ラモトリギンによる薬剤性過敏症症候群（DIHS）

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Key words 薬剤性過敏症症候群，ラモトリギン

図1 30歳台，女性．初診時臨床像
体温 38.9℃．（a）顔面は眼周囲をさけびまん性に紅斑を認め，口唇周囲に膿疱を認めた．（b）躯幹・四肢には浸潤を触れる紅色紅斑が多発・癒合していた．粘膜疹なし．リンパ節腫脹なし．

■ 症 例

30歳台，女性．
主 訴：発熱を伴う全身の紅斑．
現病歴：双極性障害に対して某年6月からオランザピン（ジプレキサ®），同年8月よりラモトリギン（ラミクタール®）による治療を行っていた．ラミクタール®は最初の2週間は1日25 mg投与，次の2週間は1日50 mg投与，5週目から1日75 mg投与に増量され，添付文書に準拠して使用されていた．1日75 mgに増量6日目（ラミクタール®開始34日目）から躯幹に皮疹を自覚し徐々に拡大した．皮疹出現2日後から39℃台の発熱を認め，皮疹出現4日後に当科を受診した．
初診時現症：顔面は腫脹し，眼周囲を避けびまん性に紅斑を認め（図1a），全身に浸潤を触れる紅色紅斑が多発・癒合していた（図1b）．体温は 38.9℃で，リンパ節腫脹は認めなかった．

■ 鑑別疾患と臨床診断

ラミクタール®漸増中に発症したため，ラミクタール®による薬剤性過敏症症候群（DIHS）を疑った．その他，多形滲出性紅斑，ウイルスや細菌などによる中毒疹が鑑別としてあげられた．

■ 検査と確定診断

初診時血液検査所見：WBC 4,600 /μL（↑）（分節核球 79.2%（↑），リンパ球 15.4%（↓），好酸球 1.5%，異型リンパ球 0%），AST 43 IU/L（↑），ALT 40 IU/L，LDH 406 IU/L（↑），CRP 1.18 mg/dL（↑），TARC 4,459 pg/mL（経過中の最高値：WBC 12,300/μL（↑），好酸球数 110，異型リンパ球 0%，AST 43 IU/L（↑），ALT 95 IU/L（↑），LDH 406 IU/L（↑））．
病理組織学的所見：基底層に液状変性を認め，真皮上層は浮腫状で，血管周囲性に好中球・リンパ球・好酸球が浸潤していた（図2）．
薬剤添加リンパ球刺激試験（DLST）：皮疹出現8日後に行った DLST はラミクタール®，ジプレキサ®どちらも陽性（SI値：ともに 2.4）だったが，16日後に行った DLST ではラミクタール®のみ陽性（SI値：2.8）であった．経過中，DIHS の診断基準（→ p.820，総論参照）の

特集 いま注目の薬疹トピックス

case 6　ラモトリギンによる薬剤性過敏症症候群（DIHS）
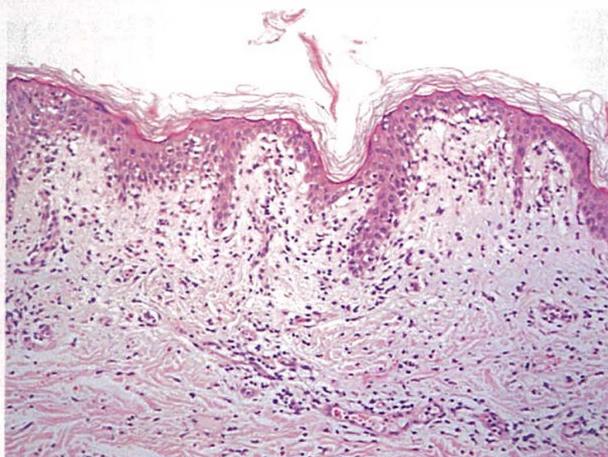
図 2　病理組織学的所見
右腰部の浸潤を触れる紅斑より生検を施行した．基底層に液状変性を認め，真皮上層は浮腫状で，血管周囲性に好中球・リンパ球・好酸球が浸潤していた．

主要所見 7 項目中 1 〜 5 項目を満たしたが，主要所見の「6. リンパ節腫脹」と「7. HHV-6 の再活性化」は認めなかったため，ラミクタール® による非典型 DIHS と診断した．

■治療と経過

プレドニゾロン 50 mg/ 日（1 mg/kg/ 日）内服により治療を開始した．加療翌日より 36℃ 台に解熱し，約 7 日間で紅斑は消褪傾向を示した．プレドニゾロンは徐々に漸減し，計 32 日で内服を終了した．以後再燃はない．

■本症例のポイント

1）ラモトリギンによる重症薬疹

本症例は病歴，臨床像，経過からラモトリギンによる非典型 DIHS と診断した．ラモトリギンは欧米で 1990 年代から使用されており，重症薬疹の報告が多い薬剤であるカルバマゼピン，フェニトイン，フェノバルビタールと同様に，使用にはその発症への注意が必要と報告されている [1]．本邦でも 2015 年 2 月にブルーレターが出され，DIHS など重篤な皮膚障害が生じうること，用法・用量を遵守し，皮膚障害の早期発見，早期治療に努めるように警告が発せられた．

ラモトリギンの皮膚障害の発現増加・重篤化の危険因子として，①用法・用量の非遵守例，②バルプロ酸併用例，③他の抗てんかん薬による薬疹既往歴，④ 13 歳以下の小児，⑤投与 8 週間以内があげられている [2]．

本症例では⑤の投与 8 週間以内が該当し，投与量の漸増に関しても用法・用量は遵守されていた．

2）ラモトリギンによる DIHS と他剤による DIHS との相違点

2008 年の承認以降，本邦でのラモトリギンによる DIHS の報告例が散見されるが，他剤による DIHS と比較して，いくつか異なる点が指摘されている．白血球数がはじめは低値を示し遅れて増加する，HHV-6 抗体の再活性化が少ない，またはあっても再活性化の時期が一定でない [3,4] といったことである．

自験例でも初診時の白血球数は基準値内であり，遅れて白血球数の増加がみられ，HHV-6 の再活性化はみられなかった．また，ステロイド投与後は比較的速やかに解熱，紅斑の消褪がみられ，治療への反応性は良好であった．このように非典型例で比較的速やかに改善が認められた症例 [5] や，ステロイド全身投与をせず保存的療法で軽快した症例 [2,4] も報告されている．

ラモトリギンにおける DLST の陽性率は海外の報告では一定していないが，本邦における報告では陽性率が高いとされている [6]．自験例も皮疹出現 8 日後と 16 日後に行った DLST が陽性であった．ラモトリギンによる薬疹では DLST が原因薬の検査法として有用と考えられる．

以上のように本剤による DIHS と他剤による DIHS との相違点が複数報告されているが，これらの点が本剤による DIHS の特徴と結論づけてよいかどうか，今後の症例集積と統計学的解析が待たれる．

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ORIGINAL ARTICLE

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms due to lamotrigine differs from that due to other drugs

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ABSTRACT

Drug-induced hypersensitivity syndrome (DIHS), also referred to as drug reaction with eosinophilia and systemic symptoms (DRESS), is a multi-organ systemic drug reaction characterized by hematological abnormalities and reactivation of human herpesvirus-6 (HHV-6). DIHS/DRESS is typically associated with a limited number of drugs, such as the anticonvulsants. Our group has treated 12 patients for DIHS/DRESS due to lamotrigine (LTG), but their presentation differed from that of patients with DIHS/DRESS caused by other drugs. The aim of the present study was to identify significant differences between DIHS/DRESS caused by LTG versus other drugs. We retrospectively reviewed data of 12 patients with DIHS/DRESS caused by LTG and 32 patients with DIHS/DRESS due to other drugs. The increase in alanine aminotransferase level was significantly milder in the LTG group than the DIHS/DRESS group due to other drugs. The percentage of atypical lymphocytes in the blood during DIHS/DRESS was lower in the LTG group. Serum levels of lactate dehydrogenase and thymus and activation-regulated chemokine were also lower in the LTG group. There were fewer DIHS/DRESS patients with HHV-6 reactivation in the LTG group than in the group treated with other drugs. Lymphocyte transformation after DIHS/DRESS onset was faster in the LTG group. The two groups did not differ with respect to the interval from first drug intake to rash, white blood cell count, blood eosinophilia or DRESS score. There were no significant histopathological differences between the two groups. The features of LTG-associated DIHS/DRESS and DIHS/DRESS due to other drugs differ.

Key words: drug reaction with eosinophilia and systemic symptoms, drug-induced hypersensitivity syndrome, human herpes virus 6, lamotrigine, thymus and activation–regulated chemokine.

