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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 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/mm^3; 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 mm^3, respectively) during the disease course.

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) Allopurinol (4) Phenobarbital (3) Mexiletine (2) Salazosulfapyridine (2) Zonisamide (2) Dapsone (1) Febuxostat (1) Phenytoin (1) Trichloroethylene (1)	Lamotrigine (12)

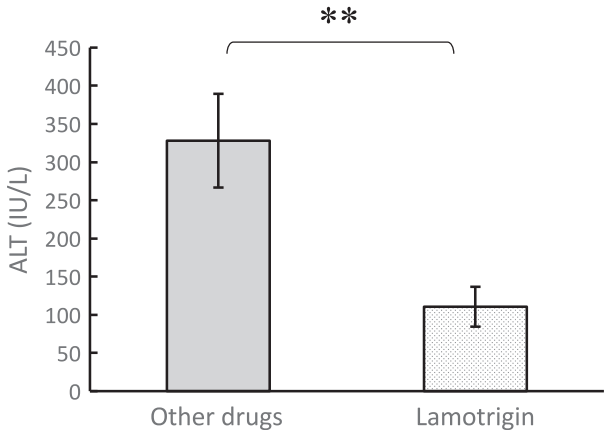

Figure 1. Serum alanine aminotransferase (ALT) levels in drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) caused by lamotrigine (LTG group) and the usual drugs (UD group). Patients in both groups had hepatic abnormalities, based on serum ALT levels 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, $^{**}P < 0.01$).

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 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.

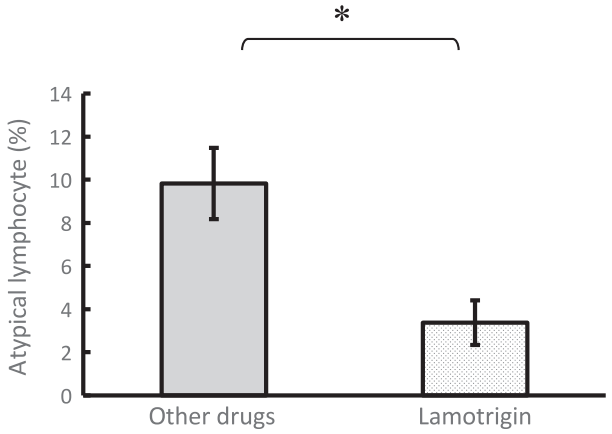

Figure 2. Atypical lymphocytes in the two groups. Atypical lymphocytes were detected in 83.3% of the patients in the lamotrigine (LTG) group and 93.8% of those in the usual drugs (UD) group. The mean percentage of atypical lymphocytes (maximum value during the disease) was significantly lower in the 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 multiorgan 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

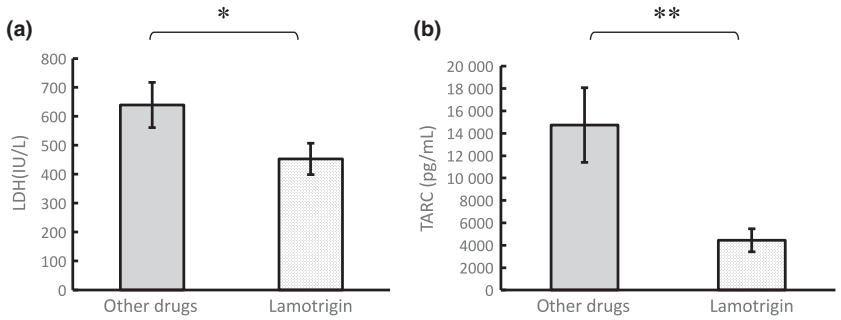

Figure 3. Serum lactate dehydrogenase (LDH) and thymus and activation-regulated chemokine (TARC) levels in drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS). (a) The mean serum LDH levels were significantly lower in patients with DIHS/DRESS caused 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.

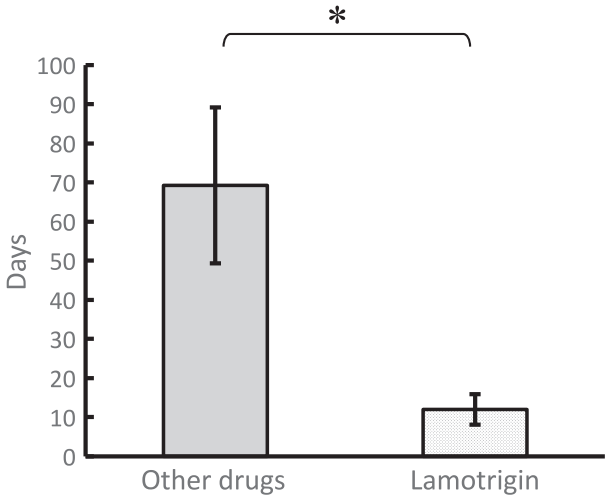

Figure 4. Lymphocyte transformation test (LTT) results. We performed LTT in all patients and obtained positive results for eight of 12 patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) caused by lamotrigine (LTG), and for 23 of 32 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

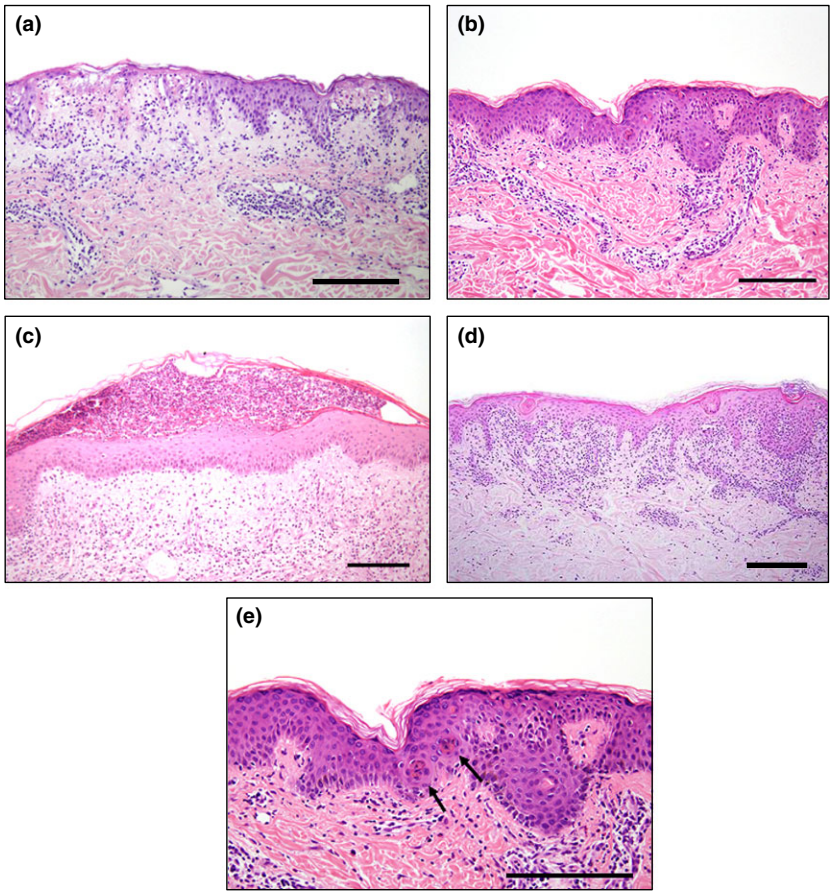

Figure 5. Histopathological features of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS). (a) Interface dermatitis. Spongiosis and ballooning degeneration are seen in the epidermis together with perivascular inflammation in the upper dermis (hematoxylin–eosin [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.

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CONCISE COMMUNICATION

Case of lamotrigine-induced drug adverse reaction under tocilizumab treatment with clinical and virological features of drug-induced hypersensitivity syndrome

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ABSTRACT

The pathological mechanisms and immunological kinetics of drug-induced hypersensitivity syndrome (DIHS), including the relevance of interleukin (IL)-6, remain unclear. We report a case of drug adverse reaction that does not fulfill the diagnostic criteria of DIHS but mimics its characteristic features. Because the patient was under anti-IL-6 therapy at the onset, some symptoms typically seen in DIHS were absent, such as fever and leukocyte count abnormalities. However, the characteristic features of DIHS were clearly observed in the subsequent course, including the repeated recurrence of skin rash, prolonged liver dysfunction and reactivation of herpes viruses. This case suggested that IL-6 role at the onset is not a main factor to determine the subsequent pathomechanism of DIHS and attention should be paid to the preceding therapy for achieving accurate diagnosis.

Key words: drug adverse reaction, drug-induced hypersensitivity syndrome, interleukin-6, lamotrigine, tocilizumab.

INTRODUCTION

Drug-induced hypersensitivity syndrome (DIHS) is a life-threatening adverse drug reaction. It is characterized by a skin rash that usually develops several weeks to months after starting one of a limited number of causative drugs including anticonvulsants, allopurinol and minocycline.[1] Typically, the patient develops a fever, lymphadenopathy and hepatic or renal dysfunction in addition to a rash. The delayed onset after starting the causative drug and prolonged relapsing clinical manifestations after discontinuing the causative drug are unique features of DIHS that differ from other cutaneous adverse drug reactions. Some clinical manifestations are considered to be related to reactivation of herpes viruses. Although DIHS is distinct because of such features, its pathological mechanisms and immunological kinetics remain unclear. Here, we report a case of lamotrigine-induced drug adverse reaction coincidentally under anti-interleukin (IL)-6 biologic therapy. This case did not fulfill diagnostic criteria of DIHS but followed its characteristic clinical features including relapsing rash and herpes virus reactivation. Based on this case, we discuss the role of IL-6 at the onset in DIHS pathogenesis.

CASE REPORT

A 29-year-old Japanese woman had been treated with tocilizumab for rheumatoid arthritis (RA). Because she developed epilepsy in addition to RA, she was started on oral lamotrigine. Three weeks later, she visited our outpatient clinic after developing a fever of 37.7°C, diarrhea, cervical lymphadenopathy, a maculopapular rash on her entire body and pustules around her mouth (Fig. 1a,b). Blood tests on the first visit (day 0) showed leukopenia (1100/μL) and a slight increase in lactate dehydrogenase (246 IU/L). Serum levels of thymus and activation-regulated chemokine (TARC) were not elevated (380 pg/mL). Histopathologically, a skin biopsy specimen on day 0 revealed mild infiltration of lymphocytes and eosinophils and liquefaction degeneration in the basal keratinocytes. Her clinical findings suggested a diagnosis of DIHS caused by lamotrigine. Before developing rash, tocilizumab had been administrated 360 mg/month for four times, and lamotrigine was administrated 50 mg/day for 18 days and subsequently 100 mg/day for 9 days.

Oral prednisone (1 mg/kg) was started immediately, and all of her other drugs were either discontinued or replaced with others (Fig. 2). The skin rash disappeared temporarily.

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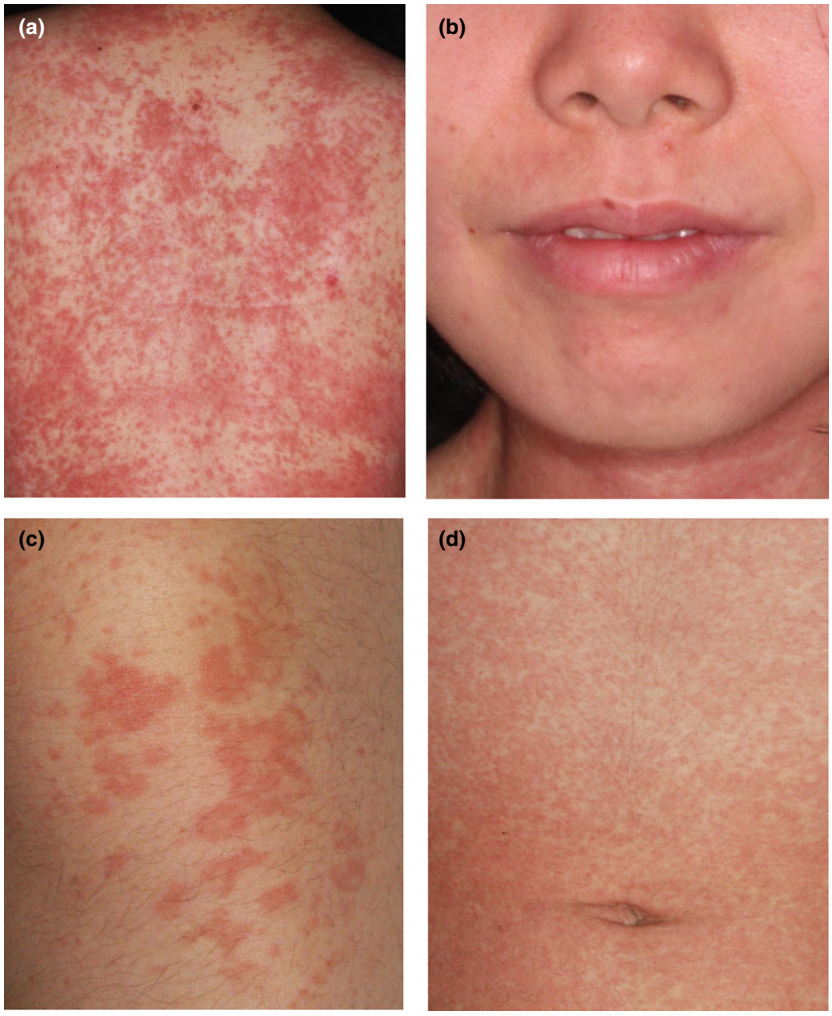

Figure 1. Skin rashes observed in this case. (a,b) Maculopapular rash on the entire body on day 0. (c) Erythema, partly arranged in an annular form, on the knee on day 10. (d) Maculopapular eruption on the entire body on day 20.

