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《アレルギー疾患ガイドラインとその使い方》

6 重症薬疹

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ポイント

- 重症薬疹にはStevens-Johnson症候群（SJS），中毒性表皮壊死症（TEN），薬剤性過敏症症候群（DIHS），急性汎発性発疹性膿疱症（AGEP）があり，現時点での診断基準が厚生労働省重篤副作用疾患別対応マニュアルに掲載されている．
- SJSおよびTENは，高熱とともに皮膚粘膜の壊死性障害をきたす重篤な疾患である．治療の第一選択はステロイド薬の全身投与（パルス療法を含む）であり，重症度を考慮しながら投与量を選択する．
- DIHSは薬剤摂取後に発症する発熱を伴う遷延性の全身性紅斑であり，末梢血異常と肝機能障害などの臓器障害を伴う．
- AGEPは高熱とともに出現する全身の浮腫性紅斑および膿疱で，血液検査所見では好中球優位の白血球増多をみる．

キーワード：Stevens-Johnson症候群，中毒性表皮壊死症（TEN），薬剤性過敏症症候群（DIHS），急性汎発性発疹性膿疱症（AGEP）

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重症薬疹は，厚生労働科学研究費補助金（難治性疾患克服研究事業）の重症多形滲出性紅斑に関する調査研究班により検討され，現時点での診断基準が厚生労働省・重篤副作用疾患別対応マニュアルに掲載されている[1]．ここで重症薬疹として扱われている疾患は，Stevens-Johnson症候群（Stevens-Johnson syndrome：SJS），中毒性表皮壊死症（toxic epidermal necrolysis：TEN），薬剤性過敏症症候群（drug-induced hypersensitivity syndrome：DIHS），急性汎発性発疹性膿疱症（acute generalized exanthematous pustulosis：AGEP）であり，このうちSJS/TENについては治療指針も作成されている[2,3]．本稿ではそれぞれの重症薬疹をガイドラインに沿って解説する．

◉Stevens-Johnson症候群および中毒性表皮壊死症

SJS/TENは，高熱とともに皮膚粘膜の壊死性障害をきたす重篤な疾患である．皮膚に紅斑と水疱・びらんの形成をみるとともに口唇・口腔粘膜，眼，肛囲・外陰部などの皮膚粘膜移行部の障害をみる．TENはSJSで発症するものが大部分である（SJS進展型）．SJS，TENともしばしば経過中に肝臓，腎臓，肺，消化管などの臓器障害を生じ，特にTENではこれらを併発する頻度が高い．骨髄抑制による白血球減少や血小板減少，血管内凝固症候群や敗血症などを併発するとさらに重篤となり，TENではいまだに高い死亡率（約20～40%）が国内外から報告されている[4]．後遺症も問題となり，重篤例では閉塞性細気管支炎による呼吸障害や結膜・角膜びらんによる視力障害をみる．

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表 1　Stevens-Johnson 症候群診断基準 2005（2011 年 1 月眼病変につき改訂）

(1) 概念
発熱を伴う口唇，眼結膜，外陰部などの皮膚粘膜移行部における重症の粘膜疹および皮膚の紅斑で，しばしば水疱，表皮剝離などの表皮の壊死性障害を認める．原因の多くは，薬剤である．

(2) 主要所見（必須）
① 皮膚粘膜移行部の重篤な粘膜病変（出血性あるいは充血性）がみられること．
② しばしば認められるびらんもしくは水疱は，体表面積の 10%未満であること．
③ 発熱．

副所見
④ 皮疹は非典型的ターゲット状多形紅斑．
⑤ 眼症状は眼表面上皮欠損と偽膜形成のどちらか，あるいは両方を伴う両眼性の急性角結膜炎．
⑥ 病理組織学的に，表皮の壊死性変化を認める．

ただし，TEN への移行があり得るため，初期に評価を行った場合には，極期に再評価を行う．

主要項目の 3 項目をすべてみたす場合 SJS と診断する．

表 2　中毒性表皮壊死症（Toxic epidermal necrolysis：TEN）診断基準 2005（2011 年 1 月眼病変につき改訂）

(1) 概念
広範囲な紅斑と，全身の 10%以上の水疱，表皮剝離・びらんなどの顕著な表皮の壊死性障害を認め，高熱と粘膜疹を伴う．原因の大部分は医薬品である．

(2) 主要所見（必須）
① 体表面積の 10%を越える水疱，表皮剝離，びらんなどの表皮の壊死性障害．
② ブドウ球菌性熱傷様皮膚症候群（SSSS）を除外できる．
③ 発熱．

(3) 副所見
④ 皮疹は広範囲のびまん性紅斑および斑状紅斑である．
⑤ 粘膜疹を伴う．眼症状は眼表面上皮欠損と偽膜形成のどちらか，あるいは両方を伴う両眼性の急性角結膜炎．
⑥ 病理組織学的に，顕著な表皮の壊死を認める．

主要 3 項目のすべてを満たすものを TEN とする．

○サブタイプの分類
1 型：SJS 進展型（TEN with spots）
2 型：びまん性紅斑進展型（TEN without spots）
3 型：特殊型

○参考所見
治療等の修飾により，主要項目 1 の体表面積 10%に達しなかったものを不全型とする．

診断基準を表 1，2 に示す．SJS/TEN の診断のポイントは壊死性，出血性の粘膜疹を伴うことである．いずれも水疱の形成をみるが，SJS では非典型的ターゲット状（標的状）紅斑の中心に水疱形成をみる．非典型的と表現される理由は，隆起性で三重の環状構造を形成する「典型的」ターゲット状紅斑とは異なり，扁平で明確な三重の環状構造を形成しないためである．眼症状については眼表面上皮欠損と偽膜形成のどちらか，あるいは両方を伴う両眼性の急性角結膜炎を診断基準としている．確定診断には紅斑部の皮膚生検が重要である．SJS では多発する表皮細胞のアポトーシスを，TEN では表皮全層にわたる壊死を認める．障害された表皮下のスリット状の列隙形成も特徴

