fever (days 15–19), skin rash
			16	14 000	40	
			20	980	1280	Hepatitis (day 20, ALT 210)
			23	0	1280	
8	45/M	Allopurinol	9	0	80	
			11	5100	80	
			12	12 000	80	Hepatitis (day 12, ALT 365)
			17	0	10 240	
9	47/F	Phenytoin	23	0	40	
			24	3400	40	
			25	7800	40	
			26	1000	40	
			28	0	640	Hepatitis (day 28, ALT 97)
10	55/M	Phenytoin	13	0	80	
			19	6600	80	Fever (day 18–21)
			23	0	1280	Hepatitis (day 22, ALT 280), skin rash
11	28/M	Salazosulfapyridine	4	0	160	
			11	6200	160	Hepatitis (day 13, ALT 250)
			21	0	1280	
12	52/F	Allopurinol	19	0	20	
			25	6000	20	Fever (days 22–27)
			32	0	2560	
13	49/F	Carbamazepine	14	6000	20	Fever (day 14)
			28	0	1280	
14	39/F	Allopurinol	22	0	< 20	
			24	200	40	
			27	2900	40	Fever (day 25)
			34	0	1280	Hepatitis (day 31, ALT 666)
15	40/F	Mexiletine	11	0	80	
			14	750	80	Fever (days 14–16)
			19	0	1280	Hepatitis (day 17, ALT 143)

Table 3 (Continued)

Patient no.	Age/sex	Causative drug	Days after onset	HHV-6 DNA copy number (copy mL^{-1})	HHV-6 IgG titre	Flaring of symptoms
16	30/M	Carbamazepine	17	0	< 20	
			21	310	20	
			24	0	1280	Hepatitis (day 26, ALT 729)
17	78/M	Allopurinol	9	0	160	
			13	300	160	Fever (days 11–14)
			18	0	1280	Hepatitis (day 16, ALT 850)
18	51/F	Carbamazepine	11	0	160	
			23	120	5120	Hepatitis (day 23, ALT 200)
			26	0	20 480	

ALT, alanine aminotransferase (IU mL^{-1}).

IgG titre occurs simultaneously or subsequently with the detection of HHV-6 DNA in serum.

Correlations between the real-time polymerase chain reaction results and clinical symptoms

To evaluate a potential role for HHV-6, we correlated clinical signs with HHV-6 DNA levels in serum samples for 18 patients. A typical case is described in detail below.

Patient 17, a 78-year-old Japanese man, suffered from chronic nephritis but no known allergy. Fever and generalized skin rash developed 39 days after the initiation of allopurinol for hyperuricaemia. Allopurinol was stopped. Laboratory findings revealed leucocytosis ($1{\cdot}55 \times 10^{10}$ L^{-1}), eosinophilia ($6{\cdot}7 \times 10^{9}$ L^{-1}) and hepatitis [alanine aminotransferase (ALT) 211 IU mL^{-1}]. Although no systemic corticosteroid was given, his clinical symptoms, including fever, skin rash, leucocytosis, eosinophilia and hepatitis started to resolve within 10 days after onset. In his serum, HHV-6 DNA was detected on day 11 after onset of the symptoms and the highest copy number was observed on day 13. The flaring of high fever and hepatitis (ALT 850 IU mL^{-1}) developed simultaneously and atypical lymphocytes appeared. HHV-6 DNA disappeared on the 16th day of illness and his symptoms improved. However, skin rash and low-grade fever subsequently relapsed on the 24th day of illness, which aggravated his general condition. He died from bacterial pneumonia 2 months after onset of the symptoms.

As in this patient, the flaring of clinical signs and symptoms almost completely coincided with the detection of HHV-6 DNA in all 18 patients (Table 3). The observed signs were fever, hepatitis and skin rash. In six patients, the flaring hepatitis was coincident with fever for 3 or 4 days. In eight patients, the flaring of hepatitis alone was noticed. However, in five of these eight patients, including patients 2, 5, 8, 9 and 11, we could not evaluate an exacerbation of fever, because they had suffered from the long-lasting high fever which continued after the onset of the disease. The flaring of fever alone, which was observed in four patients, continued for a period of 1–6 days, coinciding with the detection of HHV-6 DNA in their serum samples. These results suggest that the flaring of fever and/or hepatitis is the most common feature of HHV-6 viraemia in DIHS.