However, three recurrences of the rash were observed despite the treatment with prednisone, including erythema in a somewhat annular form at day 10 (Fig. 1c), a maculopapular eruption over her entire body on day 20 (Fig. 1d) and slight erythema on the back on day 75 (not shown). These rashes disappeared spontaneously without additional treatment. The dose of prednisone was gradually reduced from day 29 and was discontinued 1 year after onset.

Blood tests showed that serum levels of alanine transaminase and aspartate transferase were slightly elevated during each recurrence (Fig. 2). The lymphocyte transformation test (LTT) for lamotrigine was negative on day 0 but then turned positive and increased in value thereafter (stimulation index 1.7 on day 23, 2.3 on day 44 and 3.1 on day 119). Furthermore, real-time polymerase chain reaction was used to profile the peripheral lymphocytes for DNA derived from various human herpes viruses (HHV). This allowed detection of Epstein–Barr virus (EBV; 4700 copies/10^6 peripheral blood mononuclear cells [PBMC]) and HHV-7 (820 copies/10^6 PBMC) on days 35 and 43, respectively (Table 1), whereas HHV-6 DNA was not detected and anti-HHV-6 immunoglobulin G was not elevated. C-reactive protein (CRP) was also measured sequentially and the levels remained low until the skin rash recurred on day 75 (Fig. 2). Until day 40, CRP was undetectable due to tocilizumab treatment but it was subsequently detected intermittently when the rash recurred.

DISCUSSION

Drug-induced hypersensitivity syndrome has a variety of characteristic features including clinical symptoms, virological findings, immunological *in vitro* tests and so on, some of which are represented as diagnostic criteria.[1] In addition to the criteria, Kano *et al.*[2] reported as one of the characteristic DIHS features that positive LTT reactions were frequently confirmed at 5–8 weeks after the onset but not at the onset in DIHS. Another

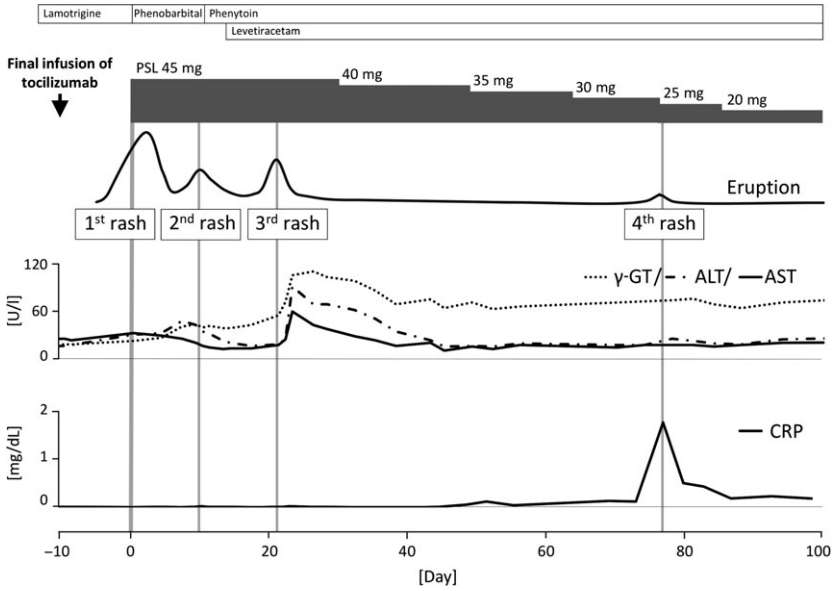

Figure 2. Schematic summary of the clinical course. The important drug history and results of blood tests are shown along the time course. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; γ-GT, γ-glutamyltransferase.

Table 1. Quantitative polymerase chain reaction results for human herpes viruses (copies/10^6 peripheral blood mononuclear cells)

Days after onset	0	7	29	35	44	94
EBV	ND	ND	ND	4700	ND	ND
CMV	ND	ND	ND	ND	ND	ND
HHV-6	ND	ND	ND	ND	ND	ND
HHV-7	ND	ND	ND	ND	820	ND

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV, human herpesvirus; ND, not detected.

characteristic of DIHS is that the reactivation of multiple herpes viruses including not only HHV-6 but others can occur during the course.[3] In this case, only four criteria were confirmed during the course that are "prolonged clinical symptoms after discontinuation of the causative drug", "maculopapular rash developed more than 3 weeks after starting with a limited number of drugs", "liver abnormalities" and "lymphadenopathy", while two criteria necessary for making diagnosis of DIHS, "fever" and "leukocyte abnormalities", were not observed probably due to tocilizumab effect at the onset. Although this case met only four of the seven diagnostic criteria, repeated recurrence of skin rash, prolonged liver dysfunction, the sequential reactivation of EBV and HHV-7 and the negative to positive conversion of the LTT results observed in our case are well consistent with the characteristic clinical course of DIHS. These clinical circumstances supported that it was reasonable to consider that this case was essentially DIHS but clinically modified by anti-IL-6 therapy at the onset.

Interleukin-6 is a key cytokine in CRP synthesis as a hepatic response to inflammatory conditions.[4] Serum levels of CRP are depressed when the serum tocilizumab concentration is 1 μg/mL or more.[5] In our study, the change in CRP levels suggested that tocilizumab had acted until approximately day 40. Therefore, we were able to evaluate the role of IL-6 at the onset of DIHS pathogenesis. Actually, our patient lacked a fever of more than 38°C as well as the leukocyte abnormalities that are usually seen in a typical case of DIHS including leukocytosis, atypical lymphocytosis or eosinophilia. In terms of fever, IL-6 is an endogenous pyrogen,[6] so it is reasonable to postulate that anti-IL-6 biologics suppressed her fever. In addition, because neutropenia is a reported side-effect of anti-IL-6 biologics,[7,8] we postulated that the use of tocilizumab might have masked leukocyte abnormality. Moreover, this case does not show elevation of serum TARC level, which is typically elevated at the onset of DIHS. Lack of elevation in serum TARC level is also considered the effect of anti-IL-6 biologics because Rachel *et al.* reported that IL-6 KO mice showed lower serum TARC level than wild-type mice in T-cell-mediated acute inflammation.[9] In addition, liver dysfunction was mild at the onset, even though it was prolonged. Considering this evidence, anti-IL-6 biologics may modify and moderate the symptoms of DIHS at the onset.

However, more importantly, the characteristic features of DIHS that usually happen several weeks after onset, including recurrent skin rash, prolonged liver dysfunction and successive reactivation of herpes viruses, were clearly observed, when the effect of tocilizumab had worn off. These features, particularly herpes virus reactivation, are important factors associated with the severity and prognosis of DIHS,[10,11] and anti-IL-6 therapy only at the onset was not able to obscure these features, at least in this case, even if it could modify some symptoms at the onset. Because the importance of IL-6 may depend on the disease phases of DIHS, anti-IL-6 therapy throughout the

course is needed to more precisely understand IL-6 roles in herpes virus reactivation and prolonged symptoms in DIHS.

This case suggests that physicians' attention should be carefully paid to the management of potential DIHS patients under biologic treatment that masks key symptoms at the onset.

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Vancomycin Mediates IgA Autoreactivity in Drug-Induced Linear IgA Bullous Dermatosis

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Vancomycin (VCM) is known to induce linear IgA bullous dermatosis (LAD). However, in contrast to conventional LAD, in which circulating IgA autoantibodies against basement membrane proteins are commonly detected, patient sera from VCM-induced LAD yields negative results in indirect immunofluorescence microscopy, and the targeted autoantigen remains undetermined. By using sera from a typical patient with VCM-induced LAD, we identified that co-incubation of sera with VCM resulted in linear IgA deposition at the basement membrane zone by indirect immunofluorescence. Patient sera reacted with the dermal side of 1 mol/L NaCl-split skin and with the recombinant noncollagenous (i.e., NC1) domain of type VII collagen by both immunoblot and ELISA in the presence of VCM. The investigation of an additional 13 patients with VCM-induced LAD showed that 10 out of the 14 sera (71.4%) reacted with the NC1 domain of type VII collagen by ELISA when spiked with VCM, whereas only 4 (28.6%) tested positive without it. The enhancement of reactivity to NC1 by VCM, as determined by optical density via ELISA, was observed in 10 out of the 14 sera (71.4%). These findings indicate that type VII collagen is a target autoantigen in VCM-induced LAD and that VCM mediates IgA autoreactivity against type VII collagen, providing an insight into mechanisms involved in drug-induced autoimmune disease.

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INTRODUCTION

Linear IgA bullous dermatosis (LAD) is an autoimmune blistering disease characterized by subepidermal blisters with linear deposits of IgA along the basement membrane zone (BMZ) on direct immunofluorescence (DIF). Clinical manifestations of LAD vary, from patients with vesicular lesions, which often appear in a herpetiform arrangement on erythemas, to those with tense blisters indistinguishable from bullous pemphigoid (Gottlieb et al., 2016). Although in most patients LAD is unassociated with an apparent triggering event, there exists a subset of patients (2.3%) in which the onset of LAD is attributed to drug administration (Horiguchi et al., 2008). LAD has been reported to be induced by a wide range of drugs (e.g., captopril, diclofenac, etc.) in more than 100 patients. Vancomycin (VCM) is the most frequent culprit, accounting for 46.2% of cases (Chanal et al., 2013; Fortuna et al., 2012; Gabrielsen et al., 1981; Kuechle et al., 1994). Drug-induced LAD is often triggered within a shorter timeframe relative to other drug hypersensitivities and drug-induced autoimmune diseases, appearing as early as 1 day after VCM administration in cases of VCM-induced LAD (vLAD) (Neughebauer et al., 2002; Richards et al., 1995; Whitworth et al., 1996; Zenke et al., 2014). Given the time required for the establishment of adaptive immune responses (Brugat et al., 2017), these observations suggest that the onset of drug-induced LAD may not involve de novo adaptive immune responses that are generated against the causative drugs. We hypothesized that the causative drugs might modify or enhance the reactivity of a preexisting immunological repertoire that is otherwise nonreactive or weakly reactive to an LAD antigen.

With the currently available technology, it is not possible to reliably distinguish between conventional and drug-induced LAD. The diagnosis of drug-induced LAD largely relies on the timing of drug administration, the onset of mucocutaneous lesions, and spontaneous resolution of the

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Abbreviations: BMZ, basement membrane zone; COL7, type VII collagen; DIF, direct immunofluorescence; IIF, indirect immunofluorescence; LAD, linear IgA bullous dermatosis; TCR, T-cell receptor; VCM, vancomycin; vLAD, vancomycin-induced linear IgA bullous dermatosis

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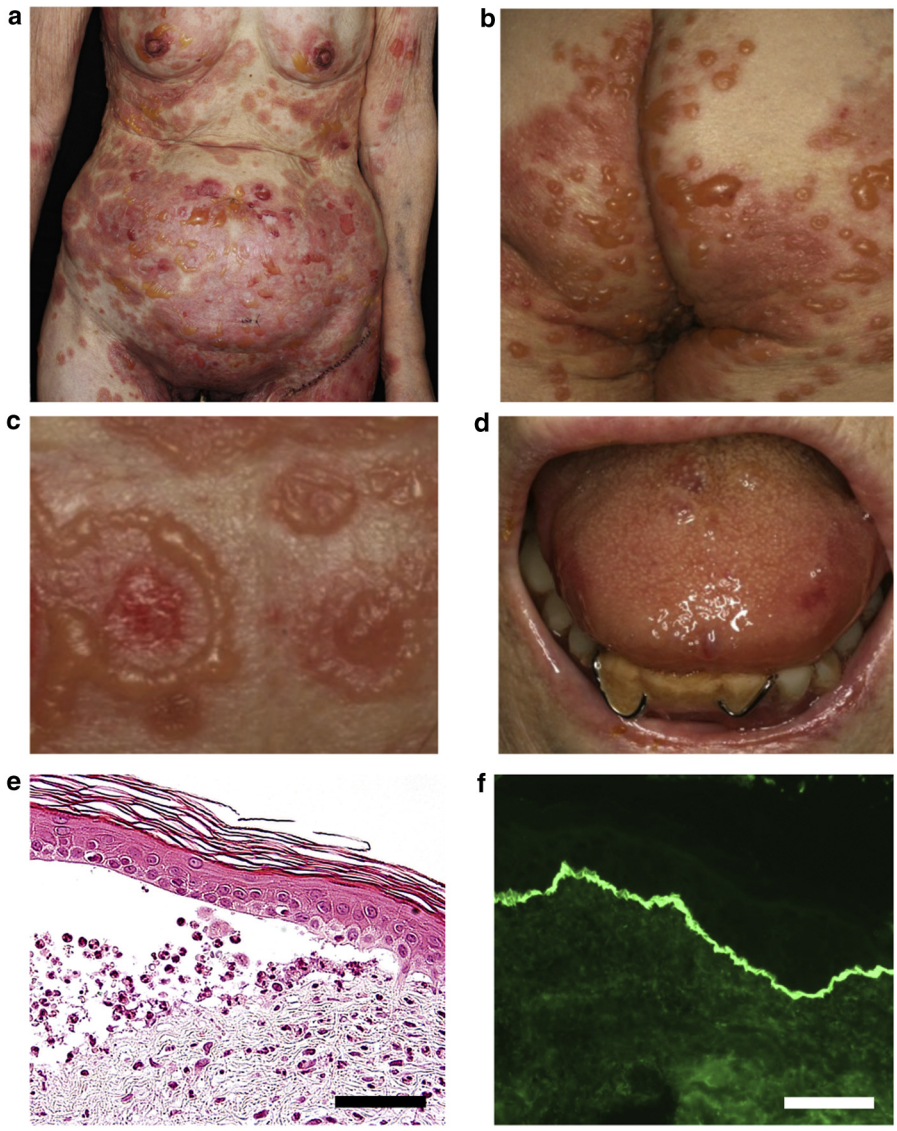

Figure 1. Clinical, histopathological, and immunological features of a typical patient with vancomycin-induced linear IgA bullous dermatosis. (**a, b**) Annular edematous erythemas with tense blisters and erosions appeared on the abdomen, buttocks, and groin. (**c**) Blisters were arranged along the edge of erythema in a ring shape. (**d**) Blisters and erosions were also seen on the oral mucosa and vulva. (**e**) Histopathological findings of the skin lesion showed a subepidermal blister filled with numerous neutrophils. (**f**) Direct immunofluorescence showed linear IgA deposition along the basement membrane zone. Scale bars = 50 μm.

lesions after drug withdrawal. Only a few cases of vLAD have been reported with supportive evidence for the cause-effect relationship, such as positive lymphocyte transformation tests and patch tests (Fortuna et al., 2012). However, such results need to be carefully interpreted, because they reflect immune responses limited to T cells, whereas IgA-mediated humoral immunity is the relevant mechanism involved in LAD.