INTRODUCTION

Drug-induced hypersensitivity syndrome (DIHS),[1–3] also referred to as drug reaction with eosinophilia and systemic symptoms (DRESS),[4–6] is characterized by severe skin eruption, fever, lymphadenopathy, hepatitis, hematological abnormalities with eosinophilia and atypical lymphocytes and, in some cases, the involvement of other organs.[1–6] Recent reports have shown that human herpesvirus-6 (HHV-6) reactivation contributes to the development of DIHS/DRESS.[1,2] Compared with other types of drug eruptions, the onset of

DIHS/DRESS tends be late (2–8 weeks or more after drug exposure).[1–7] Human HHV-6 DNA is detected in the serum 3–5 weeks after the onset, followed by a dramatic rise in anti-HHV-6 immunoglobulin (Ig)G titers.[3,7] According to published data, among patients with DIHS/DRESS, 75–95% have leukocytosis,[4,8] 18.2–90% show atypical lymphocytes,[8,9] 52–95% have eosinophilia[5,6] and 75–100% develop hepatic abnormalities.[5,8]

A limited number of drugs cause DIHS/DRESS, namely anticonvulsants, such as carbamazepine, phenytoin, phenobarbital and zonisamide, as well as allopurinol, diaphenylsulfone,

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salazosulfapyridine and mexiletine.[1–7] Lamotrigine (LTG) is an antiepileptic drug that is also effective for the treatment of bipolar disorder.[10] In Japan, LTG was approved as add-on therapy for patients with recalcitrant epilepsy in 2008. In 2011, approval was granted for its use for suppression of recurrent/relapsed mood episodes in patients with bipolar disorder, and in 2014 as monotherapy in epileptic patients.[11] The primary safety concern with LTG is drug eruption, with ordinary eruption occurring in approximately 10% of patients and serious eruption in approximately 0.1%.[12] To date, our group has treated 12 patients with DIHS/DRESS due to LTG. The presentation in these patients, such as liver dysfunction, differed from that in patients treated with other drugs. Therefore, in this study we investigated differences in DIHS/DRESS between 12 patients treated with LTG and 32 patients receiving other drugs.

METHODS

This study was approved by the ethics committees of Showa University School of Medicine, Nara Medical University School of Medicine, Kyorin University School of Medicine and Shimane University School of Medicine (all Japan), and was conducted according to the Declaration of Helsinki. Informed consent for all diagnostic and research procedures was obtained from all participating patients.

Drug-induced hypersensitivity syndrome was diagnosed according to the criteria established by the Japanese consensus group:[13] high fever, widespread eruption, lymphadenopathy, leukocytosis with atypical lymphocytosis and/or eosinophilia, and liver dysfunction. The data of 44 patients seen at our hospital for DIHS/DRESS between 1 April 2000 and 31 August 2018, and who satisfied the full criteria for DIHS, were retrospectively evaluated. There were 12 patients with DIHS/DRESS caused by LTG. There were 32 patients with DIHS/DRESS due to other drugs; data on 20 of these patients were also used in other studies.[8]

Time from disease onset to the first visit to our hospital, the results of blood examinations, the presence/absence of HHV-6 reactivation and the results of lymphocyte transformation tests (LTT) were evaluated. Biopsy specimens were available for 28 of the 44 patients.

White blood cell (WBC) counts (normal range, 3500–9000/μL) were determined both at the initial examination and at the time of maximum disease severity. Eosinophils, serum lactate dehydrogenase (LDH; normal range, 105–220 U/L) and serum alanine aminotransferase (ALT; normal range, 5–25 IU/L) levels were determined at the time of maximum disease severity. The serum thymus and activation-regulated chemokine (TARC/CCL17; normal range, <450 pg/mL) level was measured using a chemiluminescent enzyme immunoassay with the HISCL system (Sysmex, Hyogo, Japan) with a TARC assay kit (Shionogi, Osaka, Japan). The highest value of TARC during DIHS/DRESS was included in the analysis in this study.

Human herpesvirus-6 infection was evaluated by serological tests of serum samples upon patient admission and at various times thereafter. Titers of IgG and IgM antibodies to HHV-6

were determined in all DIHS/DRESS patients using an indirect immunofluorescence antibody assay. Serum HHV-6 DNA was measured using real-time polymerase chain reaction (PCR), as described previously.[1,14] HHV-6 reactivation as evidenced by the increase in HHV-6 IgG titers and HHV-6 DNA levels commonly occurs 2–3 weeks after onset.[13]

Lymphocyte transformation tests are commonly performed in Japan because the test is covered by health insurance agencies as a method for diagnosing cutaneous adverse drug reactions (ADR). All patients (12 patients with DIHS/DRESS caused by LTG and 32 patients with DIHS/DRESS due to other drugs) were examined by LTT. LTT were performed as described previously.[14] Briefly, peripheral mononuclear cells separated by density-gradient centrifugation were cultured with each possible causative drug for 7 days and the stimulation index (SI), obtained by measuring lymphocyte proliferation, was compared with that of a control. SI of more than 1.8 was considered a positive result.

The RegiSCAR scoring system[15] was developed to more clearly define DIHS/DRESS, and patient scores were evaluated in this study.

Histopathological features were investigated by hematoxylin–eosin staining of skin biopsy samples obtained from the 28 DIHS/DRESS patients for whom biopsy data were available. The histopathological features of DIHS/DRESS were classified into four patterns, as described by Ortonne et al.:[16] interface dermatitis (ID), eczematous, acute generalized exanthematic pustulosis (AGEP)-like and erythema multiforme (EM)-like. Briefly, the ID pattern was defined as basal lymphocyte exocytosis with keratinocyte vacuolization and/or apoptosis; the eczematous pattern as a grade 2 or 3 spongiosis with lymphocytes exocytosis; AGEP-like as a multilocular subcorneal or intracorneal pustulosis; and EM-like as slight to moderate acanthosis with orthokeratotic hyperkeratosis and perivascular infiltrations in the upper dermis.[16] The presence of apoptotic keratinocytes in the epidermis was also examined. The histopathological findings were examined by three experts in dermatopathology (M. I., H. S. and H. W.).

Data analysis

The Mann–Whitney U-test and Fisher's exact test were used to identify significant differences between groups. The data are expressed as means ± standard error. P < 0.05 was considered to indicate statistical significance in all tests.

RESULTS

Patient data

The data of 44 patients who satisfied all criteria for DIHS[13] were retrospectively evaluated. In 32 patients (21 males and 11 females), DIHS/DRESS occurred due to the usual causative drugs: carbamazepine (n = 15), allopurinol (n = 4), phenobarbital (n = 3), salazosulfapyridine (n = 2), mexiletine (n = 2), zonisamide (n = 2), and dapsone, febuxostat, phenytoin and trichloroethylene (n = 1 each).[17] Twelve patients (five males and seven females) developed DIHS/DRESS due to LTG use. The mean age in the group treated with the usual drugs (UD

group) and the LTG group was 49.3 and 40.9 years, respectively (Table 1). A previous report[18] showed a greater predominance of women (66.67% female and 33.33% male patients, F : M = 2:1) with DIHS/DRESS due to LTG, and 68.42% of patients were over 18 years of age. We did not observe significant sex differences, but 11 out of 12 DIHS/DRESS cases due to LTG were over 18 years of age. However, only a small number of cases were included in this report, and further studies are required.

Liver dysfunction in DIHS/DRESS is significantly milder in patients treated with LTG than with other drugs

An essential feature in the diagnosis of DIHS/DRESS is liver dysfunction,[3,7] which is the most characteristic finding of this drug eruption. In this study, all 44 patients had hepatic abnormalities, as evidenced by their serum ALT levels being above the normal range (5–25 IU/L). However, liver dysfunction was significantly milder in the LTG group (mean, 110.6 ± 26.1 IU/L) compared with the UD group (mean, 328.1 ± 61.4 IU/L; $P < 0.01$; Fig. 1). In previous reports,[18] 57.89% of DIHS/DRESS patients experienced liver dysfunction (ALT, >100 IU/L) due to LTG. In our study, liver dysfunction (ALT, >100 IU/L) was found in 33.3% of patients in the LTG group and 75% (ALT, >100 IU/L) in the UD group. Therefore, liver dysfunction from DIHS/DRESS due to LTG appears milder compared with that caused by other drugs.