Discussion

In this study, we analysed 100 patients with drug rash and systemic symptom(s) caused by drugs associated with DIHS, and demonstrated as follows: (i) a remarkable rise in HHV-6 IgG titres 14–28 days after the onset of symptoms in 62 patients; (ii) these 62 patients clearly showed severe symptoms and signs, and a prolonged course due to flaring compared with others; (iii) an active HHV-6 replication 10–27 days after the onset preceded the rise in antibody titres; and (iv) the flaring of fever and hepatitis was observed concurrently with HHV-6 reactivation.

A well-known interaction between drug hypersensitivity and viral infection is the ampicillin rash seen in patients with infectious mononucleosis.[21] Epstein–Barr virus infection is implicated in triggering the onset of drug rash after ampicillin administration. In contrast, drug allergy triggers HHV-6 reactivation in DIHS.

Bone marrow and solid organ transplant recipients sometimes develop HHV-6 reactivation within the first few weeks after transplantation.[22,23] Symptoms associated with HHV-6 reactivation after transplantation include fever, pneumonitis, meningitis, encephalitis, skin rash and hepatitis.[22,23] Interestingly, these clinical manifestations are in remarkable agreement with those observed in cases of DIHS with HHV-6 reactivation. Therefore, it is highly suggested that HHV-6 causes the reappearance of the signs seen in patients with DIHS.

Although the triad of skin rash, fever and multiple organ involvement has been generally used for diagnosis of this syndrome historically, milder cases can occur, in which symptoms rapidly improve without any treatment after drug discontinuation. This has caused confusion about the diagnosis. In 1996, Roujeau and colleagues proposed the term DRESS to define the syndrome.[5] Three criteria, including cutaneous

drug eruption, haematological abnormalities (eosinophilia or presence of atypical lymphocytes), and systemic involvement (adenopathies, hepatitis, interstitial nephritis, interstitial pneumonitis or carditis) must be fulfilled to diagnose this syndrome. Since the diagnostic criteria of DRESS still cannot exclude the milder cases, it seems to be insufficient. This drawback yields the misleading impression that DIHS/DRESS is not a distinct clinical entity. We think that prolonged course due to the flaring is the most noteworthy feature of this syndrome and should be included in the diagnostic criteria. Here, our study revealed that HHV-6 reactivation occurred in patients with severe DIHS and caused the flaring of this syndrome. Therefore, it is reasonable to define DIHS as a complex pathological condition involving both immunological reaction to a drug and HHV-6 reactivation. The definition can exclude the milder cases and make the concept of DIHS clearer. The demonstration of HHV-6 reactivation is a useful diagnostic marker of this syndrome.

It is generally believed that immunosuppressive conditions facilitate the reactivation of latent herpesviruses. Therefore, it is conceivable that systemic corticosteroid therapy induces immunosuppression, which leads to HHV-6 reactivation in DIHS. However, this possibility seems highly unlikely, for two reasons. Firstly, 12 of our 62 patients did not receive systemic corticosteroid therapy but still experienced HHV-6 reactivation. Secondly, HHV-6 reactivation was not observed in 10 patients who were diagnosed with Stevens–Johnson syndrome and toxic epidermal necrolysis, although these patients underwent intensive therapy with high doses of corticosteroid (data not shown). Nevertheless, we cannot exclude the possibility that the immune system of patients with DIHS may be suppressed by other mechanisms, because recent reports suggest that patients with DIHS show a decrease in immunoglobulin level.[24,25] The altered immune status may facilitate the reactivation of HHV-6.