Several basement membrane proteins, such as type XVII collagen (BP180) and its fragments, including LAD-1 and LABD97 (antigen with molecular weight of 97 kDa), lamin-332, and type VII collagen (COL7), have been reported as the target antigens of IgA autoantibodies in conventional LAD (Ishiko et al., 1998; Schumann et al., 2000; Tsuchisaka et al., 2015; Zenke et al., 2014). Autoantigens in conventional LAD can be determined by studying the autoantibodies that circulate in peripheral blood in approximately 70% of patients (Zone et al., 1990). Paradoxically, although DIF from vLAD patients shows linear IgA deposition, indirect immunofluorescence (IIF) results are usually negative, suggesting that circulating antibodies in LAD patients are either low in level or incapable of binding BMZ antigens in their native form. As such, targeted autoantigens, such as type XVII collagen and LAD285 (antigens with molecular weight of 285 kDa), have been identified in only a few cases (Palmer et al., 2001; Tashima et al., 2014). Thus, the lack of BMZ reactivity of patient sera and better definitions of targeted antigens are major issues that need to be explored in vLAD.

In this study, we discovered that co-incubation of VCM renders IgA from most vLAD patients reactive to COL7, as confirmed by IIF against normal human skin, ELISA, and immunoblotting analysis using recombinant COL7. Thus, COL7 is the major targeted autoantigen in vLAD, and the observation that the antigen-antibody reaction required VCM provides insight into the mechanisms involved in drug-induced autoimmune diseases.

RESULTS

Serum IgA in a typical case of vLAD acquires reactivity to the BMZ in the presence of VCM

A 73-year-old woman with mixed connective tissue disease undergoing treatment with 5 mg/day of prednisolone presented with a chronic ulcer on her left lower limb that was

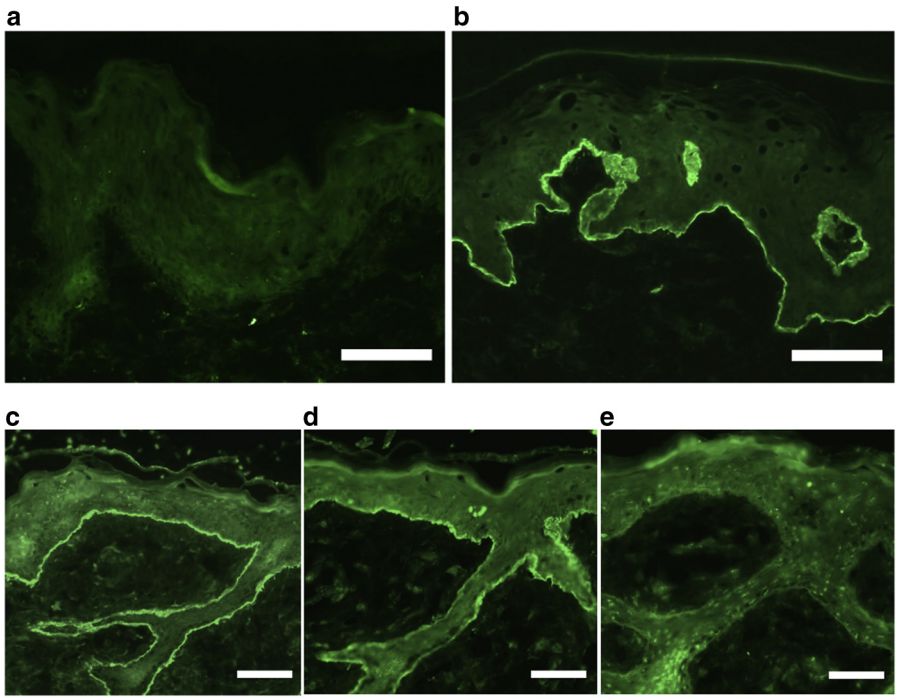

Figure 2. Serum from a patient with vancomycin (VCM)-induced linear IgA bullous dermatosis (vLAD) showed IgA deposition along the basement membrane zone (BMZ) only in the presence of VCM. (**a**) The serum of a patient with vLAD did not show IgA binding to the skin on indirect immunofluorescence with healthy human skin as a substrate. (**b**) Linear IgA deposition along the BMZ was observed when VCM was added to the serum. Scale bars = 50 μm. (**c**–**e**) IgA reactivity to the BMZ was dependent on the concentration of VCM. VCM concentrations: (**c**) 0.1 μg/ml, (**d**) 0.02 μg/ml, and (**e**) 0.004 μg/ml. Scale bars = 50 μm.

associated with subcutaneous calcinosis due to her underlying disease. Routine culture showed superinfection with methicillin-resistant *Staphylococcus aureus,* and the patient underwent treatment with intravenous VCM at a dose of 1.5 g twice daily. Six days into VCM treatment, she developed rapidly expanding annular, edematous erythemas with peripherally arranged tense blisters and erosions on the abdomen, buttocks, and groin (Figure 1a–c). Blisters and erosions were also seen on the oral mucosa and vulva (Figure 1d). Skin biopsy showed a subepidermal blister that was filled with numerous neutrophils (Figure 1e). DIF showed linear IgA deposition along the BMZ, and neither IgG nor complement component 3 deposition was observed (Figure 1f). Lymphocyte transformation test for VCM yielded a stimulation index of 3,980% (positive ≥ 180%). These findings collectively led to the diagnosis of vLAD, and VCM was immediately discontinued. The woman was further treated with systemic administration of prednisolone (1 mg/kg/day) and dapsone (75 mg/day), which led to complete resolution of skin lesions. No relapse was seen after the discontinuation of dapsone and reduction of corticosteroid to a baseline dose.

Despite positive DIF findings, IIF with normal human skin as a substrate did not show IgA binding to the skin in this case (Figure 2a). This suggested that IgA antibodies in circulation either did not exist at sufficient concentrations or were not reactive to BMZ antigens in their native form.

Recent studies on the mechanisms of T cell-mediated drug hypersensitivities proposed that causative drugs are capable of directly binding to class I MHC or to the T-cell receptors (TCRs), thereby resulting in altered specificity of the preexisting T-cell repertoire (Chung et al., 2016; Illing et al., 2012; Watkins and Pichler, 2013). We therefore hypothesized that VCM might bind to vLAD patients' IgA, modifying it to BMZ antigens. Indeed, VCM spiked into patient serum at a concentration of 2 μg/ml rendered serum reactivity against BMZ via IIF, showing IgA binding along the BMZ, a pattern identical to that obtained via DIF (Figure 2b). Serial dilution of VCM resulted in a loss of reactivity against BMZ at concentrations below 0.004 μg/ml, indicating that IgA reactivity is dependent on VCM concentration (Figure 2c–e). VCM levels peak as high as 25–50 μg/ml at 1–2 hours after administration, and trough levels are commonly maintained at 10–20 μg/ml. Thus, the concentration of VCM used for IIF reflects the in vivo condition.

COL7 as the target antigen of IgA autoantibodies in this case of vLAD

To further characterize the target of IgA autoantibodies in vLAD, we performed IIF with 1 mol/L NaCl-split skin (salt-split skin) as the substrate. A serum with 0.5 μg/ml of VCM showed IgA binding to the dermal side of the split skin (Figure 3a), indicating that the autoantibodies reacted with an autoantigen below the lamina lucida, such as COL7, which form the anchoring fibrils. We then performed immunoblot analysis using the recombinant noncollagenous (i.e., NC1) domain of COL7 to determine whether the serum bound to COL7 with or without 0.5 μg/ml of VCM. The serum showed a positive band of 150 kDa corresponding to the NC1 domain of COL7 only in the presence of VCM (Figure 3b). Furthermore, we examined serum reactivity against COL7 containing the mixture of the recombinant NC1 and NC2 domains by ELISA. VCM enhanced ELISA reactivity (optical density at 280 nm) of IgA against COL7 from 0.243 to 0.636 (2.617-fold increase) at 0.5 μg/ml concentration (Table 1, Case 1). The binding activity of circulating IgA to BMZ via IIF was lost in sera that were obtained after vLAD remission (Table 1, patient 1). However, ELISA detected the enhancement of IgA reactivity against COL7 mediated by VCM, from 0.031 to 0.125 (4-fold

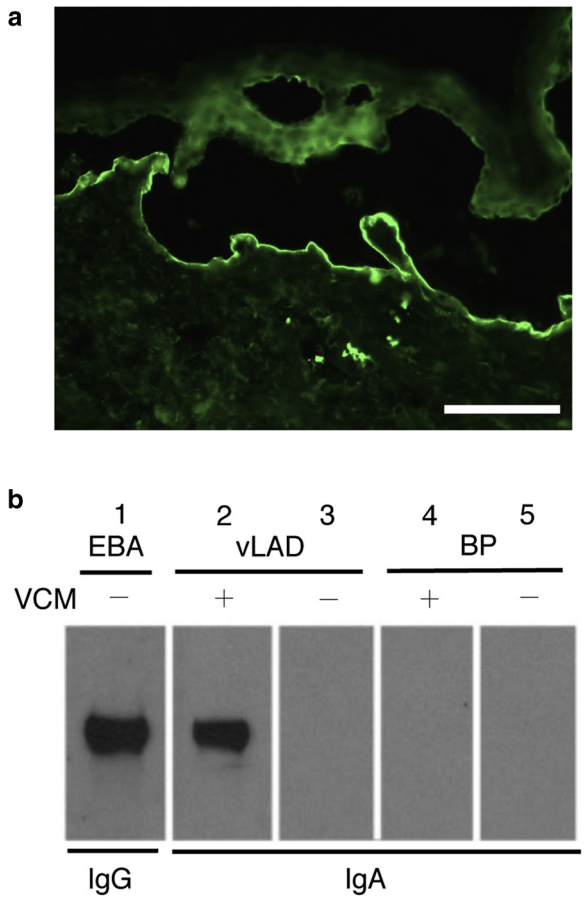

Figure 3. Identifying the target antigen of IgA autoantibody in vLAD. (**a**) The vLAD serum with 0.5 μg/ml VCM showed binding of IgA to the dermal side in indirect immunofluorescence using 1 mol/L NaCl-split skin. Scale bar = 50 μm. (**b**) Immunoblot analysis using the recombinant noncollagenous (NC1) domain of COL7. Lane 1: IgG reacting with the NC1 domain of COL7 was detected in serum from a patient with EBA (positive control). Lane 2: IgA reacting with the NC1 domain of COL7 in a vLAD serum was detected when VCM was added. Lane 3: The vLAD serum did not react with the NC1 domain of COL7 without VCM. Lanes 4 and 5: Serum from a patient with BP did not react with the NC1 domain of COL7 either with or without VCM (negative control). BP, bullous pemphigoid; COL7, collagen type VII; EBA, epidermolysis bullosa acquisita VCM, vancomycin; vLAD, vancomycin-induced linear IgA bullous dermatosis.

increase) (Table 1), suggesting that a particular repertoire of IgA that had transiently expanded during acute methicillin-resistant *Staphylococcus aureus* infection was modified by VCM. Taken together, IgA antibodies in this case of vLAD reacted with the NC1 domain of COL7, and autoreactivity was significantly enhanced by VCM.