表 3　SJS および TEN の治療指針 2009

Stevens-Johnson 症候群（SJS）および中毒性表皮壊死症（TEN）の治療には，まず被疑薬の中止を行う．厳重な眼科的管理，皮疹部および口唇・外陰部粘膜の局所処置，補液・栄養管理，感染防止が重要である．
薬物療法としては，確立されたものではないが効果を期待できる治療法として，早期の副腎皮質ステロイド薬の全身療法が第一選択となっている．症例に応じて他の治療法や併用療法を実施する．

1．副腎皮質ステロイド薬の全身投与
症例により状態が異なるため一律には決めがたいが，推奨される投与法は下記の通りである．発症早期＊）に開始することが望ましい．治療効果の判定には，紅斑・表皮剝離・粘膜疹の進展の停止，びらん面からの浸出液の減少，解熱傾向，末梢血白血球異常の改善，肝機能障害などの臓器障害の改善などを指標とする．重篤な感染症を合併している場合にはステロイド薬投与とともに抗菌薬や免疫グロブリン製剤などを併用し感染対策を十分に行う．

ステロイド療法
プレドニゾロンまたはベタメタゾン，デキサメタゾンをプレドニゾロン換算で，中等症は 0.5～1 mg/kg/日，重症は 1～2 mg/kg/日で開始する．

ステロイドパルス療法
重症例や急激に進展する症例ではパルス療法も考慮する．パルス療法は，メチルプレドニゾロン 500 mg～1,000 mg/日を 3 日間投与する（小児の場合，小児の標準的治療法に準ずる）．中等症の場合は，より少量（250 mg/日）の投与で効果がみられることがある．初回のパルス療法で効果が十分にみられない場合，または症状の進展が治まったのちに再燃した場合は，数日後にもう 1 クール施行するか後述するその他の療法を併用する．
パルス療法直後のステロイド投与量は十分量（プレドニゾロン換算で 1～2 mg/kg/日）を投与し，漸減する．減量速度は個々の症例の回復の程度により調整する．

ステロイド投与で充分に効果がみられない場合
ステロイド薬投与の効果がみられないにもかかわらず，漫然と同量のステロイド薬投与を継続することは避ける．その際には，ステロイド薬の増量や他の治療法（免疫グロブリン製剤，血漿交換療法など）も考慮する．

＊早期とは，発症後 7 日前後までを目安とする．

備考：
発症後表皮剝離が全身に及んだ段階でのステロイド薬開始は敗血症などの感染症を助長する可能性が高いため，ステロイド薬を投与する場合には感染対策を十分に行う．

皮疹が軽度でも高度の粘膜疹（例：眼表面上皮のびらん，あるいは偽膜形成）がみられる場合には，眼科受診を行い，発症初期にパルス療法など副腎皮質ステロイド薬の大量投与を行う．感染に配慮しながら，眼局所へのステロイド薬投与をあわせて行うことが望ましい．ステロイド薬全身投与の減量時に粘膜疹の悪化を生じることがあり，注意を要する．

2．その他の治療法
ヒト免疫グロブリン製剤静注（IVIG）療法
一般に 5～20 g/日，3～5 日間を 1 クールとして投与する．

血漿交換療法
ステロイド療法で症状の進行がくい止められない重症例に併用療法として，もしくは重症感染症などステロイド薬の使用が困難な場合に施行する．単純血漿交換法（PE）と二重膜濾過血症交換法（DFPP）がある．

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的である．

治療指針を表 3 に示す．治療法の選択の目安として，重症度判定の参考となる SJS/TEN 重症度スコア判定（表 4）が付記されている．治療のポイントとしては，ステロイド薬の全身投与が第一選択であること，重症例では発症早期に高用量（パルス療法を含む）で開始すること，ステロイド薬の効果が十分にみられない場合には増量また

表 4　SJS/TEN 重症度スコア判定

1	粘膜疹		
	眼病変	上皮の偽膜形成	1
		上皮びらん	1
		結膜充血	1
	口唇，口腔内	口腔内広範囲に血痂，出血を伴うびらん	1
		口唇にのみ血痂，出血を伴うびらん	1
		血痂，出血を伴わないびらん	1
	陰部びらん		1
2	皮膚の水疱，びらん		
	30%以上		3
	10～30%		2
	10%未満		1
3	38℃以上の発熱		1
4	呼吸器障害		1
5	表皮の全層性壊死性変化		1
6	肝機能障害（ALT>100 IU/L）		1

6点以上　重症　ただし，以下はスコアにかかわらず重症と判断する
1）眼球，眼瞼結膜上皮の偽膜形成，びらんが高度なもの
2）SJS/TEN に起因する呼吸障害のみられるもの
3）びまん性紅斑進展型 TEN

6点未満　中等症

（厚生労働科学研究費補助金 難治性疾患克服研究事業　重症多形滲出性紅斑に関する調査研究班）

はヒト免疫グロブリン製剤静注（IVIG）や血漿交換といった他の治療法の併用を考慮すること，十分な感染症対策を合わせて行うこと，が挙げられる．

◉薬剤性過敏症症候群

DIHS は薬剤摂取後に発症する発熱を伴う遷延性の全身性紅斑であり，末梢血異常と肝機能障害などの臓器障害を伴うものを指す．

DIHS の診断基準を表 5 に示す．診断のポイントは薬物アレルギー，および β-ヘルペスウイルスに属する human herpesvirus-6（HHV-6）再活性化のそれぞれによる症状および検査値の異常がみられることである．原因薬の多くが特定の薬剤であること，特徴的な顔貌（顔面腫脹，眼囲の蒼白や口囲を中心とした小膿疱・痂皮の形成），などが早期の診断に役立つ．診断に重要な経過中の所見は薬剤中止後の症状の遷延化や再燃と HHV-6 の再活性化であり，これには HHV-6 をはじめ cytomegalovirus（CMV）や Epstein-Barr virus（EBV），HHV-7 といった human herpesvirus の再活性化との関連が推察されている．

診断確定に至るには，全経過をとおして表 5 に示す所見がそろうことが必要である．臓器障害の大部分は肝障害であるが，腎障害，脳炎，肺炎，心筋炎がみられることがある．また，自己免疫疾患，甲状腺炎，1 型糖尿病などが発症後期の皮疹軽快後にみられることもある．病理組織所見では，真皮上層の浮腫および真皮から表皮へのリンパ球を主とする炎症細胞浸潤，軽度の表皮細胞のアポトーシスをみるが，壊死性病変はないことが重要である．