In this study, we noticed that the incidence of HHV-6 reactivation in DIHS seems to correlate to the severity of signs in the early stages of DIHS. For instance, overlapping of systemic symptoms including long-lasting high fever, leucocytosis, severe hepatitis, and renal failure facilitated the reactivation of HHV-6. The appearance of these severe signs is the key to understanding the pathology of HHV-6 reactivation. Potent immune responses from drug-reactive T cells may be required for the induction of HHV-6 replication. This characteristic of drug-reactive T cells appears to resemble that of alloreactive T cells in cases of organ transplantation. Moreover, we found that the average age of the patients who developed increased anti-HHV-6 titres was below that of the other patients. Therefore, younger patients may have a more severe immune reaction to the drug, which could result in the reactivation of HHV-6. To clarify this issue, further investigation is needed.

In conclusion, we established a distinct clinical entity of DIHS that consists of a combination of immunological reaction to a drug and HHV-6 reactivation. HHV-6 reactivation is involved in the flaring and severity of this disease. This entity is stringently in accord with a concept of DIHS as a severe drug adverse reaction.

Acknowledgments

We thank Professor Tetsuo Shiohara (Department of Dermatology, Kyorin University School of Medicine) for his kind and helpful advice. This work was partly supported by Health Sciences Research Grants for Research on Specific Diseases from the Ministry of Health, Labor, and Welfare of Japan and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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The diagnosis of a DRESS syndrome has been sufficiently established on the basis of typical clinical features and viral reactivations

DOI: 10.1111/j.1365-2133.2007.07807.x

SIR, We read with great interest the article entitled 'Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist?' by Peyrière et al.[1] Based on data collected retrospectively from the French Pharmacovigilance database, the authors concluded that the acronym 'DRESS' is both inaccurate and quite imprecise with no clear definition regarding both cutaneous and systemic signs. We believe, however, that many of the authors' considerations are in contrast to the current knowledge about this syndrome and may be based on misinterpretations of these data probably due to their unawareness of several important findings uniquely observed in this syndrome.

Thirty years ago, the rules concerning the diagnosis of this syndrome had for the most part been formulated by non-dermatologists who defined this disease in terms of each organ involvement. As a result, sufficient attention had not been paid to the cutaneous features of this syndrome. In 1996, Bocquet et al.[2] coined the term 'drug rash with eosinophilia and systemic symptoms' (DRESS) for this syndrome to encompass these diverse clinical presentations. Subsequently, during the past 10 years, the clinical spectrum of this syndrome was defined: there have been no significant differences in the clinical findings of these cases reported under the name of DRESS. The lack of a specific and sensitive diagnostic test, however, was a major obstacle to correct identification of all patients with this syndrome. In this regard, my group and Hashimoto's group independently demonstrated that human herpesvirus 6 (HHV-6) can be reactivated at a particular time point, namely 2–3 weeks after onset of rash in the vast majority of patients with this syndrome, despite the diverse clinical presentations at onset: HHV-6 reactivation as evidenced by the rise in HHV-6 IgG titres and HHV-6 DNA levels commonly occurs 2–3 weeks after onset regardless of treatment.[3–6]

In 2006, we, a Japanese consensus group, established a set of criteria for diagnosis of drug-induced hypersensitivity syndrome (DIHS; Table 1), which have stood the test of time. Diagnosis of the typical syndrome requires all seven criteria. Importantly, our series of > 60 patients diagnosed by clinical findings has consistently shown that HHV-6 reactivation can be detected in the vast majority of patients who satisfy the other six criteria and show clinical manifestations consistent with those reported by Bocquet et al.,[2] but not in those with other types of drug eruption such as papulomacular rash, Stevens–Johnson syndrome and toxic epidermal necrolysis; in contrast, HHV-6 reactivation is rarely detected in patients with a tendency toward milder disease. These results will be reported by Tohyama and Hashimoto (Tohyama M and Hashimoto K, manuscript submitted). Thus, it appears that patients fulfilling the criteria of DIHS may represent those with a more severe form of DRESS.