COL7 is a major autoimmune target in vLAD

To extend these findings, we investigated sera from an additional 13 patients with vLAD (Table 1). With the initial patient included, 12 out of the 14 patients were men, and their ages ranged from 38 to 84 (average = 67) years. The onset of vLAD since the administration of VCM ranged from 4 to 16 days. The diagnosis of vLAD in all patients was based on skin manifestations (erythema, blister, and erosion on the body), subepidermal blister formation with neutrophilic infiltration by histology, and linear IgA deposition along the BMZ by DIF. Most patients were treated with systemic corticosteroid and/or dapsone, but in two patients vLAD resolved without any treatment other than the discontinuation of VCM. The disease severities varied from mild to severe, and the duration of treatment ranged from one to several weeks.

Although IIF using normal human skin as a substrate did not show IgA binding to the skin in any of the samples tested in the absence of VCM, 3 (21.4%) out of 14 serum samples displayed IgA reactivity to BMZ with spiked VCM concentrations of 0.5 μg/ml. IIF with sodium-split skin showed that all three sera reacted with the dermal side of the skin in the presence of VCM (Table 1, patients 1, 7, and 12). We then tested the reactivity of vLAD sera to COL7 by ELISA. Only 4 of the 14 sera (28.6%) showed positive reactivity (optical density at 280 nm > 0.06) in the absence of VCM. In contrast, the addition of VCM resulted in positivity in 10 of the 14 sera (71.4%) (Table 1). Furthermore, the ratios of optical density values in the absence or presence of VCM increased in 10 of the 14 sera (71.4%) tested ($P = 0.009$) (Table 1 and Figure 4). The higher rate of positivity by ELISA than by IIF likely reflects differences in the sensitivities of the two assays.

It is possible that VCM binds to and modifies the antigenicity of COL7, rather than binding to IgA antibodies. To address this, we pretreated frozen human skin sections with 0.5 μg/ml of VCM or vehicle and then used these sections as substrates for IIF. However, incubation of VCM with the substrate did not result in IgA reactivity to BMZ with any of the three positive sera (data not shown). We also pretreated COL7-coated ELISA plates with 0.5 μg/ml of VCM, but IgA reactivity to COL7 was not observed (see Supplementary Table S1 online). These results suggest that VCM mediates IgA autoreactivity by modifying IgA rather than the antigen, COL7.

Several drugs other than VCM have been reported to cause drug-induced LAD, such as sulbactam/ampicillin and tazobactam/piperacillin, as well as teicoplanin, which belongs to the same class of glycopeptide antibiotics that share similar structures to VCM. To determine whether such drugs were also capable of mediating IgA reactivity to COL7, we co-incubated these drugs with vLAD sera and performed IIF and ELISA. However, IgA binding to the BMZ was not observed upon the addition of any of these drugs in IIF and ELISA, indicating that the acquired or enhanced reactivity of IgA to COL7 was mediated specifically by VCM in the tested cases (see Supplementary Table S2 online).

In aggregate, these findings identify COL7 as a major autoimmune target in vLAD and show that IgA antibody autoreactivity to COL7 was mediated by VCM.

DISCUSSION

Drug-induced autoimmunity is a well-recognized condition manifesting in a wide range of autoimmune diseases, but evidence for mechanisms by which drugs induce autoimmunity is scarce. Certain drugs such as hydralazine, minocycline, procainamide, and others have been associated with drug-induced lupus erythematosus. These drugs induce the production of autoantibodies, such as antinuclear antibodies, anti-single strand DNA antibody, and anti-histone

Table 1. Clinical and immunological features of vLAD patients in this study

Patient	Age in Years	Sex	Onset From VCM Administration in Days	Past History	Treatment	Duration of Treatment	Remission Period	IIF (SS)		Type VII Collagen ELISA (OD Value)			Immunoblot with NC1 of Type VII Collagen	
								VCM−	VCM+	VCM−	VCM+	Ratio	VCM−	VCM+
1								−	+ (dermis)	0.243	0.636	**2.617**	−	+
1 (Remission phase)	73	F	6	MCTD	PSL 1 mg/kg/day, dapsone 75mg	5 weeks	4 weeks	−	−	0.031	0.125	**4.032**	−	−
2	81	M	12	Unknown	Internal corticosteroid	Unknown	Unknown	−	−	0.102	0.25	**2.451**	−	−
3	76	M	12	ASO	Dapsone	Unknown	Unknown	−	−	0.035	0.093	**2.657**	−	−
4	62	M	5	Unknown	Betamethasone 4 mg/day	6 days	6 days	−	−	0.024	0.169	**7.042**	−	−
5	82	M	Within a short time	Unknown	Unknown	Unknown	Unknown	−	−	0.031	0.309	**9.968**	−	−
6	83	M	13	DM	MINO 200 mg/day	21 days	Several days	−	−	0.023	0.146	**6.348**	−	−
7	84	M	12	Unknown	Steroid pulse therapy, IVIG	Unknown	Unknown	−	+ (dermis)	0.038	0.976	**25.68**	−	+
8	67	M	Unknown	Unknown	Anti-histamine drug	1−2 months	1−2 months	−	−	0.018	0.019	1.056	−	−
9	62	M	10	DM, RF(HD)	mPSL 60 mg/day	45 days	45 days	−	−	0.042	0.031	0.731	−	−
10	47	M	4	Alcoholic pancreatitis	PSL 30 mg/day, dapsone 50 mg	2.5 months	Unknown	−	−	0.173	0.208	1.202	−	−
11	38	M	11	CHF	Topical corticosteroid	35 days	35 days	−	−	0.031	0.033	1.065	−	−
12	60s	F	11	BC, after artificial valve replacement	None	None	1 week	−	+ (dermis)	0.561	1.493	**2.661**	−	+
13	73	M	16	Cataract	PSL 30 mg/day	10 weeks	2 weeks	−	−	0.045	0.205	**4.556**	−	−
14	55	M	13	Bronchial asthma	None	None	Several days	−	−	0.023	0.952	**41.39**	−	−

Positive ELISA results are underlined. Ratios of VCM+/VCM− more than 2 are underlined and in boldface.

Abbreviations: ASO, arteriosclerosis obliterans; BC, breast cancer; CHF, chronic heart failure; DM, diabetes mellitus; F, female; HD, hemodialysis; IIF (SS), indirect immunofluorescence with salt-split skin; IVIG, intravenous immunoblobulin; M, male; MCTD, mixed connective-tissue disease; MINO, minocycline; mPSL, methylprednisolone; PSL, prednisolone; RF, renal failure; VCM, vancomycin; vLAD, vancomycin-induced linear IgA bullous dermatosis.

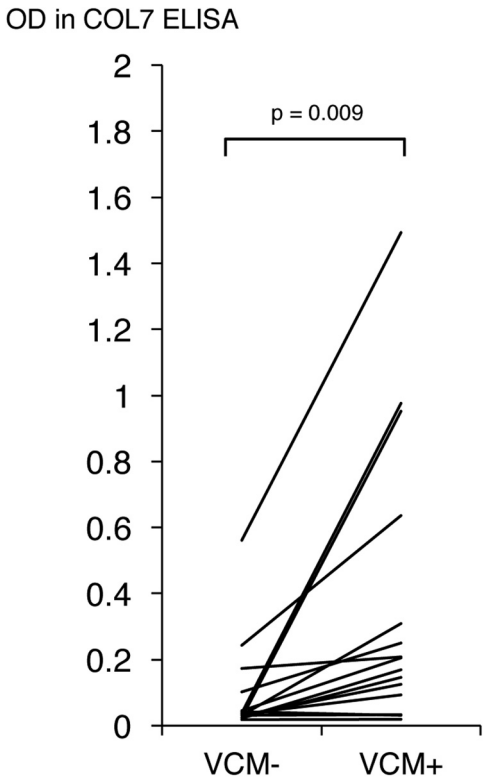

Figure 4. IgA reactivity with type VII collagen in ELISA in Japanese vLAD sera with or without VCM. A line chart representation of COL7 ELISA with 24-hour incubation. Out of 14 vLAD sera, 10 (71.4%) showed increased IgA reactivity (OD > 0.1) by the addition of VCM. Overall IgA titers (OD) against COL7 were significantly increased after adding VCM ($P = 0.009$). COL7, type VII collagen; OD, optical density; VCM, vancomycin; vLAD, vancomycin-induced linear IgA bullous dermatosis.

antibodies, and appear to be more frequently associated with autoimmunity than other drugs (Lowe et al., 2011; Vedove et al., 2009). In the field of dermatology, there is a long list of case reports on drug-associated production of autoantibodies against skin autoantigens. Pemphigus can be induced by D-penicillamine, captopril, and other drugs (Brenner et al., 1997; Korman et al., 1991; Nagao et al., 2005; Yoshimura et al., 2014), and bullous pemphigoid has been associated with dipeptidyl peptidase-IV (Izumi et al., 2016; Mendonca et al., 2016; Skandalis et al., 2012). Such drug-induced autoimmune skin diseases are reversed by the discontinuation of the culprit drug in many cases (Eisendle et al., 2007; Kuechle et al., 1994; McDonald et al., 2010; Nagao et al., 2005), resembling drug hypersensitivity in this regard. Others resulted in sustained autoimmunity that required prolonged immunosuppressive therapies (Billet et al., 2008; Fortuna et al., 2012), indicating that multiple mechanisms are involved in drug-induced autoimmunity.

In this study, we identified that VCM either mediates or enhances vLAD patient IgA reactivity against COL7. The hypothesis that vLAD patient IgA autoreactivity might be mediated by VCM was prompted by mechanistic studies on drug hypersensitivity. A series of studies on sulfamethoxazole hypersensitivity showed that direct high-affinity binding of the drug to the complementarity-determining region 2β domain of the TCR containing variable domain Vβ20-1 could result in an allosteric effect, which may directly enhance reactivity of TCR to HLAs that present endogenous peptides (von Greyerz et al., 1999; Watkins and Pichler, 2013).

The observation that a drug could directly bind to a complementarity-determining region domain of the TCR, together with the fact that immunoglobulins are similar in structure to TCRs (including complementarity-determining region domains), led us to hypothesize that VCM might have direct effects on vLAD patient IgA that results in modified antigen specificity (Illing et al., 2013; Pichler et al., 2015). This hypothesis was also inspired by the fact that onset of vLAD is usually rapid, well before adaptive immunity against VCM could take place, and by the paradoxical observation that despite IgA deposition in the BMZ, IIF with vLAD sera usually resulted in negative findings. Indeed, co-incubation of patient sera, but not co-incubation of the substrate (healthy volunteer skin), resulted in the deposition of IgA in the BMZ, showing that VCM modifies IgA and not the antigen itself. This approach enabled us to identify the targeted autoantigen in most of the studied vLAD patients as COL7, as shown by immunoblot and ELISA analyses.

After the recovery of patient 1 from vLAD, the reactivity of serum IgA to COL7 was dramatically decreased and was only minimally detectable via ELISA, even when co-incubated with VCM. This suggested that VCM does not broadly target IgA and that it may have some specificity, perhaps to a certain domain of the variable region, similar to the specific binding of sulfamethoxazole to TCR (Watkins and Pichler, 2013). Such an IgA repertoire may have transiently expanded in response to the acute methicillin-resistant *Staphylococcus aureus* infection that occurred before VCM administration, the contraction of which after infection may explain why not all sera from vLAD patients in this study showed positive results in COL7 ELISA and IIF. It remains possible that BMZ molecules other than COL7 were targeted in these cases. Because of the transient nature of vLAD, the amount of COL7-reactive sera was limited. Together with limitations in the analytical techniques that we currently have access to, the possibility of non-COL7 autoantigens will need to be further determined in future studies.

The precise mechanism by which VCM mediates or enhances IgA reactivity to COL7 remains to be determined. This may require the isolation of peripheral B cells that express IgA that VCM binds to or the generation of phage display single-chain antibodies (Payne et al., 2005) to obtain sufficient amounts of antibodies to allow determination of the specific domain(s) VCM binds to. We postulate that the mechanisms that lead to autoimmunity in conventional LAD, given the continuous autoantibody production, likely reflects a bona fide loss of tolerance and therefore is distinct from that of vLAD, in which autoimmunity is dependent on continuous presence of VCM with no true loss of tolerance against COL7.

In conclusion, we have shown that IgA from vLAD patients acquires reactivity to COL7 in the presence of VCM. This observation represents a mechanism by which a drug induces autoimmunity. Further examination on precise mechanisms may provide insight into the pathogenesis involved not only in autoimmunity but also in drug hypersensitivity.

MATERIALS AND METHODS

The study protocols were reviewed and approved by the institutional review board of the Keio University School of Medicine and were conducted following the principles established by the Declaration of Helsinki. Written informed consent was obtained from the patients.

Subjects and sera

We examined sera from a patient with vLAD treated in the Keio University Department of Dermatology and 13 patients reported by other institutes. All patients showed positive IgA reactivity along the BMZ of the skin by DIF in the active phase of the disease.

Indirect immunofluorescence

Each serum sample was subjected to IIF analysis using cryosections of normal human skin and 1 mol/L NaCl-split skin. The cryosection slides were washed in phosphate buffered saline three times for 5 minutes, and the cryosections were incubated with serial dilutions of tested sera for 1 hour at room temperature or overnight at 4°C. Deposition of human IgA antibodies was detected with a 1:100 dilution of FITC-labeled polyclonal rabbit anti-human IgA (DAKO, Copenhagen, Denmark).