Percentage of atypical lymphocytes, but not white blood or eosinophil counts, are significantly lower in patients treated with LTG than with other drugs

Because leukocytosis with atypical lymphocytes of varying amounts is a prominent feature of DIHS/DRESS,[3,7] we investigated whether the two groups differed in their blood examination results. WBC counts exceeding 11 000/μL (normal range, 3500–9000) during the clinical course were found in nine of the 12 patients (75.0%) in the LTG group and in 27 of the 32 patients (84.4%) in the UD group. There was no significant
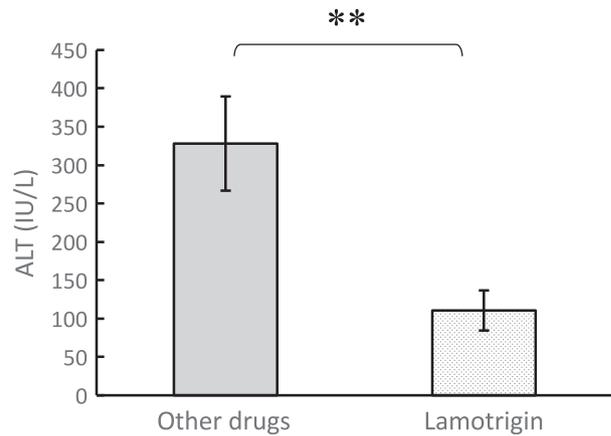
Figure 1. Serum alanine aminotransferase (ALT) levels in drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) caused by lamotrigine (LTG group) and the usual drugs (UD group). Patients in both groups had hepatic abnormalities, based on serum ALT levels that were above the normal range (5–25 IU/L). However, mean liver dysfunction was significantly milder in the LTG group than in the UD group (110.6 ± 26.1 vs 328.1 ± 61.4 IU/L, **$P < 0.01$).

difference in WBC count between the two groups during the course of the disease. Atypical lymphocytes were found in 10 patients (83.3%) in the LTG group and 30 patients (93.8%) in the UD group. The mean percentage of atypical lymphocytes (maximum value during the disease course) was significantly lower in the LTG group than in the UD group (mean, 3.38 ± 1.03% vs 9.83 ± 1.65%, respectively; $P < 0.05$; Fig. 2). Eosinophilia (≥1500/mm³; normal range: 70–440/μL) was noted in six of 12 patients (50.0%) in the LTG group and in 21 of 32 patients (65.6%) in the UD group during the clinical course of DIHS/DRESS. There was no significant difference between the LTG and UD groups in the incidence of eosinophilia or the mean eosinophil count in WBC (2391.4 ± 574.3 vs 3448.6 ± 569.4 mm³, respectively) during the disease course.

DIHS/DRESS-related serum LDH levels are significantly lower in patients treated with LTG versus other drugs

Mean serum LDH levels were significantly lower in cases with DIHS/DRESS caused by LTG (453.1 ± 54.2 U/L) than in those caused by other drugs (639.6 ± 78.2 U/L, $P < 0.05$; Fig. 3a).

DIHS/DRESS-related serum TARC/CCL17 levels are significantly lower in patients treated with LTG versus other drugs

A previous report demonstrated a correlation between serum TARC levels of patients in the acute stage of DIHS/DRESS and disease activity.[19,20] In our patients, mean serum TARC levels were significantly lower in the LTG group than in the UD group (4442.0 ± 1027.8 vs 14 736.3 ± 3334.6 pg/mL, $P < 0.05$; Fig. 3b).

Table 1. Characteristics of the patients

	Other drugs	Lamotrigine
Numbers of patients	32	12
Sex (male/female)	21/11	5/7
Age (years, mean ± SE)	49.3 ± 2.81	40.9 ± 4.37
Causative drug (numbers of patients)	Carbamazepine (15)	Lamotrigine (12)
	Allopurinol (4)	
	Phenobarbital (3)	
	Mexiletine (2)	
	Salazosulfapyridine (2)	
	Zonisamide (2)	
	Dapsone (1)	
	Febuxostat (1)	
	Phenytoin (1)	
	Trichloroethylene (1)	

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Table 2. Histological features of patients with DIHS/DRESS

Histological pattern	Lamotrigine	Other drugs	Total	Apoptotic cells	DRESS score (mean)	HHV-6 reactivation
EM	0	7	7	2	6.29	6
ID	3	5	8	3	5.38	3
Eczema	0	0	0	–	–	–
AGEP	0	1	1	–	8	1
LTR	1	1	2	1	5	1
EM+ID	0	3	3	2	6.67	1
EM+Eczema+AGEP	0	1	1	–	5	–
EM+AGEP+ID	1	1	2	–	7.5	1
EM+AGEP+LTR	0	1	1	1	7	1
ID+AGEP	0	1	1	1	7	–
ID+LTR	0	2	2	1	6.5	1

Data are numbers of patients unless otherwise stated. AGEP, acute generalized exanthematic pustulosis; EM, erythema multiforme; ID, interface dermatitis; LTR, lichenoid-tissue reaction.
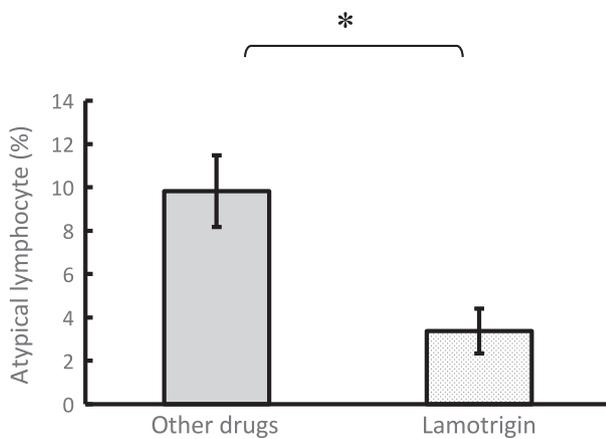
Figure 2. Atypical lymphocytes in the two groups. Atypical lymphocytes were detected in 83.3% of the patients in the lamotrigine (LTG) group and 93.8% of those in the usual drugs (UD) group. The mean percentage of atypical lymphocytes (maximum value during the disease) was significantly lower in the LTG than in the UD group ($3.38 \pm 1.03\%$ vs $9.83 \pm 1.65\%$, *$P < 0.05$).

HHV-6 reactivation

Drug-induced hypersensitivity syndrome/DRESS is a multi-organ systemic reaction closely associated with the reactivation of herpes virus, especially HHV-6.[1–3] Among the 44 patients in this study, HHV-6 reactivation was detected in one of the 12 LTG patients and 23 of the 32 UD patients with DIHS/DRESS; there were fewer DIHS/DRESS patients with HHV-6 reactivation in the LTG group than in the UD group ($P < 0.01$, Fisher's exact test). DIHS/DRESS patients with HHV-6 reactivation also had significantly higher levels of serum LDH and TARC (both $P < 0.01$).

Onset of a positive LTT

Drug-specific T-cell responses are often diagnosed using LTT. In DIHS/DRESS patients, a high rate of positive LTT results 4 weeks after disease onset (after the disappearance of eruptions) has been reported.[21] We examined all patients (12 patients with DIHS/DRESS caused by LTG and 32 patients with DIHS/DRESS due to other drugs) and observed positive results in eight of 12 patients caused by LTG and 23 of 32 patients due to other drugs. In the present study, the mean time from disease onset to a positive LTT result was shorter in the LTG group than in the UD group (12.0 ± 3.89 vs 69.3 ± 19.9 days, $P < 0.05$; Fig. 4).

Histopathological features are not associated with HHV-6 reactivation

The histopathological features of DIHS/DRESS were investigated in the 28 patients for whom skin biopsy samples were available (Table 2). The histopathological findings were classified as described in a previous study:[16] eczematous, ID, AGEP-like or EM-like. The most common histological pattern on biopsy was ID ($n = 8$, Fig. 5a), followed by an EM-like pattern ($n = 7$, Fig. 5b) and an AGEP-like pattern ($n = 1$, Fig. 5c). While an eczematous pattern alone was not seen in any of the specimens, it did occur together with other patterns. In addition to the four patterns listed above, a lichenoid-tissue reaction was seen alone in a single biopsy specimen ($n = 2$, Fig. 5d) but co-occurred with other findings in other samples.

The co-occurrence of two or more patterns in a single skin specimen was common (10/28 patients, 35.7%), similar to previous reports.[16,22,23] A report from Taiwan showed that patients with both histological patterns tended to have a higher rate of HHV-6 reactivation.[23] However, none of the histological patterns (including the coexistence of two or more patterns) was statistically associated with HHV-6 reactivation. HHV-6 reactivation was noted in six of seven patients with an EM-like pattern alone, but there was no significant difference in prevalence between an EM-like pattern and other patterns ($P = 0.0604$, Fisher's exact test). Moderately apoptotic keratinocytes were observed on the biopsies of 11 of the 28 patients (Fig. 5d), but did not correlate with HHV-6 reactivation.