Table 1 Diagnostic criteria for drug-induced hypersensitivity syndrome (DIHS) established by a Japanese consensus group[6]

1 Maculopapular rash developing > 3 weeks after starting with a limited number of drugs
2 Prolonged clinical symptoms 2 weeks after discontinuation of the causative drug
3 Fever (> 38 °C)
4 Liver abnormalities (alanine aminotransferase > 100 U L^{-1})[a]
5 Leucocyte abnormalities (at least one present)
a Leucocytosis (> 11×10^9 L^{-1})
b Atypical lymphocytosis (> 5%)
c Eosinophilia (> $1{\cdot}5 \times 10^9$ L^{-1})
6 Lymphadenopathy
7 Human herpesvirus 6 reactivation

The diagnosis is confirmed by the presence of the seven criteria above (typical DIHS) or of the five (1–5) (atypical DIHS). [a]This can be replaced by other organ involvement, such as renal involvement.

Based on these findings, we concluded that HHV-6 reactivation may be used to confirm a clinical diagnosis. Although there exists considerable clinical heterogeneity at onset even among patients in whom HHV-6 reactivations can be detected, a follow up several months after onset reveals a strikingly homogeneous clinical and biological profile: the other six criteria could be seen in sequence in all patients showing HHV-6 reactivation when they were followed up for a sufficient length of time. By the time our papers were published in 1998,[3,4] the link between this syndrome and HHV-6 reactivation was well established in Japan. However, concern has been raised about the appropriateness of the criteria as a clinical tool to identify patients with this syndrome, because the timing of sampling for detecting the rise in HHV-6 IgG levels is critical: unless sampling is performed at the right time, HHV-6 reactivation can be easily missed. Thus, the concept of an atypical syndrome can be used for patients with typical clinical presentations, in whom HHV-6 reactivation cannot be detected probably due to inappropriate timing of sampling. Following wide acceptance of the criteria, there has been little disagreement among dermatologists about the diagnosis of this syndrome in patients with obvious findings. However, we should bear in mind that the clinical criteria for this syndrome are not all present on any given day and that the severity of these clinical symptoms at onset provides only a guide to prognosis and is not absolute: usually patients initially develop two or three features of this syndrome followed by a step-wise development of other symptoms. Thus, a long-term follow up is needed to identify patients with this syndrome accurately. Because eosinophilia is seen at most in 60–70% of patients who satisfy the criteria, we propose that DRESS be replaced by the term DIHS to avoid confusion due to the lack of consensus in the literature about its terminology.[5–7] Thus, the important

criterion for the diagnosis of DIHS is the determination of HHV-6 reactivation, regardless of whether this is a causal factor or a consequence of disease. Unfortunately, however, Peyrière *et al.* did not specify this point in their patients, raising the possibility that they may have studied a very heterogeneous group of patients, presenting as a continuum from mild papulomacular rashes to full-blown DIHS.

This syndrome has several unique features that cannot be explained solely by a drug-based aetiology: they include delayed onset in relation to introduction of the causative drug and paradoxical worsening of clinical symptoms after discontinuation of the causative drug.[6,8] A major difficulty in establishing a correlation between causative drugs and the onset of this syndrome is such a long lag period before onset of clinical symptoms. However, large series of patients from Japan revealed that the drugs responsible for the development of DIHS are limited to eight drugs in the vast majority of patients: they include carbamazepine, phenytoin, phenobarbital, zonisamide, mexiletine, dapsone, sulfasalazine and allopurinol.[8] Atypical cases caused by other drugs, although reported, are much less common.

The lack of a longitudinal study including viral load evaluation in the authors' study may have made the unique clinical entity of DIHS uncertain. Once the suspicion of DIHS arises on the basis of initial history-taking and clinical presentations, a thorough investigation of viral reactivations should follow. As the recognition of this syndrome as a distinct clinical entity with highly reproducible clinical and laboratory features increases, it becomes clear that DIHS has potential long-term complications, such as type 1 diabetes mellitus,[9] even after disease-free intervals of months or years. The diagnosis is unlikely to be missed if the possibility of this syndrome is considered in the differential diagnosis of any patients with fever, rash, lymphadenopathy and hepatitis, and if HHV-6 IgG titres are routinely examined at the right time. HHV-6 reactivation would be the diagnostic marker for DIHS that is reliable and easy to determine on a routine basis. The incidence of this syndrome is much greater than previously thought. If this unique disease is viewed only as a reaction pattern and a search for viral reactivations is not made, the disease may remain idiopathic as it was in the past.