COL7 ELISA

The reactivity of IgA in sera against COL7 was measured by ELISA kit (Medical and Biological Laboratories Company, Nagoya, Japan) according to the manufacturer's protocol, instead using horseradish peroxidase-conjugated anti-human IgA antibody as the secondary antibody (DAKO). We incubated serum samples with ELISA plates at 4°C for 24 hours to increase the sensitivity. A cutoff value (optical density at 280 nm) was defined as the average value plus three standard deviations of control sera from 20 healthy individuals.

Immunoblot

Recombinant NC1 domain of COL7 was produced by mammalian expression system, and recombinant NC2 domain was produced by bacterial expression system, as previously reported (Saleh et al., 2011). The NC1 and NC2 proteins were dissolved in SDS sample buffer, fractionated by SDS-PAGE, transferred to a nitrocellulose membrane, and detected using horseradish peroxidase-conjugated anti-human IgA or IgG antibody at a dilution of 1:100. After washing the strips three times in phosphate buffered saline washing buffer, proteins were visualized using Western Lightning Chem-iluminescence Reagent (PerkinElmer LAS, Shelton, CT) and autoradiography.

Statistics

All parameters were compared by Fisher exact test, as appropriate, with P less than 0.05 considered significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.12.035.

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CONCISE COMMUNICATION

Case of thymoma-associated cutaneous graft-versus-host disease-like disease successfully improved by narrowband ultraviolet B phototherapy

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ABSTRACT

Thymoma-associated graft-versus-host disease (GVHD)-like disease is a rare paraneoplastic disease seen in patients with thymoma. Here, we describe the first case of thymoma-associated GVHD-like disease localized to the skin that was successfully improved by a combination of systemic corticosteroids and whole-body narrowband ultraviolet (UV)-B phototherapy. The patient had developed toxic epidermal necrolysis-like erosive skin lesions over the whole body. Although systemic corticosteroids were effective up to a point, we were unable to begin the steroid taper. The addition of systemic narrowband UV-B phototherapy improved the skin manifestation of this disease, allowing corticosteroids to be reduced to a third of the original dose. Histopathologically, it was confirmed that the proportion of Foxp3-positive lymphocytes in the skin increased after narrowband UV-B irradiation. We propose that whole-body narrowband UV-B phototherapy is a good therapeutic option for the skin manifestation of thymoma-associated GVHD-like disease.

Key words: Foxp3, graft-versus-host disease, narrowband ultraviolet B phototherapy, paraneoplastic disease, thymoma.

INTRODUCTION

Thymoma is often associated with a variety of autoimmune diseases such as myasthenia gravis, pure red cell aplasia and thymoma-associated graft-versus-host disease (GVHD)-like disease. Thymoma-associated GVHD-like disease is defined as a disease affecting the liver, intestine or skin. Liver dysfunction, diarrhea and erythema occur in patients with thymoma, and GVHD-like reactions are observed histopathologically in the affected organ. In some cases, a single organ may be affected, and cases in which the disease is only manifested in the skin have been reported in the published work.[1,2]

Because this disease is rare, there exists no consensus on the optimum treatment modality. Although oral corticosteroids or immunosuppressive agents have been tried, it has proven difficult to control this disease. The prognosis is generally unfavorable due to an increased risk of infection-related death.[3] Herein, we report the first case of thymoma-associated cutaneous GVHD-like disease successfully improved by whole-body narrowband UV-B (NBUVB) phototherapy and systemic corticosteroids.

CASE REPORT

A 52-year-old Japanese woman was diagnosed with invasive thymoma at the age of 45 years and developed myasthenia gravis at 48 years. Because thymoma cells had been disseminated to the pericardium in spite of thymectomy, no further treatments could be utilized on admission. One month before her admission, she developed scaly erythema and red papules with itching across the trunk and extremities, which then spread rapidly to the whole body over the course of a few weeks. Topical steroid treatment was ineffective. Several days before admission, she developed fever and widespread epidermal detachment over her whole body. At this point, she was admitted to our hospital. She was treated with tacrolimus (2 mg/day), prednisolone (4 mg/2 days), lansoprazole, minodronic acid hydrate, loxoprofen sodium and morphine sulfate hydrate for over 1 year. On admission, her body temperature was 37.8°C and widespread erythema was observed over her whole body. Widespread epidermal detachment with erythema was observed on the neck, trunk, genital area and extremities,

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involving 30% of her body surface area (Fig. 1a,b). In addition, the patient experienced crusting of the lips and oral mucosal erosion (Fig. 1c). On the upper extremities, flat atypical target lesions were observed (Fig. 1d). She had no gastrointestinal symptoms. Laboratory results, including a complete blood count, renal function and liver enzymes, were almost within the normal ranges, other than elevated C-reactive protein (9.32 mg/dL). Immunoserological examinations showed no evidence of recent infection by mycoplasma, herpes simplex virus 1 or Epstein–Barr virus.

A skin biopsy was performed on the forearm (Fig. 1d). Histopathological examination revealed necrotic changes in the upper epidermis, and moderate infiltration of lymphocytes in the epidermis and upper dermis (Fig. 2a). In the epidermis, numerous apoptotic keratinocytes were observed, accompanied by lymphocytes and satellite cell necrosis. Abundant CD8 T-cell infiltration and a few Foxp3-positive cells were confirmed by immunohistochemistry (Fig. 2b,c). In addition, direct immunofluorescence showed immunoglobulin (Ig)G deposition at the cell surfaces in the epidermis and C3 deposition at both the cell surfaces in the lower layer of the epidermis and the basement membrane zone (Fig. 2d). Because these findings suggested paraneoplastic pemphigus, we further examined the autoantibodies against epidermal components, but indirect immunofluorescence analysis revealed a negative result. Moreover, no anti-desmoglein-1 and -3 antibodies were detected in the patient's serum by enzyme-linked immunosorbent assay. Immunoblot analysis did not detect IgG antibodies for epidermal components including envoplakin and periplakin. Finally, we diagnosed the patient as having thymoma-associated cutaneous GVHD-like disease.

We began the administration of oral 50 mg (1 mg/kg) prednisolone (PSL). The erosion rapidly improved but the erythema, red papules and itching remained. Because we were unable to reduce the dose of PSL for 3 weeks, we tried whole-body NBUVB phototherapy (five times per week, maximum dose 0.72 J/cm^2). The irradiation was performed in a UV 7001 K phototherapy cabinet (Waldmann-Medizintechnik, Villingen-Schwenningen, Germany). The eruption and itching in all areas improved approximately 10 days after starting NBUVB phototherapy, after which we were able to taper PSL to 30 mg (Fig. 3a). After a total NBUVB irradiation of 14.4 J/cm^2, the patient was discharged from our hospital at 10 weeks after admission. In the outpatient department, we continued tapering oral PSL by 1–2.5 mg every month while continuing with NBUVB phototherapy once per week. During the disease course, the patient developed diffuse alopecia; however, this was eventually completely resolved. Up to the present, we have now treated her with oral 14 mg PSL for 7 months. A small amount of itchy erythema has repeatedly appeared over the whole body. A skin biopsy from the erythema on the forearm showed the features of mild interface dermatitis (Fig. 3b). Although the infiltrated CD8 T-cell level was not changed (from 33 ± 5 to 34 ± 5 cells/high-power field [HPF]), significantly more Foxp3-positive cells had infiltrated into the epidermis and dermis as compared with the results of the first skin biopsy (from 3 ± 1 to 24 ± 3 cells/HPF) (Fig. 3c,d). During the disease course, there was no progression of thymoma metastatic lesions and no new metastases.

DISCUSSION

In thymoma-associated GVHD-like disease, various types of cutaneous manifestations have been reported, including keratotic papules, scaly erythema, morbilliform eruptions and erythroderma.[3,4] The most common histological findings are

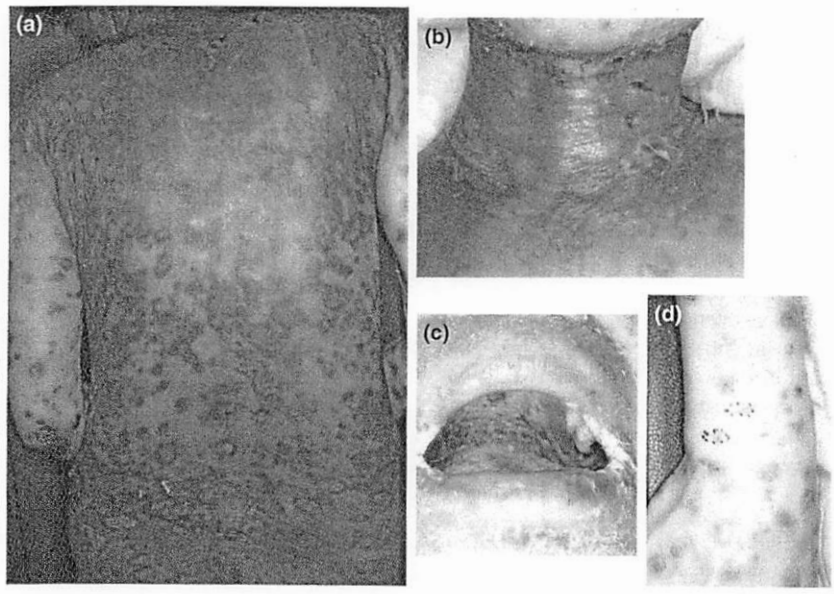

Figure 1. Clinical features on admission. Widespread erythema with (a,b) epidermal detachment and (c) oral mucosal erosions were observed. (d) Skin biopsy was taken from forearm.

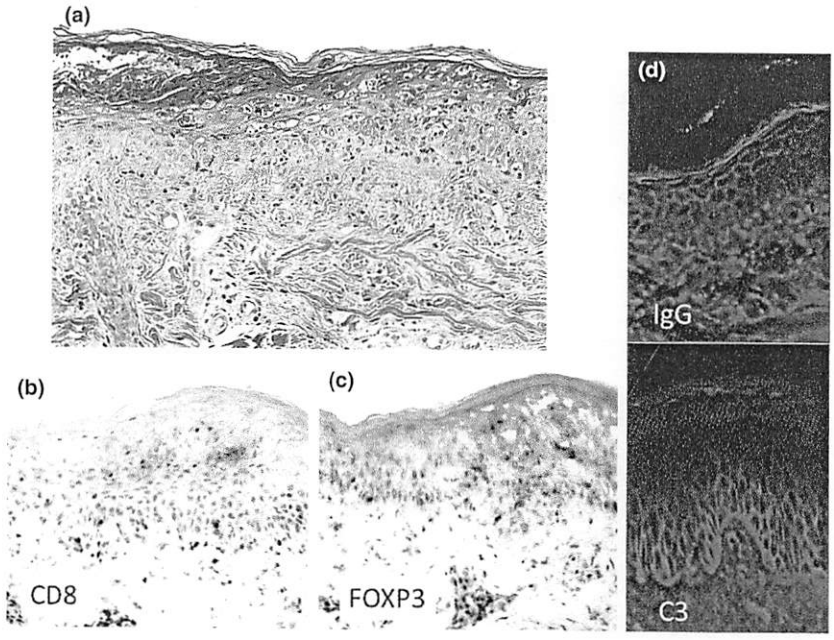

Figure 2. (a) Histopathology showed graft-versus-host disease-like reaction (hematoxylin–eosin, original magnification ×100). Immunohistochemistry showed (b) CD8 T-cell infiltration and (c) few Foxp3 positive cells (×100). (d) Direct immunofluorescence showed immunoglobulin (Ig)G and C3 deposit to the epidermis (×100).

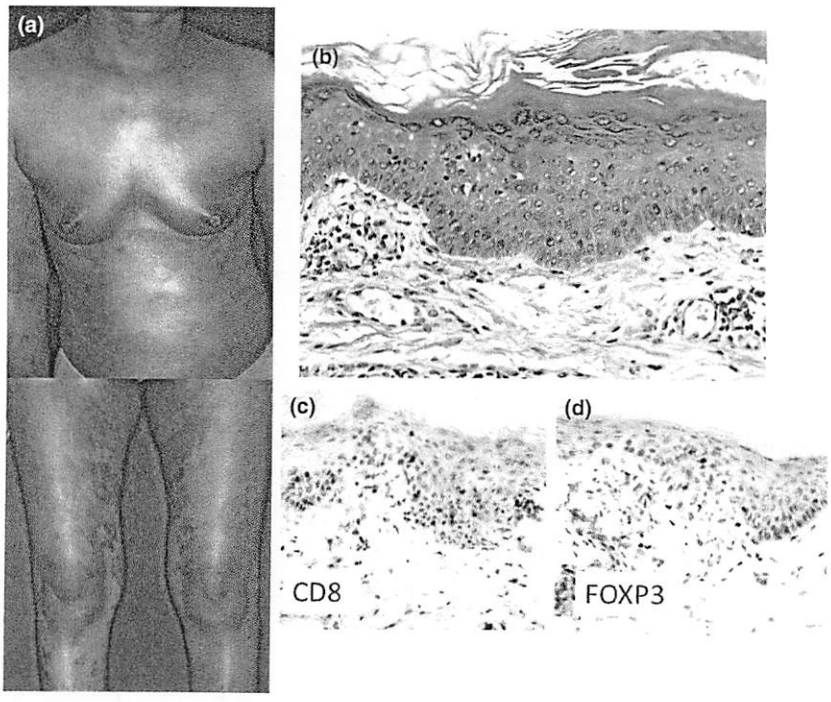

Figure 3. (a) Narrowband ultraviolet B (NBUVB) phototherapy significantly improved the skin manifestations. (b) Although lymphocytes were infiltrated in the epidermis and upper dermis, apoptotic keratinocyte was not observed (hematoxylin–eosin, original magnification ×200). In immunohistochemical analysis, (c) CD8 T cell and (d) Foxp3-positive cells were observed in the epidermis and dermis (×200).