Other findings

There was no significant difference in the interval from first drug intake to skin rash, or in skin manifestations such as a purpuric erythematous rash and/or periorbital and facial
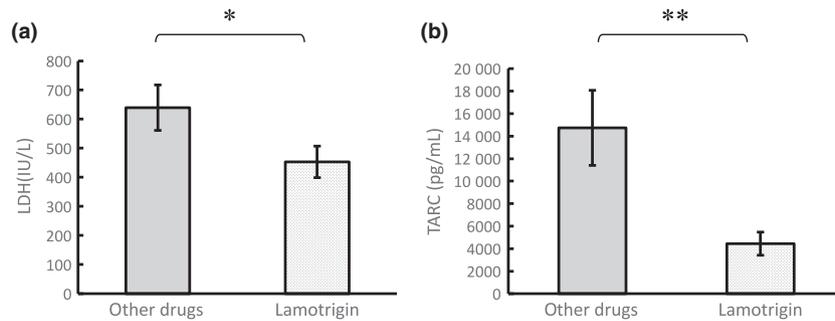
Figure 3. Serum lactate dehydrogenase (LDH) and thymus and activation-regulated chemokine (TARC) levels in drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS). (a) The mean serum LDH levels were significantly lower in patients with DIHS/DRESS caused by lamotrigine (LTG; 453.1 ± 54.2 U/L) than in those with DIHS/DRESS caused by other drugs (639.6 ± 78.2 U/L, *$P < 0.05$). (b) Mean serum TARC levels were also significantly lower in the LTG than in the usual drugs group (4442.0 ± 1027.8 vs 14 736.3 ± 3334.6 pg/mL, $P < 0.05$). DIHS/DRESS patients with HHV-6 reactivation had significantly higher serum LDH ($P < 0.01$) and TARC (**$P < 0.01$) levels.
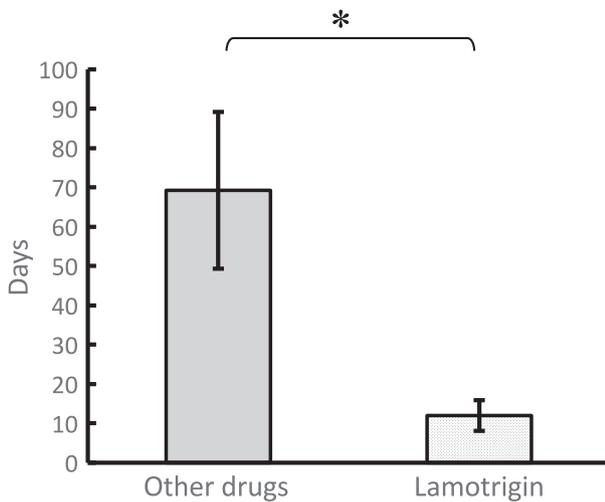
Figure 4. Lymphocyte transformation test (LTT) results. We performed LTT in all patients and obtained positive results for eight of 12 patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) caused by lamotrigine (LTG), and for 23 of 32 patients with DIHS/DRESS due to other drugs. The mean time from disease onset to a positive LTT result was shorter in the LTG group than in the usual drugs group (12.0 ± 3.89 vs 69.26 ± 19.9 days, *$P < 0.05$).

edema, which are characteristic of DIHS/DRESS, between the LTG and UD groups. In addition, there was no difference in DRESS score. The DRESS score is used for classification of DIHS/DRESS; neither DIHS caused by LTG nor DIHS caused by other drugs affected the diagnosis of DIHS/DRESS. In the UD group, three of 32 patients showed reactivations of both HHV-6 and Epstein–Barr virus (EBV) and five of 32 patients showed reactivation of both HHV-6 and cytomegalovirus (CMV). There were no patients who showed reactivation of HHV-6, EBV and CMV. In the LTG group, one patient showed only CMV reactivation and another showed only EBV

reactivation. There were no differences in the DIHS/DRESS relapse rate between LTG and other drugs.

DISCUSSION

Lamotrigine is one of the causative drugs of DIHS/DRESS, and it can also cause other types of severe drug eruptions, including Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).[10,24,25] In Japan, there is a system managed by the Pharmaceuticals and Medical Devices Agency (PMDA) designed to aid those suffering from ADR. Saeki *et al.*[11] reported that 92 out of 309 patients (29.8%) with LTG-related ADR reported to the PMDA had DIHS/DRESS. However, whether DIHS/DRESS due to LTG differs from DIHS/DRESS due to other drugs is unclear and was investigated in this study.

The main feature of DIHS/DRESS is a cutaneous rash that develops after exposure to the causative drug, and is associated with fever and organ involvement.[3,7] Hepatic failure, including elevation of serum transaminases, is a common finding.[5,8] In this study, all 44 patients had liver dysfunction, but it was milder in the LTG group than in the UD group. The reason for this difference in DIHS/DRESS due to LTG versus other drugs, including anticonvulsants such as carbamazepine and phenytoin, remains unclear. Carbamazepine and phenytoin are typical cytochrome P450 (CYP) substrates. Whereas LTG is mainly metabolized by uridine 5′-diphospho-glucuronosyltransferase (UGT), carbamazepine is metabolized to the toxic metabolite carbamazepine-10, 11-epoxide, by the enzyme CYP3A4,[26] while phenytoin is mainly metabolized to 4′-hydroxylated phenytoin by CYP2C9, and to a minor extent by CYP2C19.[27] Generally, unstable reactive metabolites metabolically activated by CYP enzymes induce hepatotoxicity. LTG contains a triazine ring that is metabolized at the 2-position by UGT to form a quaternary ammonium glucuronide.[28] A significant pharmacokinetic interaction exists between valproate and LTG that increases the risk of LTG-related drug rash due to the inhibition of UGT by valproate.[29] It has therefore been

Figure 5. Histopathological features of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS). (a) Interface dermatitis. Spongiosis and ballooning degeneration are seen in the epidermis together with perivascular inflammation in the upper dermis (hematoxylin–eosin [HE], bar = 200 μm). (b) An erythema multiforme-like pattern featuring moderate acanthosis with orthokeratotic hyperkeratosis and perivascular infiltrations in the upper dermis (HE, bar = 200 μm). (c) Acute generalized exanthematic pustulosis, characterized by subcorneal pustules, papillary dermal edema and infiltration by lymphocytes, eosinophils and neutrophils, is seen in the upper dermis (HE, bar = 200 μm). (d) Lichenoid tissue reaction, characterized by hyperkeratosis, focal acanthosis and a dense infiltration, is seen in the upper dermis, together with liquefaction degeneration between the epidermis and dermis. The rete ridges are irregularly elongated (HE, bar = 200 μm). (e) In some specimens, apoptotic keratinocytes are scattered within the epidermis. Apoptotic cells (arrows) in the epidermis were seen to some extent in samples from 11 of the 28 DIHS/DRESS patients for whom biopsy tissue was available (HE, bar = 200 μm).
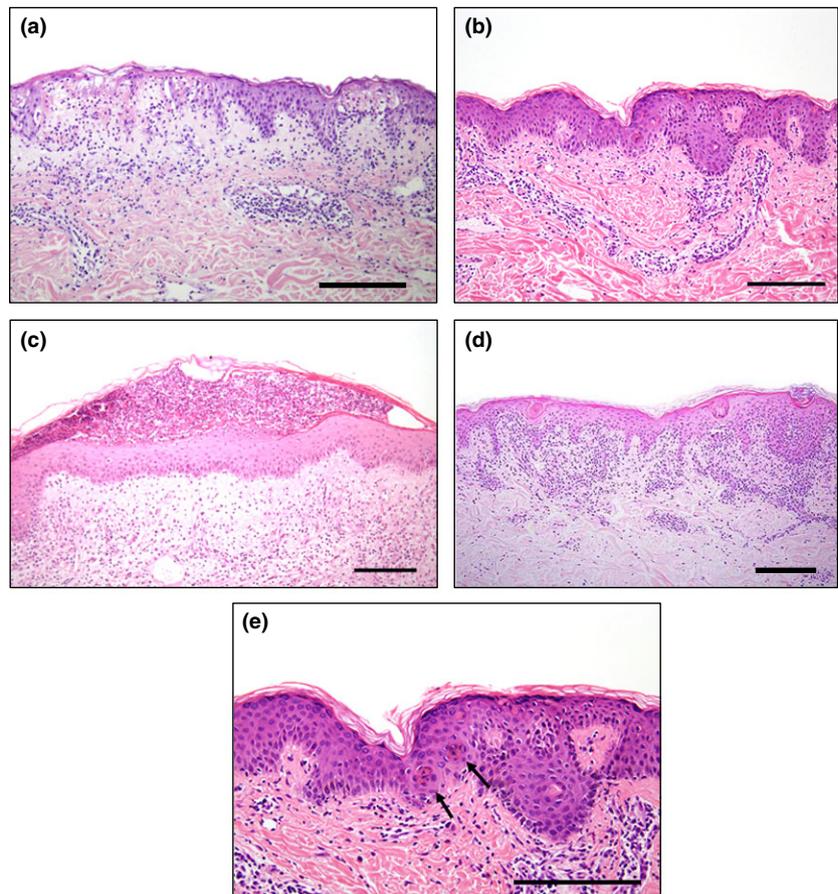
hypothesized that unmetabolized LTG is the cause of the ADR. A direct interaction between LTG and macromolecules, such as human leukocyte antigen, also triggers ADR. However, this does not explain the difference between DIHS/DRESS due to LTG versus other anticonvulsants; therefore, further studies are required.