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Conflicts of interest: none declared.

A case of herpes zoster in a child with congenital insensitivity to pain with anhidrosis

DOI: 10.1111/j.1365-2133.2007.07804.x

Sir, During primary infection with varicella-zoster virus (VZV), the virus ascends the sensory nerve from the skin sensory nerve ending to which it disseminates through viraemia, and migrates up the dorsal root and trigeminal ganglia, where it usually remains latent for the lifetime of the individual. When VZV-specific cellular immunity is reduced, the latent VZV is reactivated, descends the sensory nerve, reaches the skin, and causes herpes zoster.[1,2] In this paper, we describe a case of herpes zoster associated with congenital insensitivity to pain with anhidrosis (CIPA).

A 3-year-old boy with CIPA had developed varicella at 2 months of age. His elder brother has CIPA, and his parents and a sister are normal. Vesicles were distributed in the right alinasal region 2 days before presentation, and had gradually deteriorated. Oedematous erythema with a clear boundary and grouped vesicles were noted in the region below the right nasal foramen, over the right cheek and

Allergy 2007: 62: 1439–1444

DOI: 10.1111/j.1398-9995.2007.01553.x

Original article

Utility of the lymphocyte transformation test in the diagnosis of drug sensitivity: dependence on its timing and the type of drug eruption

Background: Lymphocyte transformation test (LTT) is a safety and reproducible test to assess activation of drug-specific T cells *in vitro*; however, there are several practical concerns such as the time of testing and the influence of treatment. Our aim was to define the right timing to perform LTT for determining the causative agent in various types of drug reactions.
Methods: Lymphocyte transformation test was performed at different time points during the evolution of three types of drug reactions, maculo-papular type of drug eruptions (MP), Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), and drug-induced hypersensitivity syndrome/drug rash and eosinophilia with systemic symptoms (DIHS/DRESS).
Results: Positive LTT reactions were obtained when the test was performed at the acute stage but not the recovery stage in MP and SJS/TEN, while positive LTT reactions were obtained at the recovery stage but not the acute stage in DIHS/DRESS, regardless of treatment with systemic prednisolone.
Conclusions: Lymphocyte transformation test is a reliable method to define the causative agent, when LTT is performed at the right timing depending on the type of drug reactions. Lymphocyte transformation test should be performed within 1 week after the onset of skin rashes in patients with MP and SJS/TEN; and 5–8 weeks after in patients with DIHS/DRESS, respectively.

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Key words: DIHS; DRESS; drug reaction; lymphocyte transformation test; SJS; toxic epidermal necrolysis.

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Accepted for publication 18 August 2007

Activation of drug-specific T cells is generally thought to play a central role in mediating adverse drug reactions, such as Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-induced hypersensitivity syndrome/drug rash and eosinophilia with systemic symptoms (DIHS/DRESS) (1–5). In clinical settings, patch tests and lymphocyte transformation tests (LTTs) have been often used for the diagnostic assessment of drug-specific T cell responses (6–19). Laboratory-based *in vitro* technology such as LTT offers numerous advantages including absolute safety, simultaneous assessment of T cell responses to multiple drugs, and a lack of risk of developing additional drug allergies. In fact, the LTT has been shown to have a better diagnostic value than skin tests to identify causative agents (11, 12). Nevertheless, a number of practical concerns, including the time of testing and the influence of therapy, such as systemic corticosteroids, continue to limit their frequent application to clinical diagnosis. Because blood samples sequentially obtained from a sufficient number of patients with various types of severe adverse drug reactions are simply not available, whether the timing of sampling or the type of drug reactions could influence the outcome of the test remains to be determined.