GVHD-like reactions consisting of liquefaction degeneration and apoptotic keratinocytes accompanied by lymphocytic infiltration in the epidermal layer. It has been demonstrated immunohistopathologically that CD8-positive T cells are dominantly infiltrated in the epidermis, and that the frequency of Foxp3-positive regulatory T cells (Treg) is reduced in the dermis.[5]

In the case of our patient, the skin manifestation resembled toxic epidermal necrolysis, and histopathological findings revealed numerous apoptotic keratinocytes. To the best of our knowledge, no case of thymoma-associated cutaneous GVHD-like disease with toxic epidermal necrolysis-like features has been reported. Paraneoplastic pemphigus, one of the cutaneous complications of thymoma, was excluded because no autoantibodies were detected in the patient's serum. However, because direct immunofluorescence showed the existence of autoantibodies against the intercellular component of the epidermis, it is possible that some kind of autoantibody was involved in facilitating the widespread erosions in this case.

The pathological mechanisms of this disease remain unclear. In the normal thymus, autoreactive T cells are eliminated by apoptosis in a process termed negative selection by medullary thymic epithelial cells (mTEC).[6] This process depends largely on autoimmune regulator (*Aire*) gene expression in mTEC, which controls the ectopic expression of a wide range of peripheral tissue-specific antigens.[5] The lack of *Aire* in thymoma increases the number of autoreactive T cells.[2] Moreover, mTEC plays a critical role in the generation of Treg.[7,8] It is suggested that inadequate T-cell selection and insufficient Treg generation in the tumor environment of the thymus may cause thymoma-associated GVHD-like disease.[5] Because surgical excision of the thymoma and chemotherapy are unable to restore the function of the thymoma cells, systemic corticosteroids and/or immunosuppressive agents such as cyclosporin have been used.[9] However, patients with this disease usually suffer infection-related death due to long-term high-dose immunosuppressive treatments.[3,4] Therefore, the development of an effective treatment is required to improve the prognosis.

Recently, the effectiveness of NBUVB phototherapy for cutaneous GVHD after transplantation has been reported in many cases.[10] The direct effect of NBUVB on lymphocytes infiltrating into the skin is the most plausible mechanism.[11] In addition, it has been demonstrated recently that NBUVB increased the proportion of Treg in GVHD patients' peripheral blood[11] and skin.[12] Based on these findings, we used systemic NBUVB irradiation on our patient. This treatment was effective and allowed us to taper the dose of PSL. In addition, we confirmed that the proportion of Foxp3-positive lymphocytes in the skin was increased after NBUVB irradiation (Figs 2c,3d).

To the best of our knowledge, this is the first reported case of thymoma-associated cutaneous GVHD-like disease successfully treated with systemic NBUVB irradiation. More recently, Nakayama *et al.*[13] reported one case in which targeted NBUVB phototherapy used on a limited area of the lower leg improved the skin eruptions of this disease. Thus, whole-body NBUVB phototherapy may be helpful to allow tapering of the dose of corticosteroids in thymoma-associated cutaneous GVHD-like disease.

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CONFLICT OF INTEREST: None declared.

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SHORT COMMUNICATION

A Case of Perforating Folliculitis Induced by Vemurafenib

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Vemurafenib, an oral BRAF kinase inhibitor, has been approved for the treatment of late-stage metastatic malignant melanoma. Although vemurafenib prolongs progression-free and overall survival, numerous cutaneous side-effects have been reported (1, 2). We present here a case of perforating folliculitis (PF) associated with vemurafenib. To the best of our knowledge, this is the first report of vemurafenib-associated PF.

CASE REPORT

A 62-year-old man was treated with vemurafenib (960 mg twice daily) for metastatic malignant melanoma. Two months after initiating therapy, numerous disseminated keratotic follicular papules developed on his scalp, face, trunk and legs (**Fig. 1**A, 3A). Each papule contained a central, cone-shaped, keratotic plug (Fig. 1B). Differential diagnoses included a perforating disorder, suppurative folliculitis, hyperkeratotic folliculitis, and keratosis pilaris. Histopathological examination showed a dilated follicular infundibulum filled with a mixture of keratotic material, basophilic debris and inflammatory cells (**Fig. 2**A). The follicular epithelium showed a perforation through which degenerated collagen fibres entered into the follicular cavity (Fig. 2B). The follicles were surrounded by inflammatory infiltrate, mainly comprising lymphocytes. Elastic Masson and Azan staining revealed invasion or penetration of collagen fibres into the follicular epithelium (Fig. 2C, 2D). A diagnosis of PF was established. Because of the clinical efficacy against metastatic melanoma, vemurafenib was maintained at the same dosage. Combined treatment with topical corticosteroid ointments and antibiotic ointments (nadifloxacin) did not improve the folliculitis. We initiated minocycline at 100 mg/day, but the skin lesions did not disappear (**Fig. 3**B). After vemurafenib was discontinued and changed to nivolumab due to tumour recurrence, follicular papules rapidly began to improve. One month after stopping vemurafenib, the skin lesions disappeared with residual pigmentation (Fig. 3C). We concluded that PF was a cutaneous adverse drug reaction due to vemurafenib.

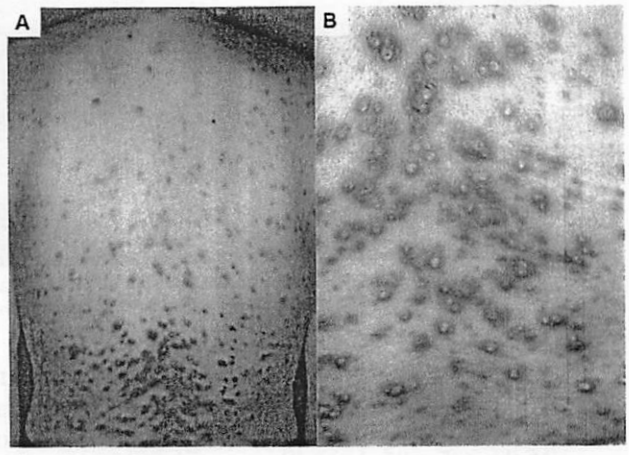

Fig. 1. (A) Numerous disseminated keratotic follicular papules on the trunk. (B) Each papule contained a central, cone-shaped, keratotic plug.

DISCUSSION

Vemurafenib is a small molecule that belongs to the group of protein kinase inhibitors. The drug has been approved for the treatment of metastatic melanoma harbouring the BRAF mutation. Although vemurafenib prolongs progression-free and overall survival, numerous cutaneous side-effects, including photosensitivity, alopecia, xerosis, squamous cell carcinoma, keratoacanthomas, palmar-plantar keratoses, and keratosis pilaris-like eruptions, have been reported (1, 2).

Acquired perforating dermatosis is an uncommon cutaneous perforating disorder characterized by transepidermal elimination of dermal tissue materials, such as keratin, collagen and elastic fibres. PF is an acquired perforating dermatosis together with Kyrle disease,

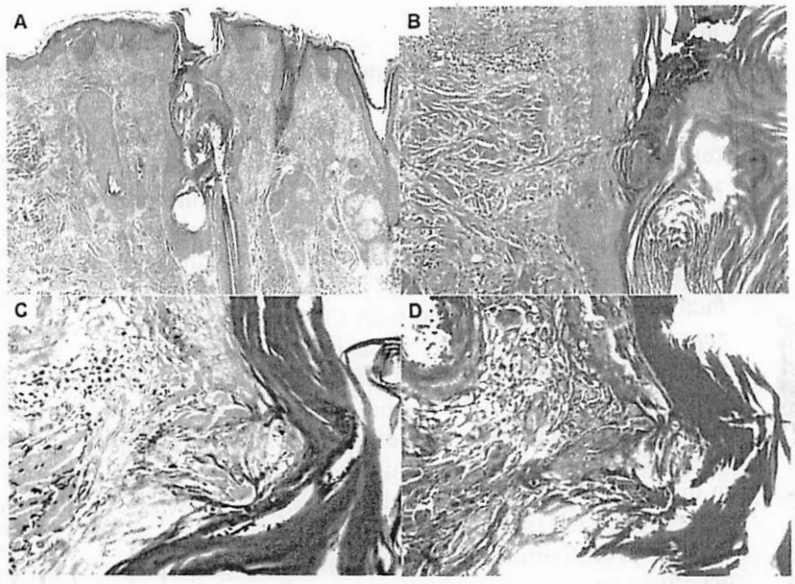

Fig. 2. (A) Dilated follicular infundibulum filled with a mixture of keratotic material, basophilic debris and inflammatory cells. Haematoxylin and eosin staining; original magnification ×40. (B) Degenerated collagen fibres enter the follicular cavity through the follicular epithelium. Haematoxylin and eosin staining; original magnification ×100. (C) Elastic Masson staining reveals invasion or penetration of collagen fibres (green); original magnification ×200. (D) Azan staining detects collagen fibres (blue) invading into the follicular cavity; original magnification ×200.

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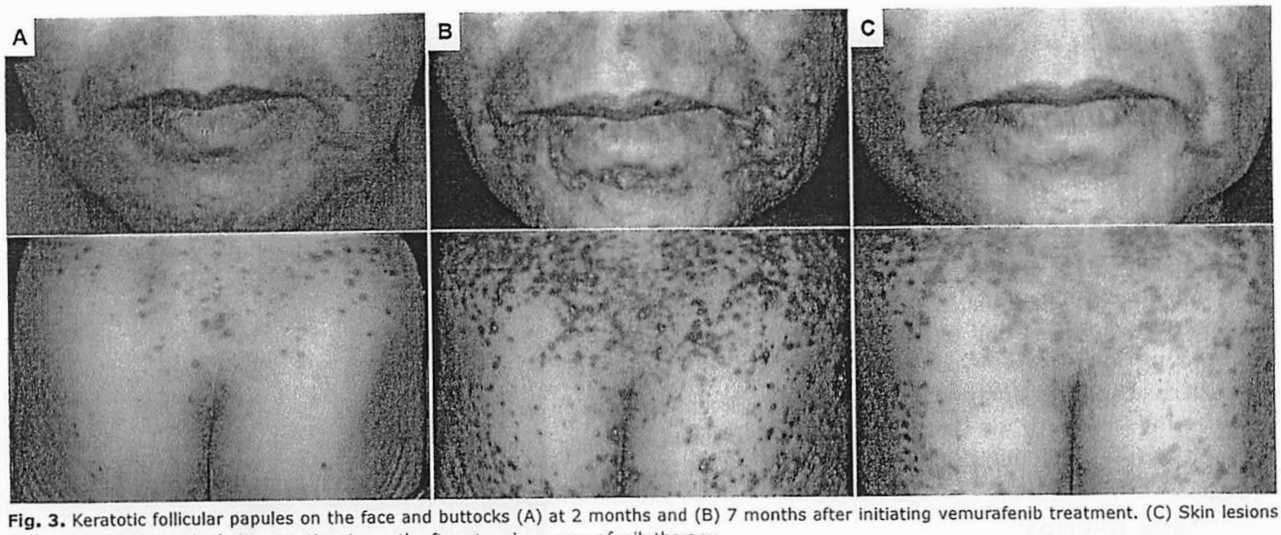

Fig. 3. Keratotic follicular papules on the face and buttocks (A) at 2 months and (B) 7 months after initiating vemurafenib treatment. (C) Skin lesions disappeared with residual pigmentation 1 month after stopping vemurafenib therapy.

reactive perforating collagenosis and elastosis perforans serpiginosa (3). PF is characterized by asymptomatic to severe pruritic keratotic follicular papules with disruption of the infundibular follicular wall. These skin lesions appears more frequently in patients with severe renal insufficiency, poorly controlled diabetes mellitus or arterial hypertension, as well as in sclerosing cholangitis (4). Scratching and deficiency of bile acids or vitamin A have been considered as possible causes (5). In this case, asymptomatic lesions and absence of excoriations ruled out chronic scratching as a causal factor. During vemurafenib therapy, PF developed gradually (Fig. 3A, B) and did not disappear with any treatments, including corticosteroid ointments, antibiotic ointments, and minocycline. After discontinuing vemurafenib, the skin lesions improved rapidly (Fig. 3C). We therefore concluded that the PF skin lesions were a cutaneous adverse drug reaction to vemurafenib.

The pathogenesis of PF induced by vemurafenib remains unknown. Interestingly, some cases of PF associated with sorafenib, another Raf kinase inhibitor, have been reported (3, 5–7). Sorafenib inhibits not only Raf-1 kinase, but also vascular endothelial growth factor receptor (VEGFR) 2, VEGFR3, platelet derived growth factor (PDGF) receptor and c-kit (3, 7). Some authors suggest that the pathogenic mechanisms could be explained by a possible direct toxic effect on follicular cells due to the inhibition of Raf and other kinases, and an indirect effect due to c-kit inhibition (5, 8). Inhibition of the PDGF receptor by sorafenib could also play a role in the pathogenesis of PF (3). Furthermore, a previous report suggested that EGFR blockade increases expression of proinflammatory chemokines and p27kip, a negative growth regulator that enhances apoptosis and promotes keratinocyte differentiation. This may lead to a thin stratum corneum and inflammatory infiltration of the follicles, resulting in dilation and plugging by excessive keratin (9). Moreover, our patient had chronic renal insufficiency, diabetes mellitus and arterial hypertension. This background may also have supported or promoted the development of PF by vemurafenib.