◉急性汎発性発疹性膿疱症

高熱とともに出現する全身の浮腫性紅斑および膿疱で，血液検査所見では好中球優位の白血球増多をみる[5]．多くはペニシリン系やマクロライド

表 5　薬剤性過敏症症候群（Drug-induced hypersensitivity syndrome：DIHS）診断基準 2005

(1) 概念
高熱と臓器障害を伴う薬疹で，薬剤中止後も遷延化する．多くの場合，発症後 2 から 3 週間後に HHV-6 の再活性化を生じる．

(2) 主要所見
1．限られた薬剤投与後に遅発性に生じ，急速に拡大する紅斑．しばしば紅皮症に移行する．
2．原因薬剤中止後も 2 週間以上遷延する．
3．38 度以上の発熱
4．肝機能障害
5．血液学的異常：a，b，c のうち一つ以上
　a．白血球増多（11,000/mm^{3}以上）
　b．異型リンパ球の出現（5%以上）
　c．好酸球増多（1,500/mm^{3}以上）
6．リンパ節腫脹
7．HHV-6 の再活性化

典型 DIHS：1〜7 すべて
典型 DIHS：1〜5 すべて，ただし 4 に関しては，その他の重篤な臓器障害をもって代えることができる．

○参考所見
1．原因薬剤は，抗けいれん剤，ジアフェニルスルフォン，サラゾスルファピリジン，アロプリノール，ミノサイクリン，メキシレチンであることが多く，発症までの内服期間は 2 週から 6 週間が多い．
2．皮疹は，初期には紅斑丘疹型，多形紅斑型で，後に紅皮症に移行することがある．顔面の浮腫，口囲の紅色丘疹，膿疱，小水疱，鱗屑は特徴的である．粘膜には発赤，点状紫斑，軽度のびらんがみられることがある．
3．臨床症状の再燃がしばしばみられる．
4．HHV-6 の再活性化は，① ペア血清で HHV-6IgG 抗体価が 4 倍（2 管）以上の上昇，② 血清（血漿）中の HHV-6DNA の検出，③ 末梢血単核球あるいは全血中の明らかな HHV-6DNA の増加のいずれかにより判断する．ペア血清は発症後 14 日以内と 28 日以降（21 日以降で可能な場合も多い）の 2 点にすると確実である．
5．HHV-6 以外に，サイトメガロウイルス，HHV-7，EB ウイルスの再活性化も認められる．
6．多臓器障害として，腎障害，糖尿病，脳炎，肺炎，甲状腺炎，心筋炎も生じうる．

系などの抗生剤やテルビナフィン，ジルチアゼム，カルバマゼピン，アセトアミノフェンなどの薬剤による遅延型アレルギー反応である．

診断基準を表 6 に示す．診断のポイントは，顔や腋窩，鼠径部などの間擦部に始まる紅斑と無菌性の小膿疱が全身に急速に拡大することである．通常，粘膜疹や重度の臓器障害は伴わないことも，他の重度薬疹との鑑別点となる．病理組織所見では角層下膿疱が特徴的であり，表皮内の海綿状膿疱もみられる．基礎疾患として好中球の増加をみる乾癬，関節リウマチ，潰瘍性大腸炎，掌蹠膿疱症や，糖尿病，骨髄性白血病をしばしば伴うことも特徴といえる．原因薬剤の中止から約 2〜3 週間で治癒することから，他の重症薬疹と比較して予後は格段によいが，高熱の持続による消耗やときに肝機能障害などの臓器障害をみることがあり，注意深い観察が必要である．

◉診断基準ガイドラインの課題

重症薬疹では SJS から TEN への移行だけでなく，それぞれの移行や overlap した臨床像がみられることもある．そのため，診断基準に固執することなく日々の観察により得られる情報から適切な検査・治療計画を立てることが重要である．

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表 6　急性汎発性発疹性膿疱症（acute generalized exanthematous pustulosis：AGEP）の診断基準

(1) 概念
薬剤摂取後，発熱とともに急速に出現する多数の無菌性小膿疱を有する汎発性の紅斑で，末梢血の好中球増多を伴う．
(2) 主要所見
① 急速に出現，拡大する紅斑
② 紅斑上に多発する無菌性の非毛孔性小膿疱
③ 末梢血の好中球増多（7,000/mm^{3}以上）
④ 発熱
(3) 副所見
⑤ 皮膚病理組織学的に角層下膿疱あるいは表皮内膿疱
⑥ 除外疾患：膿疱性乾癬，角層下膿疱症，中毒性表皮壊死症，汗疹，敗血疹

主要項目のすべてをみたすものを急性汎発性発疹性膿疱症とする．

(4) 参考所見
・皮疹は間擦部や圧迫部に出現しやすい．
・膿疱は 5 mm 大以下のことが多い．
・多くで粘膜疹は認めない．
・ウイルスや細菌感染が先行あるいは増悪因子となることがある．
・基礎疾患（乾癬，関節リウマチ，骨髄性白血病，潰瘍性大腸炎，掌蹠膿疱症，糖尿病など）が存在していることが多い．

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gene-related peptide, which is the mediator of pruritus, and modulate the μ-opioid receptor making it possible to change the sensorium of pruritus.[1,3,4] Pregabalin shows faster treatment response than gabapentin and it does not bind to plasma protein, so has the advantage of use in patients with low plasma protein and hepatic failure.[3]

Our study has several limitations. First, this was an open-label uncontrolled study and the effect of pregabalin could be attributed to a placebo effect. Second, we used pregabalin in a fixed dose of 150 mg/day. It is known that pregabalin may be used in higher doses up to 300 mg/day within 1 week based on efficacy and tolerability. Third, pregabalin also works for underlying neuropathic pain and anxiety, and so it could affect these accompanying symptoms and may lead to an overall improvement.