Thymus and activation-regulated chemokine/CCL17, a member of the CC family of chemokines,[30] is a ligand for CC chemokine receptor (CCR)4, expressed on type 2 helper T (Th2) lymphocytes.[31–33] TARC plays important roles in Th2-type immune responses, by selectively incorporating CCR4+ Th2-polarized memory/effector T cells into inflamed tissues, such as those seen in atopic dermatitis.[34] Ogawa et al.[19] determined a correlation between serum TARC levels and disease activity in patients in the acute stage of DIHS/DRESS, consistent with our finding of significantly lower serum TARC levels in DIHS/DRESS due to LTG than that due to other drugs. Moreover, both previous investigations[19,20] and our own suggest that elevated serum TARC levels during the early stage of disease is a useful marker for early recognition of HHV-6 reactivation. Our results also showed higher serum LDH levels in patients with DIHS/DRESS than in those without HHV-6 reactivation, in agreement with a previous study.[8] Thus, both serum TARC and serum LDH levels in patients with DIHS/DRESS may be biomarkers of HHV-6 reactivation.

Moreover, serum TARC levels may be an indicator of DIHS/DRESS severity.

Lymphocyte transformation test positivity after disease onset occurred significantly earlier in the LTG group than in the UD group in this study. Previous reports noted that positive LTT reactions during the acute, but not the recovery, stage of maculopapular drug eruptions and SJS/TEN, while the opposite situation characterized DIHS/DRESS.[21] In this study, a positive LTT was also observed in patients during the recovery stage of DIHS/DRESS. The time to LTT was faster in the LTG group than in the UD group, although patients in both groups suffered from the same syndrome, DIHS/DRESS. Thus, it may be possible to identify causative drugs by performing LTT at an early stage when DIHS/DRESS is suspected due to LTG. Hanafusa et al.[35] detected drug-specific CD8+ cytotoxic T lymphocytes in the acute stages of DIHS/DRESS and SJS, whereas CD4+ T-cell proliferation predominated in most patients in the recovery stage of DIHS/DRESS, and in those with maculopapular-type drug eruption or EM. Moreover, during the course of DIHS/DRESS, there was a dramatic switch in the predominant drug-specific proliferating T-cell population, in which first CD8+ cytotoxic T cells, but later CD4+ T cells, predominated, followed by proliferation of drug-specific CD4+ CD25+ Foxp3+ regulatory T cells during the recovery stage of DIHS/DRESS.[35] These findings are suggestive of a

predominant drug-specific proliferating T-cell population in the acute stage of LTG-related DIHS/DRESS. LTT are also used to diagnose drug-induced liver injury; therefore, we examined the relationship between LTT positivity and liver dysfunction. There were no significant differences in LTT results between patients with ALT of 100 IU/L or more and those with ALT of less than 100 IU/L. In addition, there was no significant difference in ALT value between the LTT-positive and LTT-negative groups.

Among the four histopathological patterns of DIHS/DRESS identified by Ortonne *et al.*,[16] namely eczematous, ID, AGEP-like and EM-like patterns, ~54% of our patients had ID or an EM-like pattern. Only one patient had an AGEP-like pattern. In addition, the eczematous pattern also occurred together with one or more of the other types of pattern. We further identified lichenoid tissue reaction as a characteristic feature of DIHS/DRESS, occurring alone and with other histopathological patterns. A recent study demonstrated that patients with certain histological patterns tended to have a higher rate of HHV-6 reactivation.[23] However, none of the histological patterns (including cases with coexistence of two or more patterns) was statistically associated with HHV-6 reactivation. Interestingly, among the seven biopsy specimens with only an EM-like pattern, six were obtained from patients with HHV-6 reactivation, but the incidence did not differ between study groups. Two studies reported a correlation between apoptotic keratinocytes in skin biopsies and severe DIHS/DRESS,[16,22] whereas in our study scattered apoptotic keratinocytes were seen in 39.3% of the DIHS/DRESS samples. However, there was no correlation between the presence of these cells in the epidermis and DIHS/DRESS severity. Histological differences between the LTG and UD groups were not observed.

In conclusion, DIHS/DRESS due to LTG seems to be characterized by symptoms that are milder than those occurring in DIHS/DRESS due to other drugs, including liver dysfunction and the percentage of atypical lymphocytes, but there was no difference in the DRESS score between our UD and LTG groups. Fewer patients in the LTG group had HHV-6 reactivation than was the case in the UD group, with both TARC and LDH levels correlating with HHV-6 reactivation. Moreover, the time to LTT positivity after DIHS/DRESS onset was significantly faster in the LTG group. However, histological differences between the two groups were not observed.

CONFLICT OF INTEREST: None declared.

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SHORT COMMUNICATION

Check for updates

Up-regulation of Human Herpesvirus 6B-derived microRNAs in the Serum of Patients with Drug-induced Hypersensitivity Syndrome/Drug Reaction with Eosinophilia and Systemic Symptoms

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Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a life-threatening multi-organ hypersensitivity reaction. Reactivation of human herpesvirus 6B (HHV-6B), which typically occurs 2–3 weeks after its onset, has been implicated in DIHS/DRESS (1). Reactivation of HHV-6 has been reported to correlate with flaring of symptoms such as fever and hepatitis (2) and renal failure (3) in patients with DIHS/DRESS, indicating that virus reactivation could contribute to some symptoms or complications in DIHS/DRESS. However, it has also been reported that reactivation of HHV-6 could be merely a result of a strong drug-specific immune response and not contribute to DRESS symptoms and severity (4).

MicroRNAs (miRNAs) play important roles in biological processes such as immune responses and cell differentiation. Herpesviruses express their own miRNAs and may regulate key viral genes (5). HHV-6A encodes miR-U86 that regulates viral lytic replication (6), while HHV-6B encodes at least 4 miRNAs: hhv6b-miR-Ro6-1, -2, -3 and -4 (7). However, the precise roles of these 4 miRNAs in the regulation of HHV-6B latency and reactivation remain largely unknown. Moreover, the roles of individual miRNAs in DIHS/DRESS have not yet been elucidated. The present study investigated the expression levels of the 4 HHV-6B miRNAs in the serum of patients with DIHS/DRESS during the acute and subacute stages.

MATERIALS AND METHODS (see Appendix S1[1])

RESULTS

The maximum levels of hhv6b-miR-Ro6-1, -2, -3, and -4 in serum were significantly higher in patients with DIHS/DRESS than in those with MPE and healthy controls ($p < 0.05$, respectively) (**Fig. 1**a).

The time course of HHV-6B miRNA expression was examined in the serum of patients with DIHS/DRESS. In case 1, HHV-6B reactivation was confirmed by detecting HHV-6B DNA in peripheral blood mononuclear cells (PBMCs) on day 25 after onset. The expression of hhv6b-miR-Ro6-2 in serum was detected on day 19, while hhv6b-miR-Ro6-4 and -1 were detected on days 25 and 33, respectively (Fig. S1a[1]).

In case 2, HHV-6B reactivation was detected on day 16 after onset. Hhv6b-miR-Ro6-2 was expressed on day 10, while hhv6b-miR-Ro6-3 and -1 were expressed on the same day as HHV-6B DNA was detected (Fig. S1b[1]).