In this study, LTT was sequentially performed during the evolution of the disease in patients with various types of drug reactions and repeated several months to 1 year later. Despite having been widely believed that false negative LTT reactions are observed in the acute stage regardless of type of drug reactions, our results indicate that positive LTT reactions can be observed when tests are performed in the acute stage except for DIHS/DRESS, in which positive LTT reactions can be exclusively observed 5–8 weeks after onset.

Methods

Patients

Between 1998 and 2006, 27 patients (16 male and 11 female; age range 1–87 years, mean age 48.0 ± 21.8 years) who developed adverse drug reactions and treated in our hospital were enrolled in this study. This study has approved by the Institutional Review Board at Kyorin University School of Medicine. The adverse drug reactions were divided into three groups according to clinical presentation. Maculo-papular type of drug eruptions (MP, eight patients); SJS/TEN (SJS, six patients and TEN, two patients); and DIHS/DRESS (11 patients). Maculo-papular type drug eruptions was diagnosed based on the clinical manifestations, showing

Table 1. Characteristics of patients

Type of drug eruption	MP	SIS/TEN	DIHS/DRESS
Numbers of patient	8	8 (SIS 6; TEN 2)	11
Sex (male/female)	5/3	3/5	8/3
Age (years) (mean ± SD)	49.0 ± 17.0	56.3 ± 25.3	41.0 ± 21.9
Time to onset (days)* (mean ± SD)	4.7 ± 4.5#	11.1 ± 8.2#	34.0 ± 11.0
Causative drags (numbers of patient)	Acetaminophen (2) Amoxicillin (1) Scopolamine butylbromide (1) Tiaprofenic acid (1) Loxoprofen sodium (1) Meropenem (1) Sultamicillin (1)	Acetaminophen (3) Minocycline (2) Bromhexine (1) L-carbocicsteine (1) Phenytoin (I)	Carbamazepine (5) Phenobarbital (2) Phenytoin (2) Mexiletine (1) Dapson (1)

MP, maculo-papular type drug eruptions; SJS/TEN, Stevens–Johnson syndrome/toxic epidermal necrolysis; DIHS/DRESS, drug-induced hypersensitivity syndrome/drug rash and eosinophilia with systemic symptoms; *Duration between first intake of the drug and onset of skin rashes and #$P < 0.005$ *vs* in DIHS/DRESS.

generalized morbilliform eruptions. All patients analyzed met full criteria for SJS/TEN or DIHS/DRESS described below. The clinical criteria used for the diagnosis of SJS were widespread erythematous macules or flat atypical targets and detachment below 30% of the body surface area; those for TEN were widespread erythematous macules or flat atypical targets and detachment above 30% of the body surface area (13): and those for DIHS/DRESS were high fever, a widespread maculo-papular and/or diffuse erythematous eruption, lymphadenopathy, leukocytosis with atypical lymphocytosis and/or eosinophilia, and liver dysfunction (14–18). The clinical features and some laboratory findings of these patients are summarized in Table 1. The causative drugs were withdrawn when the diagnosis of drug reactions were made, usually followed by significant improvement in patients with MP, but neither in those with SJS/TEN nor DIHS/DRESS. Because of the risk of reproducing severe reactions, these patients were not challenged with the causative drug. The duration between the first intake of the drug and onset of skin rashes in DIHS/DRESS was 34.0 ± 11.0 days, which was significantly longer than the others ($P < 0.005$). After informed consent was obtained, heparinized blood was obtained via venipuncture at the time of each visit from each patient; at least two occasions, the first during the acute stage and the second long after recovery from the disease. The acute stage measurement of stimulation index (SI) levels in LTT was performed within 1 week after the skin rashes in all but two patients, while the recovery stage measurement of those with MP, SJS/TEN and DIHS/DRESS was performed 86.7 ± 84.2 (range: 20–244), 61.7 ± 39.1 (range: 34–140), and 79.6 ± 53.7 (range: 21–165) days after the onset, respectively. All patients with MP were treated with supportive therapy alone. Six out of eight patients with SJS/TEN were treated with systemic prednisolone 0.8–1 mg/kg daily; two out of the eight patients had been treated with prednisolone before admission to our hospital. In contrast, six out of 11 patients with DIHS/DRESS were treated with systemic prednisolone 1 mg/kg daily after admission, but in four out of the six patients, the acute stage measurement of LTT was performed before the administration of systemic prednisolone. The other patients with DIHS/DRESS were treated with supportive therapy alone. There were no patients with human immunodeficiency virus infection.