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Case of carbamazepine-induced hypersensitivity syndrome associated with human leukocyte antigen-A*3101

Dear Editor,

In 2008, Mallal *et al.*[1] reported that a hypersensitivity reaction to abacavir, a reverse transcriptase inhibitor of HIV, was strongly associated with human leukocyte antigen (HLA)-B*5701. In addition, it was indicated that HLA-B*5701 screening can reduce the risk of a hypersensitivity reaction to abacavir and a pharmacogenetic test was found to be useful for preventing a specific toxic effect of the drug.[1] Furthermore, carbamazepine (CBZ)-induced cutaneous adverse drug reactions (cADR), including Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS), have been shown to be closely associated with HLA.[2,3] Ozeki *et al.*[4] demonstrated that HLA-A*3101 is significantly associated with susceptibility to DIHS induced by CBZ in the Japanese population. It remains unclear how DIHS develops but these reports shed light on the pathogenesis of DIHS and are expected to promote the development of a genetic test for identifying individuals at risk for this potentially life-threatening condition caused by CBZ.

The patient was a 62-year-old female. One month after CBZ for trigeminal neuralgia, she became ill with fever, a sore throat and an erythematous maculopapular eruption. Three weeks after becoming ill, the patient was admitted to our hospital. At that time her temperature was 38.6°C and she had gained 5 kg of bodyweight. Her neck lymph nodes were enlarged and erythroderma was evident. Leukocytosis, eosinophilia and liver dysfunction were present. A comparison of virus antibody values on the 1st day (human herpesvirus [HHV]-6 immunoglobulin [Ig]G, 10; cytomegalovirus [CMV] IgG, 13.3) and the 30th day (HHV-6 IgG, 80; CMV IgG, >128), indicated revitalization of HHV-6 and CMV. The drug lymphocyte stimulating test (DLST) of CBZ was positive (SI = 281%). The diagnosis was DIHS-induced CBZ on the basis of physical examination, blood tests, medication with CBZ and a positive reaction to DLST. First, we suspended treatment with CBZ and administrated 60 mg/day of prednisolone by drip infusion for 3 days. We then administrated 40 mg/day of prednisolone tablets for 7 days with subsequent tapering. Four weeks after admission, all symptoms disappeared, and 114 days after admission, the prednisolone course was complete. Eighty-three days after completion of the course of prednisolone, the patient acquired indolent thyroiditis (Table 1, Fig. 1). After providing consent, high-resolution HLA serum typing of this case was performed by using a reverse sequence-specific oligonucleotide polymerase chain reaction (PCR-rSSO) method (Mitsubishi-Chemical BCL Laboratory, Tokyo, Japan). The HLA-A*3101 allele was confirmed to be present.

Carbamazepine is a frequently used anticonvulsant agent, which occasionally induces drug eruption. It is one of a few drugs that produce various cADR such as DIHS. The pathogenesis of the drug reaction is unclear because pathological analysis still has not been established due to the diverse forms of reactions, factors and modifiers and the absence of a suitable animal model. Therefore, even

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Table 1. Examinations of thyroid function

F-T3 4.38 (2.1–4.1 pg/mL)
F-T4 1.80 (1.0–1.7 pg/mL)
Thyroid-stimulating hormone 0.05 (0.436–3.78 μU/mL)
Anti-thyroid antigen (–)

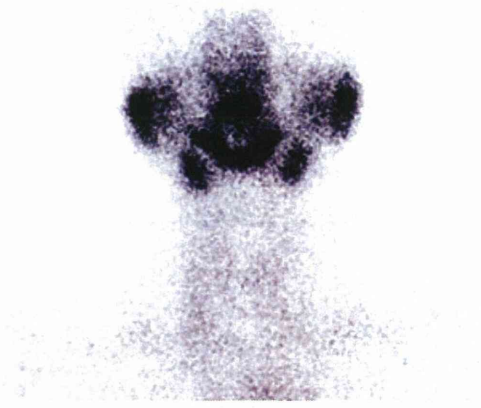

Figure 1. No uptake of technetium-99*m* was identified in the thyroid. F-T3 and FT-4 were high and thyroid-stimulating hormone was suppressed. Furthermore, anti-thyroid antigen did not appear. We then made a diagnosis of indolent thyroiditis.

though drug eruption is a common disorder, it tends to get short shrift and potentially life-threatening conditions such as DIHS are not well recognized.

In 2011, Ozeki *et al.* found that 12 single nucleotide polymorphisms significantly associated with CBZ-induced cADR are located within a 463-kb region on chromosome 6p (21,33). It is notable that this region corresponds to the major histocompatibility complex (MHC) class I region containing the HLA-A locus.[4] The individual HLA-A alleles were genotyped for 61 cases that developed cADR and 376 cases that did not develop cADR with administration of CBZ. It was found that the HLA-A*3101 allele was present in 60.7% (37/61) of the cases with CBZ-induced cADR, but in only 12.5% (47/376) of the CBZ-tolerant controls (odds ratio = 10.8). This implies that the allele has 60.7% sensitivity and 87.5% specificity when applied as a risk predictor for CBZ-induced cADR.[4] This report suggested that for certain drugs, HLA alleles which code MHC class I molecules are significantly associated with cADR with respect to pathogenesis.

In therapy for HIV infection, pharmacogenetic tests are useful for preventing specific toxic effects of drugs.[1] If pharmacogenomics tests had been administered for our case in the same manner before onset of disease, other anti-neuralgia agents may have been chosen and the complications would have been avoided. It would be ideal if profiling analysis of HLA alleles could be performed in certain geographical areas on a mass scale and if clinical applications of gene analysis could be generalized from this point forward.