[1]https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-2925
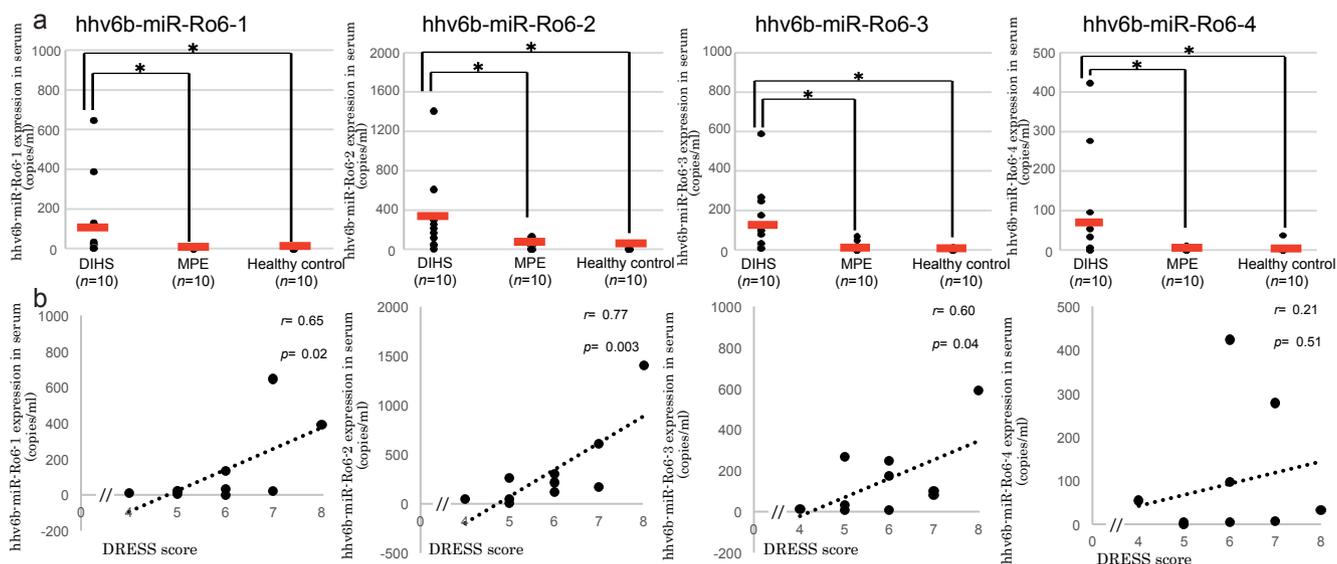
Fig. 1. (a) **Up-regulation of human herpesvirus 6B (HHV-6B)-derived miRNAs in the serum of patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS).** The maximum levels of HHV6b-miR-Ro6-1, -2, -3, and -4 in serum were significantly higher in patients with DIHS/DRESS than in those with maculo-papular eruption (MPE) and healthy controls. *$p < 0.05$. (b) **Correlation between DRESS scores and HHV-6B miRNAs in the serum of patients with DIHS/DRESS.** DRESS scores correlated with the serum levels of hhv6b-miR-Ro6-1, -2, and -3, respectively.

In case 6, the expression of HHV-6B DNA and hhv6b-miR-Ro6-2 and -3 was detectable on day 9 after onset, while hhv6b-miR-Ro6-4 was detected on day 21 following hhv6b-miR-Ro6-2 expression (Fig. S1c[1]).

It was then investigated whether HHV-6B miRNA levels correlated with clinical symptoms and laboratory data. The RegiSCAR scoring system (DRESS score) was used to evaluate the severity of clinical symptoms in patients with DIHS/DRESS. Ten patients with DIHS/DRESS (4 men and 6 women) were graded according to DRESS scores as "probable" ($n=4$) or "definite" ($n=6$) (Table SI[1]). As shown in Fig. 1b, DRESS scores correlated with the serum levels of hhv6b-miR-Ro6-1 ($r=0.65$, $p=0.02$), hhv6b-miR-Ro6-2 ($r=0.77$, $p=0.003$), and hhv6b-miR-Ro6-3 ($r=0.60$, $p=0.04$). DRESS scores were weakly associated with the serum levels of hhv6b-miR-Ro6-4 ($r=0.21$, $p=0.51$).

Relationships between the serum levels of HHV-6B miRNAs and each variable in the clinical and laboratory data were examined. The expression levels of HHV6B-derived miRNAs were not associated with liver function test results, eosinophil counts, the percentage of atypical lymphocytes, cervical lymphadenopathy, or the HHV-6B DNA levels of PBMC (data not shown). However, as shown in Fig. S2[1], the duration of fever (>38.0°C) correlated with serum levels of hhv6b-miR-Ro6-2 ($r=0.72$, $p=0.01$) and hhv6b-miR-Ro6-3 ($r=0.69$, $p=0.01$). The duration of fever was weakly associated with the serum levels of hhv6b-miR-Ro6-1 ($r=0.30$, $p=0.34$), but not with those of hhv6b-miR-Ro6-4 ($r=0.005$, $p=0.99$).

Serum levels of hhv6b-miR-Ro6-2 were associated with the severity of skin lesions (Table SII[1]). When the expression levels of hhv6b-miR-Ro6-2 in DIHS/DRESS patients were listed in descending order, the first 8 patients with higher levels of hhv6b-miR-Ro6-2 had erythroderma, while the last 2 patients with lower levels of hhv6b-miR-Ro6-2 had diffuse MPE. hhv6b-miR-Ro6-2 may reflect the type of skin eruption. Neither Hhv6b-miR-Ro6-1, -3, nor -4 were associated with the type of skin eruption.

DISCUSSION

HHV-6B encodes at least 4 miRNAs: hhv6b-miR-Ro6-1, -2, -3 and -4. These 4 HHV-6B-derived miRNAs were identified in Sup-T-1 cells infected with HHV-6B using a deep sequencing approach and expressed during lytic infection (7). Hhv6b-miR-Ro6-2 and -3 are detectable very early after infection and are encoded antisense to the immediate-early (IE) genes (8). Hhv6b-miR-Ro6-1 is detected 2 days after the expression of hhv6b-miR-Ro6-2 and -3, and is encoded antisense to IE (9) or early genes (8). Hhv6b-miR-Ro6-4 is detected 4 days after HHV-6B infection (7). As shown in Fig. S1[1], our results showed that the serum levels of hhv6b-miR-Ro6-2 were increased before or at the same time as the detection of HHV-6B DNA, while those of hhv6b-miR-Ro6-1 and/or -4 were significantly increased a few weeks later than hhv6b-miR-Ro6-2 expression in some patients with DIHS/DRESS. The kinetics of the emergence of hhv6b-miR-Ro6-2, -1, and -4 in DIHS/DRESS in the present study were mostly consistent with the *in vitro* findings reported by Tuddenham et al. (7). These results suggest that hhv6b-miR-Ro6-2 and hhv6b-miR-Ro6-1/-4 have distinct functions in the regulation of HHV-6B reactivation.

We also demonstrated that the expression of hhv6b-miR-Ro6-1, -2, and -3 was associated with DRESS scores, while that of hhv6b-miR-Ro6-2 and -3 was associated with the duration of fever. These results suggest that the serum levels of HHV-6B miRNAs may be useful indicators of the severity of DIHS/DRESS.

In conclusion, the detection of the miRNAs of HHV-6B in DIHS/DRESS may reflect the reactivation of HHV-6B, and hhv6b-miR-Ro6-2 may be an early and specific biomarker for predicting the reactivation of HHV-6B. We consider these results, which were obtained by identifying a number of differentially expressed HHV-6B miRNAs in the course of DIHS/DRESS, to provide novel insights into the molecular pathogenesis of DIHS/DRESS.

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Research letter

Serum thymus and activation-regulated chemokine is associated with the severity of drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome

DEAR EDITOR, Drug reaction with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS) is a severe adverse drug-induced reaction with reactivation of human herpesvirus (HHV)-6.[1–3] We previously reported that serum thymus and activation-regulated chemokine (TARC) levels were markedly increased in patients with DIHS and suggested that TARC is a useful diagnostic marker of DIHS in the early stage.[4,5] In this study, we determined whether serum TARC levels correlate with the severity of clinical symptoms and laboratory data in patients with DRESS/DIHS.

We evaluated 16 patients with DRESS/DIHS (eight male and eight female, median age 44 years and 68·5 years, respectively) for their clinical symptoms and laboratory data. Recorded data included copy numbers of HHV-6 and human cytomegalovirus (CMV) DNA in peripheral blood mononuclear cells, serum cytokines and soluble interleukin-2 receptor (sIL-2R), and serum TARC. This was carried out under the approval of the ethics committee at Nara Medical University. Serum TARC levels increased in the acute stage and decreased upon remission of the skin eruption. We evaluated the peak levels of serum TARC in the acute stage.