In conclusion, we have described vemurafenib-associated PF for the first time. Alterations in keratinocyte differentiation and/or proliferation pathways induced by vemurafenib could induce PF. This observation is important for elucidating the pathogenesis of PF, which remains unknown. Further prospective studies are needed to clarify the precise mechanisms.

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Correspondence

Pemphigus foliaceus induced by topical imiquimod treatment

Imiquimod (BESELNA®) is a topical immunomodulatory agent with potent antiviral and antitumor activity. The most common adverse events are itching, pain, erythema, exudation, and erosions at the application site, but secondary systemic effects also arise. Here, we describe a patient who developed pemphigus foliaceus (PF) at the application site and at distant locations after applying imiquimod cream. Possible mechanisms involved in the induction of PF by topical imiquimod are discussed.

A 61-year-old man presented with hyperkeratotic lesions on the left side of his face that clinically resembled actinic keratosis. He refused biopsy and surgery and was treated with topical 5% imiquimod (BESELNA® cream) 3 days/week. However, this therapy was discontinued after 2 weeks as he developed intolerable irritation and redness at the application site. Painful erythema that persisted for 1 month were treated with topical corticosteroid, but the skin lesions did not improve, and erosive erythema developed on his trunk. He presented at our department, with large, erythematous scaly patches on the left side of

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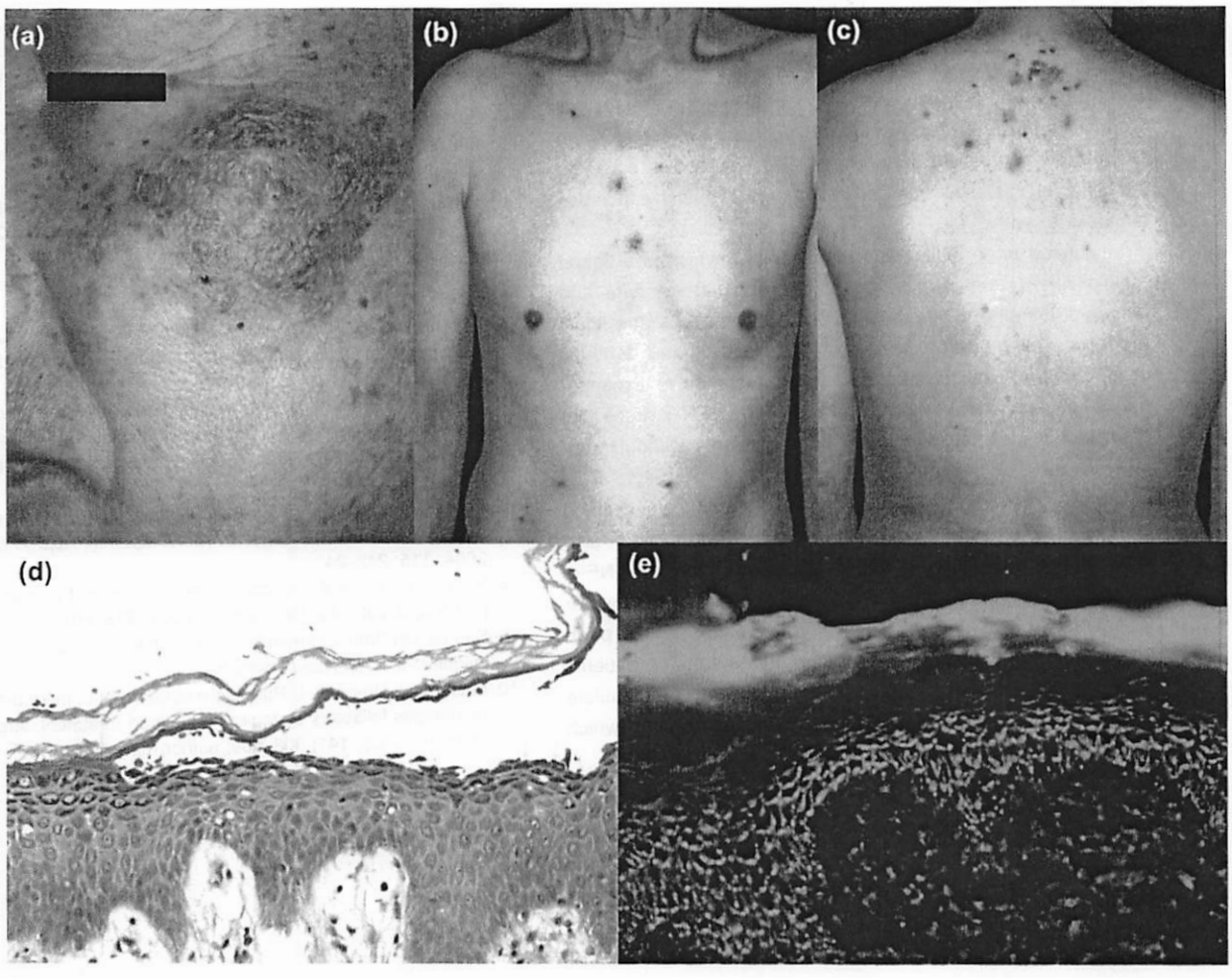

Figure 1 (a) Large, erythematous scaly patches on the left side of patient's face. (b, c) Multiple exudative erythematous erosions on chest, abdomen (B), and upper back (C) of the patient. (d) Biopsy specimen shows subcorneal acantholytic cleavage (hematoxylin-and-eosin, ×100). (e) Direct immunofluorescence is positive for IgG on keratinocyte surface, with typical fishing net appearance (original magnification ×100)

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his face (Fig. 1a) and multiple erythematous erosions on his chest, abdomen (Fig. 1b), and upper back (Fig. 1c). A biopsy specimen obtained from a lesion on the left of his face, where he had applied imiquimod, displayed subcorneal acantholytic cleavage (Fig. 1d). Direct immunofluorescence (DIF) was positive for IgG and C3 on keratinocyte surface, with the typical fishing net appearance (Fig. 1e). Anti-desmoglein 1 (anti-Dsg1) antibodies were detected by ELISA at a titer of 72.5, and anti-desmoglein 3 (anti-Dsg3) antibodies were absent. The clinical and laboratory findings indicated a diagnosis of PF induced by topical imiquimod. He was treated with oral prednisone (20 mg initial dose, then tapered by 5 mg per day), which obviously improved the skin lesions and prevented recurrence.

Brenner *et al.* first described pemphigus at locations that were local and distant from a topical contact site (contact pemphigus)[1]. Some chemicals can induce pemphigus, but little is known about pemphigus induced by imiquimod[2–6]. The present report describes the third known instance of PF induced by imiquimod. One published report was limited to the application site[5], whereas another described PF relapse at locations near and far from an application site[2]. In our patient, the skin lesions were generalized and the epidermis was positive for IgG and C3. Furthermore, the finding of anti-Dsg1 antibodies could be explained by systemic drug absorption or by a massive increase in cytokine synthesis.

Imiquimod is an imidazoquinolone amine, which is a ligand for Toll-like 7 and 8 receptors and it stimulates innate and acquired immune responses[3]. Imiquimod induces the local production of interferon (IFN)-α by keratinocyte and dendritic cells, and IFN-α is associated with the induction and maintenance of autoreactive B-cells[2]. Long-term IFN-α treatment often induces pemphigus antibodies in patients without pemphigus[2] as well as the onset of true pemphigus[7,8]. This might be one of the mechanisms through which imiquimod induces pemphigus. Furthermore, topical Imiquimod also induces the production of other cytokines, such as TNF-α, interleukins-1, 6, 8, 10, and 12, and interleukin-1 receptor antagonist[9]. Most of these cytokines can either induce pemphigus or circulate at high levels in the serum of patients with pemphigus[10]. The capacity of imiquimod to stimulate these factors may provide additional pathways through which imiquimod induces pemphigus[10].

In conclusion, we report the rare case of imiquimod-induced PF with lesions near far from the application site. We suggest that repeated applications of imiquimod led to the onset of PF by altering local or generalized immune responses, possibly because of the increased synthesis of many cytokines.

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◇DIHS＜臨床例＞——⑤

〈抄録はp.262に掲載〉

loop-mediated isothermal amplification（LAMP）にて *HLA-A**3101*を迅速検出したカルバマゼピンによるDIHSの2例

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Key words
カルバマゼピン，薬剤性過敏症症候群，*HLA-A*3101*

症例のポイント

・本邦においてカルバマゼピン(Carbamazepine：CBZ)による薬疹患者は，*HLA-A*3101*の保有率がオッズ比約10倍で薬疹非発症群と比較して高いとのエビデンスが後ろ向き調査にて報告され，薬疹発症のマーカーとしての臨床応用が模索されている．
・HLAタイピングは保険適用外で，高価かつ結果返却まで1週間以上を要するため，早期診断・治療介入には有用とはいえない．
・CBZによる薬疹2症例に対して，既報告のLAMP(loop mediated isotheramal amplification)を用いたin houseでの*HLA-A*3101*検出を行い[1]，早期の被疑薬同定，治療介入が行えた．

症例1　63歳，男．

初診　2013年9月．

主訴　全身の紅斑．

家族歴　父：糖尿病，兄：脳梗塞．

既往歴　高血圧，脂質異常症．

現病歴　2013年7月に左上葉原発肺癌にて化学療法＋放射線治療が開始された．同月に痙攣発作が出現し，症候性てんかんの診断でCBZが開始された．9月より37℃台の微熱と同時に腹部を中心として淡い紅斑が出現．発疹出現から14日後に当科紹介となる．

現症　体温 38.8℃，血圧 115/76 mmHg，脈拍 105回/分，呼吸数 20回/分．体幹，四肢に浮腫性紅斑(図1)，口腔内粘膜にびらんを認めた．頸部および腋窩リンパ節腫脹を認めた．

鑑別診断　以下の疾患と鑑別を要する．

リケッチア症：肝障害・異型リンパ球が出現している点で鑑別にあがるが，感染好発地域への発症約2週間前の立ち入りや居住歴がない．

伝染性単核球症：好発年齢が異なる．皮疹に瘙痒は通常なし．

単純ヘルペス再活性化による多形紅斑：単純ヘルペス再活性化既往なし．

入院時検査所見(下線部は異常値)　WBC 8,800 /μl(Neutro 76.8%，Eos 20.3%，Baso 0.3%，Mono 6.9%，Lymph 10.2%)，RBC 3.60×10^6 /μl，Hb 10.7 g/dl，Hct 33.2%，CRP 27.79 mg/dl，IgE 2.5 IU/ml，AST 139 U/l，ALT 101 U/l，LDH 309 U/l，γ-GTP 111 U/l，CK 98 U/l，Amylase 31 U/l，尿素窒素 18.8 mg/dl，Crea 0.83 mg/dl．

LAMP　採血検体からDNA抽出して，*HLA-A*3101*特異プライマーを使用し，LAMPを行ったところ，1時間以内で遺伝子増幅が確認された(図2)．

CBZを用いたDLST　S.I.：120(陰性)(第14病日)，S.I.：134(陰性)(第20病日)

Title：Two cases of rapid detection of *HLA-A*3101* for the diagnosis of carbamazepine-induced adverse drug reaction

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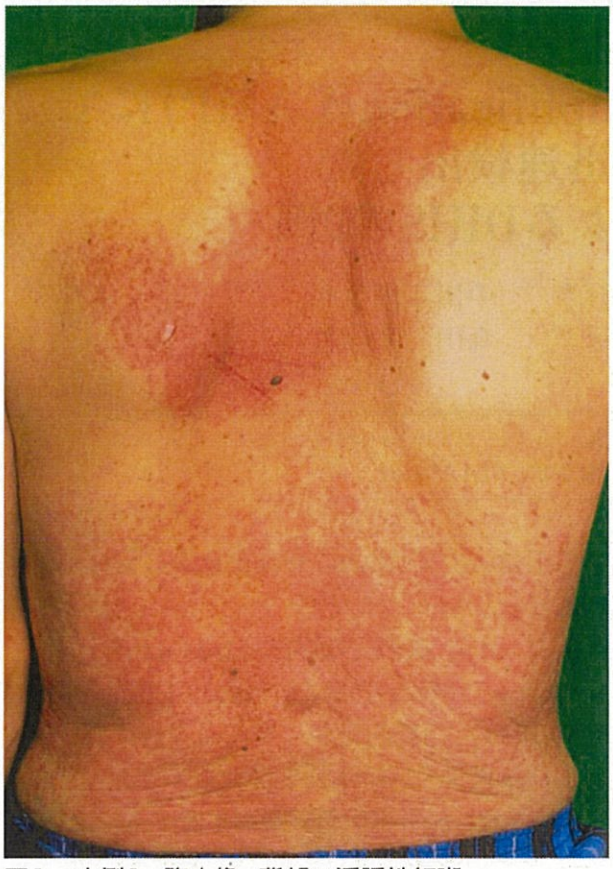