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Successful treatment with adapalene of cetuximab-induced acneiform eruptions

Dear Editor,

The epidermal growth factor receptor (EGFR) has been identified as a new target for the treatment of many human solid tumors. EGFR is involved in normal cell growth and differentiation in non-malignant cells such as epidermal keratinocytes, sebocytes and hair follicles. Cetuximab is a chimeric monoclonal antibody that selectively binds to the EGFR, blocking its activation and signal transduction. Cetuximab is approved for use in EGFR-expressing colorectal cancer in patients previously resistant to chemotherapy.[1] The most common adverse effects of cetuximab are cutaneous

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ORIGINAL ARTICLE

HLA-A31 strongly associates with carbamazepine-induced adverse drug reactions but not with carbamazepine-induced lymphocyte proliferation in a Japanese population

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ABSTRACT

Carbamazepine (CBZ) is the most frequent culprit drug for severe cutaneous adverse drug reactions (ADR), such as Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS). A strong association between human leukocyte antigen (HLA)-B*1502 and CBZ-induced SJS/TEN has been reported in Han Chinese, Thai, Malaysian and Indian populations, but not in Caucasian or Japanese populations. Recent studies showed an association between HLA-A*3101 and CBZ-induced ADR in Caucasian and Japanese populations. We conducted a case–control study to determine HLA genotyping of patients with CBZ-induced ADR in a Japanese population. Fifteen patients with CBZ-induced ADR and 33 subjects who had taken CBZ for more than 3 months without evidence of any ADR as a control were enrolled. In addition, the results of a CBZ-induced lymphocyte stimulation test were compared between the groups. A strong association was found between HLA-A31 and CBZ-induced ADR ($P < 0.001$), and a weak association was found between HLA-A11 and HLA-B51 with CBZ-induced ADR. No HLA-B*1502 was found in either patients or control subjects. The mean CBZ-induced lymphocyte stimulation index was significantly high in patients with CBZ-induced ADR compared with CBZ-tolerant patients ($P < 0.001$); however, no significant difference was seen between HLA-A31-positive subjects and HLA-A31-negative subjects in either group. These findings suggest that HLA-A31 is strongly associated with CBZ-induced ADR in the Japanese, but does not determine CBZ-induced lymphocyte proliferation.

Key words: adverse drug reaction, carbamazepine, drug-induced hypersensitivity syndrome, human leukocyte antigen A31, lymphocyte stimulation test.

INTRODUCTION

Carbamazepine (CBZ) is an antiepileptic drug that has been widely used for treating not only seizures, but also neuropathic pain. CBZ is the most frequent culprit drug for severe cutaneous adverse drug reactions (ADR), including Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS). Since a strong association between human leukocyte antigen (HLA)-B*1502 and CBZ-induced SJS/TEN was reported in Han Chinese residing in Taiwan,[1] intensive studies have focused on the association between HLA class I allele and ADR.[2–16] The results are summarized in Table 1. An association between HLA-B*1502 and CBZ-induced SJS/TEN has been confirmed in Han Chinese in Hong Kong and in Chinese, Thai, Malaysian and Indian populations,[5,6,8,9,11–14] but an association has not been seen in Caucasians.[3,17,18] In Japanese studies, no CBZ-induced SJS/TEN patients carried HLA-B*1502.[7,10] In addition, no HLA-B*1502 carriers were detected in drug-unspecified SJS/TEN patients in a Japanese population.[19,20] Instead, HLA-B*1511 was found to be associated with CBZ-induced SJS/TEN patients in the Japanese.[10] Interestingly, HLA-B*1502 was found to be specific to CBZ-induced SJS/TEN, and no association was seen in patients with CBZ-induced hypersensitivity syndrome (HSS) or maculopapular eruption (MPE) in Han Chinese residing in Taiwan.[2] In addition, no association with HLA-B*1502 was confirmed in Caucasian patients with HSS.[16] An association between HLA-A*3101 and CBZ-induced ADR was recently reported in both Japanese and Europeans by genome-wide approaches.[15,16] These observations indicate a diversity in HLA class I association with CBZ-induced ADR.

We conducted a case–control study to determine HLA types associated with CBZ-induced ADR in a Japanese population. In addition, CBZ-induced lymphocyte proliferation was evaluated to

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Table 1. Reported HLA associated with CBZ-induced cutaneous ADR

HLA	Race	Diagnosis	Selectivity*	References
B*1502	Han Chinese (Taiwan)	SJS/TEN	44/44	1
	Han Chinese (Taiwan)	SJS/TEN	59/60	2
	Asian in Europe	SJS/TEN	4/4	3
	Caucasians	SJS/TEN	0/8	3
	Caucasians	DIHS	0/56	4
	Han Chinese (Hong Kong)	SJS/TEN	4/4	5
	Thai	SJS	6/6	6
	Thai	MPE	0/5	6
	Japanese	ADR	0/22	7
	Indians	SJS	6/8	8
	Thai	SJS	37/42	9
	Japanese	SJS/TEN	0/14	10
	Han Chinese (Central China)	SJS/TEN	8/8	11
	Han Chinese (Central China)	MPE	3/28	11
	Malaysian	SJS/TEN	12/16	12
	Chinese	SJS/TEN	3/3	12
	Indians	SJS/TEN	2/2	12
	Han Chinese (Southern China)	SJS/TEN	9/9	13
	Han Chinese (Southern China)	MPE	10/39	13
	Han Chinese	SJS/TEN	16/17	14
	Han Chinese (Taiwan)	HSS	0/13	2
A*3101	Han Chinese (Taiwan)	SJS/TEN	1/60	2
	Han Chinese (Taiwan)	HSS	2/13	2
	Han Chinese (Taiwan)	MPE	6/18	2
	Japanese	ADR	11/22	7
	Japanese	SJS/TEN	5/6	15
	Japanese	DIHS	21/36	15
	Caucasian (Northern European)	SJS	5/12	16
	Caucasian (Northern European)	HSS	10/27	16
	Caucasian (Northern European)	MPE	23/106	16
B*1511	Japanese	SJS	4/14	10

*HLA-positive patients/examined patients. ADR, adverse drug reactions; CBZ, carbamazepine; DIHS, drug-induced hypersensitivity syndrome; HLA, human leukocyte antigen; HSS, hypersensitivity syndrome; MPE, maculopapular eruption; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

determine whether HLA types are associated with lymphocyte activation, because T-cell-mediated allergic reaction is likely to be involved in the pathogenesis of ADR.[19]

METHODS

Patients

All patients were recruited from Shimane University Hospital between April 2005 and February 2011. These included 15 patients with CBZ-induced ADR and 33 patients who had been receiving CBZ for more than 3 months without drug eruption. The backgrounds of the patients with ADR are shown in Table 2. CBZ-induced ADR was determined by medical history indicating that symptoms occurred within 3 months after starting CBZ administration and that the symptoms resolved upon withdrawal of this drug. The diagnosis was confirmed by a positive stimulation index (>180%) in the CBZ-induced lymphocyte stimulation test (n = 12), positive patch testing (n = 4) and/or challenge test (n = 1). Diagnoses of SJS/TEN and DIHS were made according to the diagnostic criteria established by Roujeau and Shiohara.[21,22] All patients were interviewed by investigators regarding the histories of their biological parents and grandparents. Those with both biological parents and both sets of grandparents born in Japan were classified as native Japanese. This study was approved by the ethics committee of Shimane University Faculty of Medicine (approval no. 221).