We first evaluated the association of clinical symptoms with TARC using a severity score of skin and mucosal lesions that we developed. The severity of skin and mucosal lesions and the duration of fever ($\geq$ 38 °C) showed positive correlations with serum TARC levels (Fig. 1a, b). All 11 patients with a high level of serum TARC ($\geq$ 10 000 pg mL^{-1}) developed erythroderma, and three of five patients with a lower TARC level (< 10 000 pg mL^{-1}) developed erythroderma. The serum TARC levels also correlated with the DRESS score (r = 0·35, P = 0·18), as previously reported.[4,5] Moreover, the patient with the highest serum TARC level (105 300 pg mL^{-1}) died of renal failure, suggesting that TARC is related to severe complications.[6]

We next determined the correlations of complete blood count and blood biochemistry with TARC. The percentage of atypical lymphocytes, and alanine transaminase and creatinine levels were positively correlated with serum TARC levels (Fig. 1c, d; for ALT: r = 0·45, P = 0·08). In contrast, platelet counts had a negative correlation with serum TARC levels (Fig. 1e). There was no significant relationship between TARC and eosinophil counts. These data also suggest that TARC may reflect the severity of the disease.

We then investigated whether serum TARC levels correlated with the levels of HHV-6 and CMV DNA. HHV-6 and CMV DNA in the peripheral blood were assessed by real-time quantitative polymerase chain reaction as previously reported.[7] The maximum copy numbers of HHV-6 and CMV DNA during the clinical course had positive correlations with TARC levels (Fig. 1f; for CMV: r = 0·46, P = 0·18). These data suggest that TARC may be associated with the extent of reactivation of HHV-6 and CMV. Tohyama et al. reported that HHV-6 reactivation is involved in the flaring, such as fever and hepatitis, and severity of DIHS.[3] Their results are in accordance with our findings that serum TARC levels correlate with the clinical severity and HHV-6 reactivation in patients with DRESS/DIHS. TARC may influence the pathological condition of DRESS/DIHS via HHV-6 reactivation. The correlation of the extent of CMV reactivation with TARC suggested that CMV reactivation should be carefully monitored in patients with high levels of TARC.

Finally, we investigated the association of serum cytokines and sIL-2R with TARC. We previously reported that T helper (Th)2-associated chemokines (TARC and macrophage-derived chemokine) were markedly upregulated in DRESS/DIHS, while Th1-associated chemokines (interferon-inducible protein 10) and monokine induced by interferon-γ predominated in Stevens–Johnson syndrome/toxic epidermal necrolysis.[8] We therefore examined the Th2 cytokines IL-10, IL-5 and IL-4. The levels of IL-10 and IL-5 correlated with TARC (Fig. 1g; for IL-5: r = 0·47, P = 0·07), but IL-4 had no correlation. These results suggest that serum TARC may selectively induce certain Th2 cytokines. In contrast, the Th1 cytokine interferon-γ showed no correlation with serum TARC. The levels of sIL-2R showed a correlation with serum TARC levels (Fig. 1h). The results for sIL-2R and atypical lymphocytes, together with those for Th2 cytokines, might suggest that Th2 cell activation is involved in DRESS/DIHS, as sIL-2R and atypical lymphocytes are related to T-cell activation.

In conclusion, the serum TARC levels in DRESS/DIHS were correlated with the severity of skin and mucosal lesions; fever; dysfunction of liver and kidney; levels of HHV-6 and CMV DNA; and IL-5, IL-10 and sIL-2R. Our results suggest that TARC might be not only a diagnostic marker but also a useful
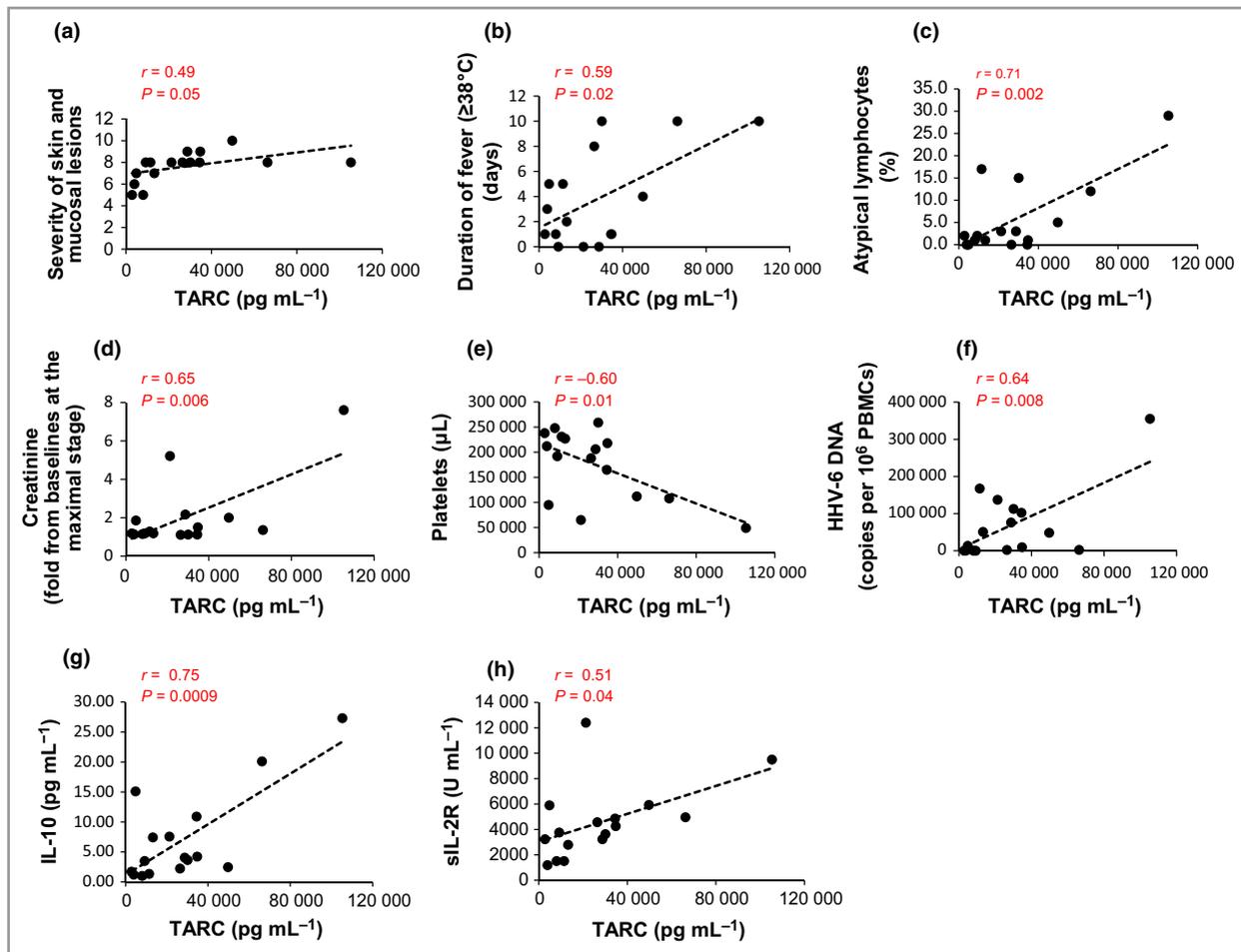
Fig 1. (a) Correlation between the severity of skin and mucosal lesions and serum thymus and activation-regulated chemokine (TARC) levels. The severity score was calculated as the sum of each of the following scores at the peak of disease activity: the extent of erythema (score 1–3); the presence or absence of facial oedema (score 0, 1), purpura (score 0, 1); the number of pustules or scales (score 0–3) and the extent of mucosal lesions (score 0–2). (b–h) Correlations between serum TARC levels and (b) the duration of fever (≥ 38 °C); (c) the percentage of atypical lymphocytes; (d) elevated creatinine levels; (e) blood platelet counts; (f) the peaks of human herpesvirus (HHV)-6 DNA copy level in the acute phase; (g) interleukin (IL)-10 and (h) soluble IL-2 receptor (sIL-2R). PBMC, peripheral blood mononuclear cell.

marker for assessing the clinical and immunological condition of patients.

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Research letter

Epidermal growth factor receptor (EGFR) inhibitory monoclonal antibodies and EGFR tyrosine kinase inhibitors have distinct effects on the keratinocyte innate immune response

DEAR EDITOR, Epidermal growth factor receptor inhibitors (EGFRIs) are a well-established targeted therapy for several cancers. Two categories of EGFRIs are known, EGFR tyrosine kinase inhibitors (TKIs) and EGFR monoclonal antibodies (mAbs). These EGFRIs frequently cause cutaneous adverse effects, such as papulopustular eruptions, xerosis and chronic paronychia. These cutaneous toxicities can result in reduction or even cessation of anti-EGFR therapy and have been shown to compromise patients' quality of life.