Lymphocyte transformation test

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood utilizing a Ficoll Hypaque (Sigma Chemical, St Louis, MO, USA) gradient. *In vitro* proliferation assays were performed as previously described by Pichler and Tilch (1), with some modifications. Briefly, PBMC were cultured in wells containing 200 µl RPMI-1640 medium supplemented with 20 mM HEPES, 1 mM nonessential acids, 5×10^{-5} M 2-mercaptoethanol, and 10% autologous plasma obtained from the density-gradient centrifugation, in a round-bottom microtiter plate (Becton-Dickinson, Lincoln Park, NJ, USA). The culprit drugs used for LTT assays were unmodified parent drug compounds dissolved in culture medium and they were sonicated to solve in the mediums. Dilutions of the drug used for LTT were generally started with 40–120 µg/ml that correspond to a 1/50 dilution of each known therapeutic dose and the drug was further diluted in fivefold steps up to 0.32–0.96 µg/ml that correspond to a 1/6250 dilution because our preliminary experiments showed that these concentrations of drugs were nontoxic for the lymphocytes and that positive LTT reactions were mostly obtained within these concentrations of a variety of drugs. Cultures were performed in triplicate at 37°C and 5% CO_2 for 5 days. As positive and negative controls, cells in triplicate were also incubated in the presence of 5 µg/ml phytohemagglutinin (PHA), and in the absence of these agents, respectively. Twenty-four hours before harvesting 1 µCi ^{3}H-thymidine (Amersham, Arlington Heights, IL, USA) was added. After harvesting radioactivity was measured in a liquid scintillation counter (Pharmacia LKB Nuclear, Gaithersberg, MD, USA) and the results were expressed as the SI; SI was calculated as follows: SI = counts per minute (c.p.m.) with drug/c.p.m. without drug. An SI of more than 1.8 was regarded as positive, based on previous studies preformed in Japan (6, 10, 19–21). In most cases, the optimal drug concentration was found to be 1/50–1/100. In some experiments, 10 control patients without a previous history of allergic drug reactions but with some cutaneous diseases were included in this study. The LTT was also performed in control patients who were taking the drugs used for this analysis for ≥3 months without any clinical symptoms. The *t*-test was used to assess the analyses of variable values of LTT, with $P < 0.05$ considered statistically significant.

Results

The 12 patients (MP, two patients; SJS, two patients; and DIHS/DRESS, eight patients) out of the 27 patients were followed for a total of 37 visits (at least three visits per patient). The results of analysis performed on the 37 visits are shown in Table 2. In patients with MP and SJS/TEN,

Table 2. Time-course analyses of SI in LTT

Type of drug eruption	Time from the onset of skin rashes					
Number of causative drug	1 week	2–4 weeks	5–8 weeks	12–16 weeks	1 year	>1 year
MP						
1. Scopolamine butylbromide	1.89 (1484)	1.73 (1907)	1.65 (3376)			
2. Amoxicillin	5.45 (1020)		2.12 (2704)	1.75 (1462)		
SIS						
3. Minocycline	2.47* (191)	1.29 (212)	1.11 (310)			
4. Acetaminophen	1.96 (658)	1.56 (202)	0.90 (194)			
DIHS/DRESS						
5. Carbamazepine		0.93* (1383)	1.09* (1250)	3.03 (1988)		
6. Carbamazepine	1.88 (299)				5.54 (1401)	8.95 (413)
7. Carbamazepine	1.28 (1156)	1.46 (613)	4.82 (276)			
8. Carbamazepine	2.66 (273)	1.46 (1224)		1.91 (1396)		
9. Phenobarbital	1.19 (870)	1.18* (346)	2.49* (598)			5.00 (918)
10. Phenytoin	1.68 (450)	4.42 (375)	6.16 (346)			
11. Mexiletine	0.74 (852)	1.28* (229)	2.88* (1228)			
12. Dapsone	1.08 (887)			3.94* (1102)		9.75 (2628)