HLA typing

DNA was extracted from peripheral blood. Low-resolution HLA typing was performed using the reverse sequence-specific oligonucleotide with polymerase chain reaction (PCR-rSSO) method, which is also called the Luminex method.[23,24] The area of exons 2 and 3 of the HLA-A, -B and -DR genes was amplified using HLA-A, -B and -DR locus-specific primers. The amplicon sizes of HLA-A and HLA-B loci were 400–500 bp (exon 2) and 300–400 bp (exon 3), and that of HLA-DRB1 locus was 250–300 bp (exon 2). Amplified PCR products were treated with alkali and turned to single chains. After being neutralized, PCR products were hybridized with a carboxylated fluorescent microbead-coated voluntary sequenced oligonucleotide primer. After a centrifugal wash of the microbead mixtures, reaction outcomes from biotin-labeled PCR amplicons

Table 2. Clinical characteristics of CBZ-induced ADR patients

No.	Age/sex	Type of ADR	CBZ indications	Latency (days)*	SI (%)	Measurement (day)†	Total systemic steroid (mg)‡	PT	Challenge test
1	42/F	TEN	Trigeminal neuralgia	32	398	52	8185	ND	ND
2	68/F	SJS	Peripheral neuropathy	13	132	15	950	+	ND
3	74/M	SJS	Peripheral neuropathy	32	137	9	952	−	+
4	30/M	DIHS	Epilepsy	28	259	28	0	ND	ND
5	22/F	DIHS	Depression	28	373	103	4560	+	ND
6	61/F	DIHS	Epilepsy	28	293	112	4860	ND	ND
7	67/F	DIHS	Neuropathic pain	20	1336	19	0	+	ND
8	49/M	DIHS	Peripheral neuropathy	47	642	78	6800	ND	ND
9	51/M	DIHS	Peripheral neuropathy	75	246	24	2200	ND	ND
10	75/F	DIHS	Trigeminal neuralgia	27	281	50	2700	ND	ND
11	61/F	DIHS	Epilepsy	38	324	54	2940	ND	ND
12	26/F	DIHS	Depression	24	410	247	6240	ND	ND
13	61/F	EEM	Epilepsy	4	380	10	0	ND	ND
14	45/M	EEM	Trigeminal neuralgia	24	149	21	0	+	ND
15	28/F	MPE	Trigeminal neuralgia	10	457	32	0	ND	ND

*Latency: days after starting CBZ administration. †Measurement day from onset. ‡Hydrocortisone titer conversion. EEM, erythema exsudativum multiforme; ADR, adverse drug reactions; CBZ, carbamazepine; DIHS, drug-induced hypersensitivity syndrome; ND, not done; PT, patch test; SI, stimulation index in drug-induced lymphocyte stimulation test; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis..

were measured by the Luminex 100 flow cytometer (GMI, Inc., Ramsey, Mn, USA), which is equipped with two types of lasers. The bead populations were detected and identified using the 635-nm laser. The phycoerythrin fluorescence of the streptavidin–phycoerythrin–biotin-labeled amplicons (Genosearch ver. 2) that had hybridized to the oligobeads was quantitated using the 532-nm laser. The median fluorescence intensity (MFI) of phycoerythrin was used to quantify the amount of DNA bound to the oligobeads. The measured data were read using dedicated software. The fluorescence intensity of negative controls was subtracted as background from each of the MFI values to determine the true intensity. The preset cut-off value for each fluorescing oligobead set was used to discriminate between positive and negative controls. The sequence-specific oligonucleotide probe (SSOP) hybridization results were matched with the Pattern File database using Genosearch HLA typing software to determine the HLA alleles.

High-resolution HLA-B genotyping was determined using the PCR sequence-based typing (SBT) method.[25] Exons 1–5 of the HLA-B gene were amplified using a HLA-B locus-specific primer by PCR using DNA extract kits (Qiagen, Tokyo, Japan). By using amplified products, the areas of exons 2–4 were each sequenced using a sequence kit (Abbott Japan, Tokyo, Japan). The sequenced products of exons 2–4 were directly read using a DNA analyzer (ABI 3730 DNA analyzer; Life Technologies, Carlsbad, CA, USA) and the type of gene was determined.

Drug-induced lymphocyte stimulation test (DLST)

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood and *in vitro* proliferation assays were performed as previously described by Pichler and Tilch.[26] The CBZ used for DLST assays were unmodified compounds dissolved in culture medium and they were sonicated to solve in the medium. Cultures were performed in triplicate at 37°C and 5% CO_2 for 3 days. As positive and negative controls, cells in triplicate were also incubated in the presence of 5 μg/mL phytohemagglutinin and in the absence of these agents, respectively. Twenty-four hours before harvesting, 1 μCi ^{3}H-thymidine (Amersham, Arlington Heights, IL, USA) was added. After harvesting, radioactivity was measured in a liquid scintillation counter (Pharmacia LKB Nuclear, Gaithersburg, MD, USA) and the results were expressed as the stimulation index (SI) (%); SI was calculated as follows: SI (%) = counts per minute (c.p.m.) with drug/c.p.m. without drug × 100. An SI (%) of more than 180 was regarded as positive based on previous studies preformed in Japan.[27–31] The DLST was also performed in control patients who were taking CBZ for more than 3 months without any clinical symptoms. DLST of the patients with CBZ-induced ADR was tested several times after consultation and the maximal SI was presented. The maximal SI was observed at day 56 ± 61 (mean ± SD) from onset.