図1　症例1．臨床像．背部の浮腫性紅斑

S.I.：257(陽性)(第89病日)．

HLA-A遺伝子タイピング　*HLA-A*3101*, *HLA-A*2601*(検体提出後7日目に結果報告)．

HHV-6再活性化　ペア血清にてIgG抗体価の上昇あり(急性期に20→回復期に640)．

診断確定　発症約2カ月前からCBZの服用が開始されていた．眼球周囲が白く抜けた薬剤性過敏症症候群(drug-induced hypersensitivity syndrome：DIHS)に比較的特徴的とされる紅斑が出現していたことから，CBZ誘導型薬疹を疑いCBZ中止し，発疹は2週間程度で改善した．*HLA-A*3101*特異プライマーを用いたLAMPが陽性で，治癒に至るまで遷延化した点からCBZ誘導型DIHSと診断した．複数回測定のDLSTにて回復期に陽性が認められ，被疑薬同定とした．HHV-6再活性化があり，リンパ節腫脹も認め典型DIHSと確定診断した．

治療と経過　肺癌に対する薬物加療目的で定期通院中の当院の呼吸器内科を発疹，発熱にて受診し，同日入院．当科へも紹介受診となった．採血検体からDNA抽出して，LAMPを行い，即日陽性反応が得られた．先行研究にて*HLA-A*3101*を保有していることでCBZ誘導型薬疹の可能性が約10倍上昇するとされており，被疑薬をCBZとして中止した．軀幹，四肢に浮腫性紅斑，頸部リンパ節腫脹，口腔内粘膜びらんを認めるも，意識清明で重篤感は乏しかったので，ステロイド投与はせずに補液，肺炎に対して抗菌薬のみで経過フォローした．約2週間で平熱に戻り，皮疹も消褪していった．採血結果は正常範囲内に回復するまで2週間以上要し，ペア血清によるHHV-6 IgG抗体価上昇が確認された．

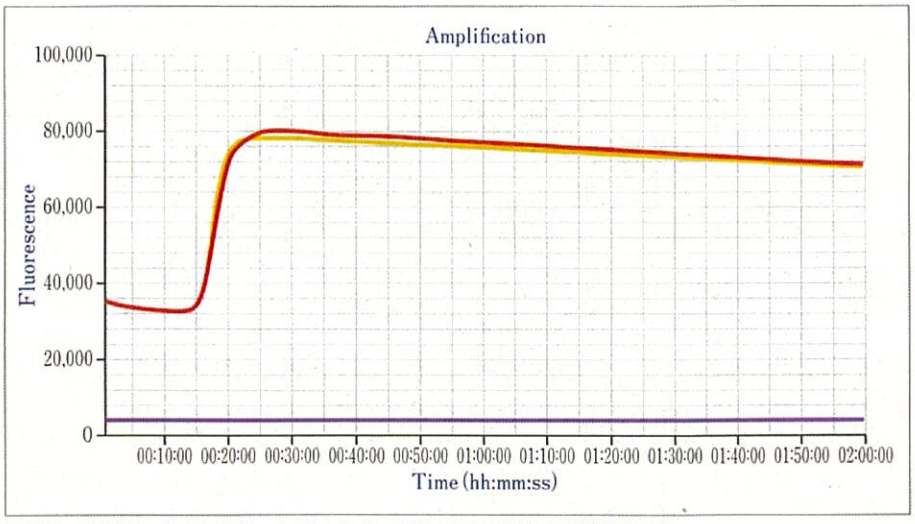

図2　症例1．LAMPによる*HLA-A*3101*遺伝子増幅反応

症例2　80歳，女．

初診　2015年6月．

家族歴　とくになし．

既往歴　甲状腺腫，左卵巣腫瘍手術，直腸穿孔後腹膜穿通手術，人工肛門造設術，弁膜症に対して弁輪形成術あり．慢性腎臓病．

現病歴　5月下旬より三叉神経痛に対してCBZを開始された．6月下旬，CBZ開始28日後から体幹，四肢に紅斑が拡大し当院

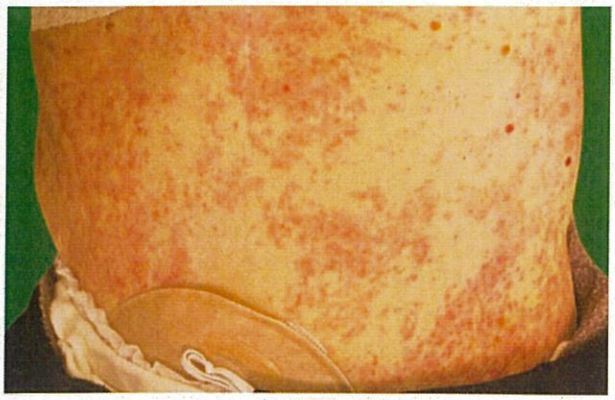

図3　症例2. 臨床像. 腹部の浸潤を触れる小紅斑

救急外来を受診. 薬疹を疑われ当科へ紹介となった.

現症　体温 37.1℃, 血圧 141/69 mmHg, 心拍 83 bpm, 呼吸数16回/分.

軀幹, 四肢に浸潤を触れる小紅斑が散在(図3). 口腔内や眼球・瞼結膜に粘膜疹なし. 頸部リンパ節腫大あり.

鑑別診断　単純ヘルペス再活性化による多形紅斑：膿疱を混じる紅斑である点が非典型である. また, 単純ヘルペス再活性化既往なし.

伝染性単核球症：好発年齢が異なる.

麻疹・風疹：CRP上昇から可能性があるが抗菌薬使用なく改善している点が異なる.

臨床検査所見(下線部は異常値)　WBC 4,380 /μl(Neutro 58.7%, Eos 23.3%, Baso 0.2%, Mono 5.9%, Lymph 11.9%), RBC 3.68×10^6 /μl, Hb 10.7 g/d*l*, Hct 34.3%, CRP 1.74 mg/d*l*, TARC 9319.3, IgE 2.5 IU/m*l*, AST 75 U/*l*, ALT 109 U/*l*, LDH 351 U/*l*, γ-GTP 517 U/*l*, CK 16 U/*l*, Amylase 133 U/*l*, 尿素窒素 58.8 mg/d*l* Crea 3.71 mg/d*l*.

LAMP　採血検体からDNA抽出して, *HLA-A*3101*特異プライマーを使用し, LAMPを行ったところ, 1時間以内で遺伝子増幅が確認された(図4).

CBZを用いたリンパ球幼弱化試験(DLST)　S.I.：120(陰性)(第5病日), S.I.：134(陰性)(第12病日), S.I.：257(陽性)(第96病日).

HLA-A遺伝子タイピング　*HLA-A*3101*, *HLA-A*2603*(検体提出後7日目に結果報告).

HHV-6再活性化　ペア血清にて4倍のIgG抗体価上昇あり(急性期に20倍→回復期に640倍).

診断確定　発症1カ月前からCBZの服用が開始されており, 紅斑および膿疱を混じる皮疹が出現していたこと, *HLA-A*3101*特異プライマーを用いたLAMPが陽性であることから, CBZ誘導型薬疹を疑いCBZを中止した. 複数回測定のDLSTにて回復期に陽性が認められ, 被疑薬同定とした. HHV-6再活性化あり, リンパ節腫脹も認め典型DIHSと確定診断した.

治療と経過　CBZを中止後も皮疹が増悪傾向であったので, ステロイドパルス加療追加し, プレドニゾロン1 mg/kg/日から開始して漸減し, 症状は寛解した.

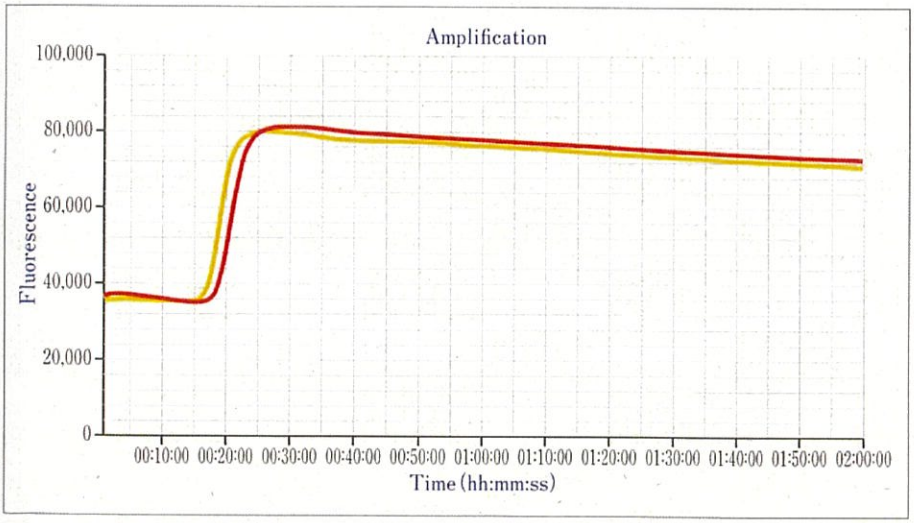

図4　症例2. LAMPによる*HLA-A*3101*遺伝子増幅反応

考　按

急性発症する急性発疹症は, それが薬疹であるのか, 薬疹であれば原因薬剤は何であるのかについて速やかに判断し, 原因薬剤を中止して, 必要な加療を行わなければならない.

薬疹は問診情報が重要で被疑薬決定の参考となるが, 高齢になれば複数薬剤をときに同時期に開始することがある. その

際，DLST結果が被疑薬検討の参考になるが，本邦では外注依頼で結果返却に1週間程度要することや正確な感度・特異度が十分検討されておらず，急性期の被疑薬決定の参考には寄与しにくい．

近年，国内外から特定の薬剤による薬疹と特定のHLAが関連することが報告されている．

重症薬疹の原因薬剤で高頻度な薬剤にCBZがあるが，これも特定のHLAと薬疹との関連が報告されている．カルバマゼピンは漢民族で*HLA-B*1502*とオッズ比約1500で関連があり[1]，*HLA-A*3101*は，日本人でオッズ比約10程度で関連が報告されている[2,3]．近年，薬疹発症リスク因子保有により，カルバマゼピン代替薬を使用する前向き試験が行われた．漢民族では，*HLA-B*1502*の投与前スクリーニングにより，カルバマゼピンの重症薬疹の発症が有意に抑えられたと報告されている[4]．本邦においても，投与前スクリーニングで重症薬疹の発症を有意に抑えられたことが報告されている[5]．

カルバマゼピン誘導型薬疹の回避または原因薬剤同定のために*HLA-A*3101*保有について検査を行うことが有用であることが報告されているが，本邦においてHLAタイピングは外注依頼で結果返却に約1週間程度要し，保険適用でなく1検体当たりのコストが数万円程度かかるため検査としての日常臨床での有用性は十分とはいえない．近年，等温条件下で1時間で10の9乗倍に目的遺伝子を増幅して検出できるLAMPが開発され，とくに感染症領域で臨床応用されつつある[6]．本法は，特定の一塩基多型(single nucleotide polymorphisms：SNP)の検出にも有用であることが報告されており[7]，*HLA-A*3101*の同定に有用であったことが報告されている[8]．今回，当科で経験した2症例について臨床応用した．検査方法はまず患者採血検体からDNAを抽出して，*HLA-A*3101*検出用に作成した6種類のプライマーを使用して反応を進めた．いずれの症例も約1時間以内でSNPを含む遺伝子領域を増幅することができ，*HLA-A*3101*保有を確認できた．*HLA-A*3101*を保有しており，発症の数週間前からカルバマゼピンを服用開始していることから，カルバマゼピン誘導型薬疹と同定して，カルバマゼピンをいったん中止した．発熱はあるものの，全身状態が比較的良好で食事摂取可能であり，1例目は補液による対症療法で徐々に解熱傾向となり，2例目はステロイドの投与で治癒に至った．

LAMPは等温条件下においてPCR増幅反応を行うので，増幅装置もコンパクトな装置となっている．増幅される遺伝子量が膨大であり，増幅された遺伝子が反応液内の濁りとして目視で確認することも可能である．比較的短時間のうちに大量に標的遺伝子を増幅でき，必要とする試薬も安価であることから，1検体あたりの費用が安く抑えられる．LAMPで*HLA-A*3101*保有について検査することは，今回の2例においてCBZ誘導型薬疹の早期診断に寄与したものと考える．

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Two cases of rapid detection of *HLA-A*3101* for the diagnosis of carbamazepine-induced adverse drug reaction

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Abstract

A 63-year-old man with symptomatic epilepsy developed diffuse erythema on his trunk and extremities 2 months after being administered carbamazepine. He was referred to our department 14 days after the rash appeared. Loop-mediated isothermal amplification (LAMP)was performed for the detection of *HLA-A*3101*. A positive result on LAMP confirmed the diagnosis within one hour. His symptoms disappeared on withdrawal of carbamazepine. Furthermore, an 80-year-old woman with trigeminal neuralgia developed diffuse erythema on her trunk and extremities 28 days after being administered carbamazepine. The causative drug was determined with a positive LAMP reaction for *HLA-A*3101*. Her symptoms disappeared after methylprednisolone pulse therapy was administered. Overall, the LAMP assay for the detection of *HLA-A*3101* was useful for the diagnosis of carbamazepine-induced adverse drug reactions in these two cases.