We previously reported that EGFR TKIs suppressed the expression of human β-defensins (hBDs) induced by the secreted products of *Staphylococcus epidermidis*, but not by those secreted by *Staphylococcus aureus*.[1] Other groups also reported that
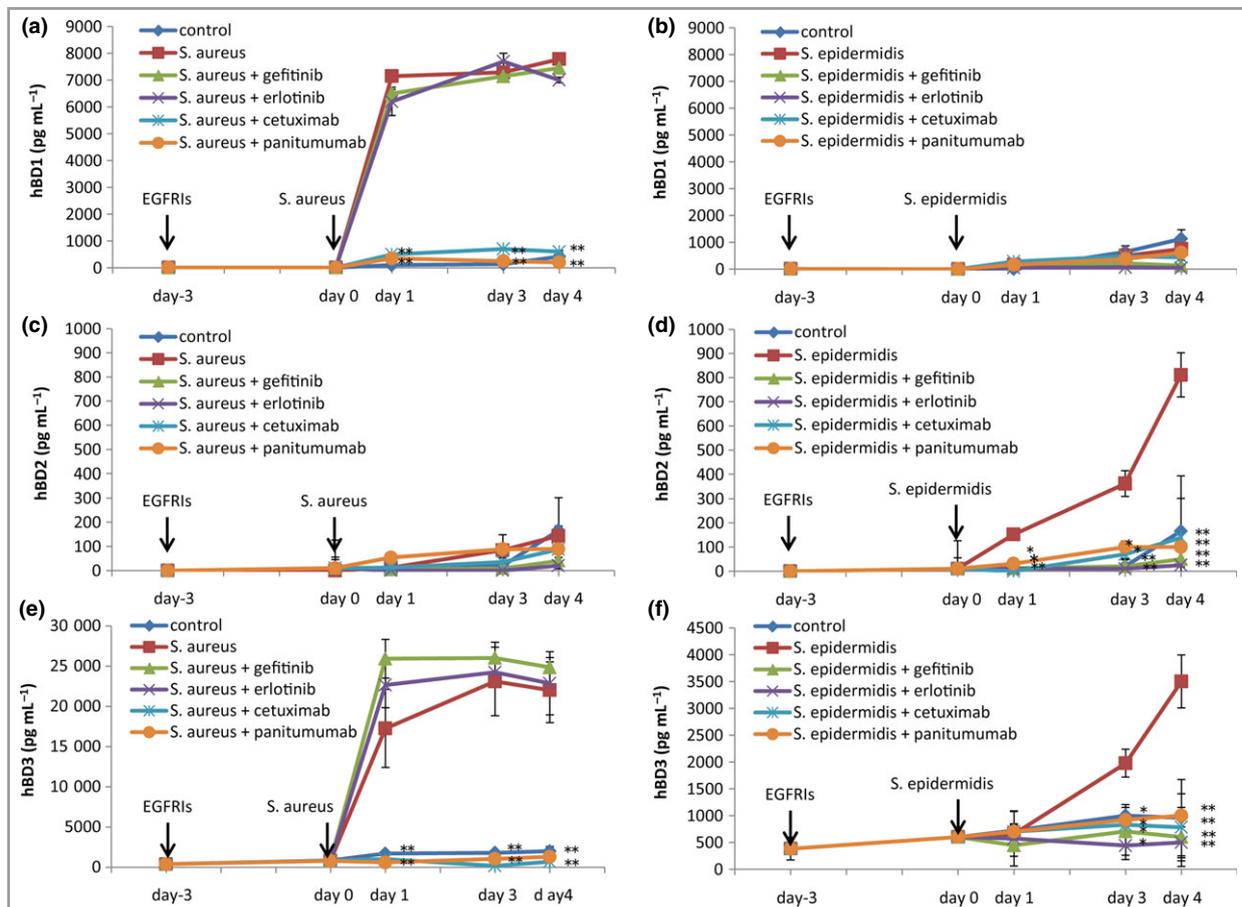
Fig 1. Suppressive effects of epidermal growth factor receptor inhibitors (EGFRIs) on human β-defensin (hBD) expression induced by staphylococci. Normal human epidermal keratinocytes were cultured with or without EGFRIs for 3 days before stimulation with the bacterial supernatants, and the keratinocytes were cultured for an additional 4 days. The expression levels of (a, b) hBD1, (c, d) hBD2 and (e, f) hBD3 in the culture supernatants were evaluated by enzyme-linked immunosorbent assay. Data represent the mean ± SD from three experiments. P-values (bacteria-stimulated keratinocytes cultured with vs. without EGFRIs) were evaluated using Student's t-test. *P < 0·05; **P < 0·01.

EGFRIs impaired the expression of antimicrobial peptides.[2,3] It is known that papulopustular eruptions caused by EGFR mAbs are more severe (5·2% at least grade 3) than those caused by EGFR TKIs (1·6% at least grade 3).[4,5] In addition, S. aureus is frequently detected in papulopustular eruptions caused by EGFRIs.[4] This led us to hypothesize that EGFR mAbs block hBD expression by keratinocytes in a way different from EGFR TKIs. In this study, we demonstrated that this is actually the case; EGFR mAbs blocked the expression of all hBDs induced by both S. aureus and S. epidermidis.

Normal human epidermal keratinocytes (NHEKs) were cultured in the presence or absence of the EGFRIs for 3 days before secreted products of S. aureus and S. epidermidis were added into the culture. We used the EGFR TKIs gefitinib (1·25 μg mL^{-1}) or erlotinib (5 μg mL^{-1}), and the EGFR mAbs cetuximab (0·2 mg mL^{-1}) or panitumumab (0·16 mg mL^{-1}). The final concentrations of these drugs were adjusted to the maximum concentrations of each drug in the human blood. The filtrated bacterial culture supernatants, prepared as described previously,[6] were used as the source of the secreted products of staphylococci. The NHEKs stimulated with the secreted products of staphylococci were cultured with or without EGFRIs for an additional 4 days. The expression levels of hBDs in the culture supernatants were then evaluated by enzyme-linked immunosorbent assay, as described previously.[6]

Consistently with the findings of our previous study, the secreted products of S. aureus and S. epidermidis induced the expression of hBD1 and hBD3 (Fig. 1a, c, e) and hBD2 and hBD3 (Fig. 1b, d, f), respectively.[6] We next examined the effects of EGFR mAbs on the expression of hBD. We found that both EGFR mAbs, cetuximab and panitumumab, suppressed the expression of hBD1 and hBD3 induced by the secreted products of S. aureus (Fig. 1a, e), in addition to the expression of hBD2 and hBD3 induced by the secreted products of S. epidermidis (Fig. 1d, f). This is in sharp contrast to the effects of EGFR TKIs, which suppressed the expression of hBD2 and hBD3 induced by the secreted products of S. epidermidis (Fig. 1d, f), but not expression of hBD1 and hBD3 induced by the secreted products of S. aureus (Fig. 1a, e), as previously reported.[1]

Keratinocytes serve as the front line of defence against the invasion of pathogenic microbes, presumably by exhibiting different responses depending on the types of microbes, thereby acting as a crucial site for innate immune response. hBDs are secreted from epithelial cells, including keratinocytes, and function as immunoreactive agents when stimulated by microorganisms.[7] We previously reported that S. aureus and S. epidermidis induced the expression of distinct subtypes of hBD by keratinocytes.[6] In this study, we demonstrate that hBD production by keratinocytes is differentially regulated by EGFR mAbs and EGFR TKIs when stimulated with staphylococci. In the presence of EGFR mAbs, keratinocytes did not respond to the secreted products of S. epidermidis or S. aureus, whereas in the presence of EGFR TKIs, keratinocytes demonstrated a significant response to the secreted products of S. aureus by producing a certain hBD.

Currently, the mechanism of differential effects between EGFR TKIs and EGFR mAbs is unknown, although their signalling pathways are well characterized.[8] There may be an additional signalling pathway other than the known tyrosine kinase-dependent pathway. Our results suggest that EGFR mAbs and EGFR TKIs have distinct effects on the keratinocyte innate immune response. Marked suppression of S. aureus-induced hBDs by EGFR mAbs, but not EGFR TKIs, may cause more severe papulopustular eruptions induced by S. aureus. The precise mechanisms of cutaneous adverse effects caused by each EGFRI are still unknown, although they are thought to be related to EGFR blockade in the skin. However, these differential effects of each EGFRI on hBD production may be associated with the severity of the cutaneous adverse reaction. Further study is needed to verify whether the effects of EGFRIs on the innate immune response induced by commensal bacteria are involved in cutaneous adverse reactions in patients.

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