Statistical analysis

Statistical analysis of the differences in each allele frequency among patients with ADR and control subjects was performed by Fisher's exact test. The strength of association was estimated by calculating the odds ratio (OR). The OR was determined using Haldane's modification, which adds 0.5 to all cells to accommodate possible zero counts. Differences in SI of DLST, the mean measurement day and total systemic steroid between subject groups were compared by the Mann–Whitney *U*-test. SAS ver. 9.2 software was used for statistical analysis. All reported *P*-values were two-sided. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Table 3 shows HLA DNA typing of CBZ-induced ADR patients. One of the three SJS/TEN patients had A31, eight of the nine DIHS patients had A31, and one of the three MPE/erythema exsudativum multiforme had A31 when low-resolution HLA DNA typing was performed. Comparing the frequency of each type between the

Table 3. HLA DNA typing of CBZ-induced ADR patients

No.	HLA low-resolution						HLA high-resolution	
	HLA-A		HLA-B		HLA-DR		HLA-B	
1	A26	A24	B62	B55	DR4	DR14	B*1507	B*5502
2	A2	A11	B75	B61	DR12	DR15	B*151101	B*400201
3	A31	A31	B56	B60	DR4	DR4	B*4001	B*5601
4	A24	A31	B60	B51	DR9	DR12	B*4001	B*510101
5	A31	A24	B51	B52	DR9	DR15	B*5101	B*5201
6	A2	A31	B46	B61	DR8	DR11	B*400201	B*4601
7	A2	A11	B51	B67	DR14	DR15	B*510101	B*670101
8	A11	A31	B7	B51	ND	ND	B*070201	B*510101
9	A31	A24	B61	B52	DR12	DR15	B*400201	B*520101
10	A24	A31	B54	B61	DR8	DR14	B*400201	B*5401
11	A31	A33	B44	B51	DR13	DR14	B*440301	B*510101
12	A24	A31	B51	B52	DR14	DR15	B*5101	B*5201
13	A24	A26	B13	B44	DR12	DR3	B*1310	B*440301
14	A11	A31	B51	B62	DR4	DR8	B*150101	B*510101
15	A11	A26	B55	B55	DR8	DR14	B*5502	B*5502

ADR, adverse drug reactions; CBZ, carbamazepine; HLA, human leukocyte antigen; ND, not done.

CBZ-induced ADR patients and the CBZ-tolerant patients, we found that the OR of A11, A31 and B51 were individually significantly high in the CBZ-induced ADR patients, as shown in Table 4. In particular, the OR of A31 was the highest ($P = 0.001$). Although the *P*-value of A11 was more than 0.05, we considered that the OR of A11 was significantly high because the 95% confidence interval (CI) of A11 did not range across 1.000. On the contrary, the OR of A2 was significantly low in the CBZ-induced ADR patients ($P = 0.04$). Table 5 shows the high-resolution HLA-B typing and their frequencies in the CBZ-induced ADR patients and CBZ-tolerant patients together with those reported for a general Japanese population.[32] The HLA-B*5101 genotype appeared significantly higher in the CBZ-induced ADR patients ($P = 0.031$). The OR of HLA-B*5101 was 4.900 and the 95% CI was 1.219–19.689. None of the 15 CBZ-induced ADR patients, including three SJS/TEN patients and 33 CBZ-tolerant patients, possessed the HLA-B*1502 genotype.

We also investigated the CBZ-induced proliferation of PBMC in each patient. The mean SI of CBZ-induced ADR patients ($382.1 \pm 295.1\%$, $n = 15$) was significantly high compared with that of CBZ-tolerant patients ($125.3 \pm 29.5\%$, $n = 32$, $P < 0.001$). Table 6 shows a comparison of DLST values, mean measurement day from onset and mean systemic steroid dose from onset to DLST measurement day between subjects with and without the HLA-A31 allele in CBZ-induced ADR patients. DLST values were also compared between subjects with and without the HLA-A31 allele in CBZ-tolerant patients. The mean SI was not significantly different between subjects with and without the HLA-A31 allele in both CBZ-induced ADR patients and CBZ-tolerant patients. The mean measurement day and the mean systemic steroid dose were not significantly different between subjects with and without the HLA-A31 allele in CBZ-induced ADR patients. No significant difference was seen in DLST values between subjects with or without the HLA-A11 allele or between subjects with or without A51 in CBZ-induced ADR patients and CBZ-tolerant patients (data not shown).

DISCUSSION

On the basis of previous reports of HLA associated with CBZ-induced ADR in a Japanese population,[7,15] we confirmed the association between HLA-A*3101 and CBZ-induced ADR, especially CBZ-induced DIHS. HLA-B*1502 was not found in either CBZ-induced ADR patients or CBZ-tolerant patients, compatible with previous results obtained from Japanese populations.[7,10,15,19,20] Altogether, HLA-B*1502 is strongly associated with SJS/TEN in Asians, but not in Japanese or Caucasians. On the other hand, HLA-A*3101 is well associated with SJS/TEN and DIHS in Japanese and Caucasians and, though to a somewhat lesser extent, associated with HSS/MPE in Asians.

The reason for the diversity of HLA association in CBZ-induced ADR among races is unclear. Similar diversity in HLA associated with rheumatoid arthritis (RA) has been observed. Since Isomäki *et al.*[33] first reported an association between RA and HL-A27 by mixed lymphocyte culture in 1974, associations between RA and HLA-DR4 have been reported in various races[34,35] but not in Spanish, Abrahamidae and Indian, in which an association with HLA-DR1 and DR10 was shown.[36,37] Compared with the amino acid sequence of HLA-DRB1 in HLA-DR4, -DR1 and -DR10, common amino acid sequences were found in positions 67–74 in the HLA-DRB1 molecule.[38] The common amino acid sequence is situated in the third super-variable area of the DRβ chain and constitutes a part of the α-helix,[38] which plays an integral role in antigen presentation. Thus, this common structure has been considered to be involved in the development of RA. Accordingly, we compared the amino acid sequences of HLA-A*3101, HLA-B*1502, and HLA-A*240201 which is one of the major Japanese HLA alleles. Table 7 shows the amino acid sequences of positions 61–80 in the α1-helix structure of HLA-B*1502, HLA-A*3101 and HLA-A*240201, whose areas have a huge variety of amino acid sequences, although other areas have relatively conserved amino acid sequences. Although six amino acid compositions are common between HLA-B*1502 and HLA-A*3101