SI, stimulation index; LTT, lymphocyte transformation test; MP, maculo-papular type drug eruptions; SJS/TEN, Stevens–Johnson syndrome/toxic epidermal necrolysis; DIHS/DRESS, drug-induced hypersensitivity syndrome/drug rash and eosinophilia with systemic symptoms. *Treated with systemic prednisolone; values in the parentheses denotes the background counts per minute without drug.

positive LTT reactions were obtained when the test was performed within 1 week after onset; however, the levels of SI decreased with time. In contrast, negative results were observed in five out of the seven patients with DIHS/DRESS at the 1 week measurement, at which time no systemic prednisolone was given. Even when the test was repeated at 2–4 weeks, only one patient showed a positive result. At the 5–8 week measurement, all but one patient with DIHS/DRESS exhibited positive LTT reactions. The positive LTT reactions remained detected even 1 year after recovery in all three patients examined. These positive reactions observed after complete recovery was not related to the drug used. The use of prednisolone during the acute stage did not have an impact on SI (Table 2).

When the analysis was performed at two occasions, the acute and recovery stages, in 27 patients including the remaining 15 patients, we found very similar results in three groups, as demonstrated in Table 2. In eight patients with MP there was no significant difference in SI levels of LTT between the acute stage and recovery stage (SI: 2.41 ± 1.26 *vs* 1.94 ± 0.63, $P = 0.420$), although positive LTT reactions were obtained in 7seven out of the eight patients at the acute stage and in three out of the eight at the recovery stage, respectively. In the vast majority of patients with SJS/TEN, positive LTT reactions were obtained only when the test was performed at the acute stage. Of note, SI levels of LTT were higher in two patients with TEN than those in patients with SJS (Fig. 1A). The positive LTT reactions at the acute stage became negative at the recovery stage; there was a significant difference between the two stages (SI: 2.43 ± 1.30 *vs* 1.19 ± 0.24, $P = 0.049$). The SI in all patients with SJS/TEN at the recovery stage did not exceed 1.80, a cut off value employed here. In contrast, negative results of LTT were constantly observed in patients with DIHS/DRESS independently of drugs given, when the tests were performed at the acute stage. At the recovery stage, the majority of patients with DIHS/DRESS exhibited high levels of SI (Fig. 1B). In contrast, the SI ≥ 1.80 was never observed in control patients who received the same drug for ≥3 months but not have clinical symptoms ($n = 4$), confirming that an SI ≥ 1.80 can be regarded as positive.

To determine whether the loss of LTT reactions to the causative drug during the acute stage of DIHS/DRESS and the recovery stage of SJS/TEN was associated with global loss of responsiveness to the mitogen stimulation, we also examined the capacity of PBMC from the same patients to respond to stimulation by the mitogen PHA. The reason for using PHA rather than tetanus toxoid frequently used as a positive control for LTT was to reveal full proliferative potential of T cells and try to minimize variability in kinetics resulting from antigen processing and/or presentation by antigen-presenting cells which would be variably present in the PBMC analyzed. Responses to PHA were somewhat higher in patients with SJS/TEN than those with DIHS/DRESS regardless of the stage examined. Nevertheless, there was no significant difference in responses to PHA in three groups between the acute and recovery stage.

Because our studies were not initiated as prospective studies of the effect of systemic prednisolone on the LTT, the choice of treatment with systemic prednisolone did not follow a predetermined protocol, but was dependent on the judgment of the individual dermatologist. On