Table 4. Statistical analysis in HLA typing of CBZ-induced ADR patients and CBZ-tolerant patients

HLA low-resolution	CBZ-induced ADR patients (n = 15)	CBZ-tolerant patients (n = 33)	OR	95% CI	P-value	HLA gene frequencies in Japanese (n = 371)
A1	0	2	0.103	0.004–2.418	1.000*	0.009
A2	3	17	0.235	0.056–0.991	0.040	0.222
A11	5	3	5.000	1.009–24.773	0.088*	0.083
A24	7	18	0.729	0.214–2.480	0.613	0.38
A26	3	8	0.781	0.175–3.483	1.000*	0.13
A30	0	1	0.699	0.027–18.157	1.000*	0.001
A31	10	5	11.200	2.668–47.105	0.001*	0.071
A33	1	4	0.518	0.053–5.074	1.000*	0.097
B7	1	4	0.518	0.053–5.074	1.000*	0.065
B13	1	2	1.107	0.093–13.248	1.000*	0.018
B27	0	1	0.699	0.027–18.157	1.000*	0.004
B35	0	7	0.114	0.006–2.136	0.082*	0.076
B37	0	1	0.699	0.027–18.157	1.000*	0.013
B39	0	1	0.699	0.027–18.157	1.000*	0.05
B44	2	1	4.923	0.410–59.112	0.227*	0.075
B46	1	3	0.714	0.068–7.493	1.000*	0.039
B48	0	2	0.103	0.004–2.418	1.000*	0.037
B51	7	5	4.900	1.129–19.689	0.031*	0.101
B52	3	8	0.781	0.175–3.483	1.000*	0.028
B54	1	5	0.400	0.043–3.760	0.650*	0.036
B55	2	2	2.385	0.303–18.788	0.579*	0.022
B56	1	1	2.286	0.133–39.203	0.532*	0.006
B58	0	2	0.103	0.004–2.418	1.000*	0.004
B59	0	1	0.699	0.027–18.157	1.000*	0.018
B60	2	4	1.115	0.181–6.878	1.000*	ND
B61	3	6	1.636	0.385–6.951	0.703*	ND
B62	2	4	1.115	0.181–6.878	1.000*	ND
B67	1	2	1.107	0.093–13.248	1.000*	0.003
B71	0	2	0.103	0.004–2.418	1.000*	ND
B75	1	1	2.286	0.056–0.991	0.532*	ND
DR1	0	5	0.168	1.009–24.773	0.167*	0.065
DR4	3	14	0.339	0.214–2.480	0.132	0.225
DR7	0	1	0.699	0.175–3.483	1.000*	0.003
DR8	4	6	1.636	0.027–18.157	0.703*	0.121
DR9	2	11	0.308	2.668–47.105	0.182*	0.012
DR11	1	2	1.107	0.107–15.598	1.000*	0.034
DR12	3	3	2.500	0.520–17.316	0.360*	0.051
DR13	2	4	1.115	0.211–8.249	1.000*	0.084
DR14	6	6	3.000	0.676–11.695	0.152*	0.09
DR15	5	12	0.875	0.291–4.109	0.839	0.185
DR16	0	2	0.103	0.021–10.386	1.000*	0.009

*Fisher's exact test, no mark; Pearson's χ^2-test. ADR, adverse drug reactions; CBZ, carbamazepine; CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio (determined using Haldone's modification, which adds 0.5 to all cells to accommodate possible zero counts); ND, no data.

(nos. 61, 64, 68, 72, 75 and 78), each amino acid sequence of HLA-A*240201 is also the same. These same amino acid compositions are commonly preserved in other HLA types and would not affect structural difference among the types. In addition, we found no common amino acid compositions of amino acids with polar characters (nos. 71, 80) or non-polar characters (nos. 62, 63, 65, 66, 67, 69, 70, 73, 74, 76, 77 and 79), which can affect 3-D conformation, between HLA-B*1502 and HLA-A*3101. Furthermore, no single amino acid was commonly present in alleles of both HLA-A*3101 and HLA-B*1502, except the amino acids present at the 61, 64, 68, 72, 75 and 78 positions. Therefore, HLA-B*1502 and HLA-A*3101 have no structural commonality for the common antigen presentation. We found no 3-D commonality between the two HLA from amino acid sequence, and significant difference in DLST values between subjects with and without the HLA-A31 allele in CBZ-induced ADR patients and CBZ-tolerant patients, which can mean severe ADR are independent of HLA structure and antigen presentation.

Another possibility for an association between the two HLA types and ADR is a linkage disequilibrium phenomenon in the HLA locus.

Table 5. Statistical analysis of HLA-B DNA typing of CBZ-induced ADR patients and CBZ-tolerant patients

HLA-B high-resolution	CBZ-induced ADR patients (n = 15)	CBZ-tolerant patients (n = 33)	OR	95% CI	P-value*	HLA gene frequencies in Japanese (n = 371)
07020	1	4	0.518	0.053–5.074	1.000	0.011
1301	0	1	0.699	0.027–18.157	1.000	0.065
1302	0	1	0.699	0.027–18.157	1.000	0.015
1310	1	0	6.931	0.266–180.436	0.313	0.003
1501	0	4	0.518	0.053–5.074	1.000	ND
1507	1	0	6.931	0.266–180.436	0.313	0.087
1511	1	1	2.286	0.133–39.203	0.532	0.007
1518	0	2	0.467	0.021–10.386	1.000	0.004
2704	0	1	0.699	0.027–18.157	1.000	0.015
3501	0	7	0.114	0.006–2.136	0.082	0.003
3701	0	1	0.699	0.027–18.157	1.000	0.076
3904	0	1	0.699	0.027–18.157	1.000	0.013
4001	2	4	1.115	0.181–6.878	1.000	0.001
4002	3	4	2.636	0.560–12.421	0.236	0.042
4006	0	2	0.103	0.004–2.418	1.000	0.086
4403	2	1	4.923	0.410–59.112	0.227	0.039
4601	1	3	0.714	0.068–7.493	1.000	0.087
4801	0	2	0.103	0.004–2.418	1.000	0.036
5101	6	5	4.900	1.219–19.689	0.031	0.030
5201	3	8	0.781	0.175–3.483	1.000	0.077
5401	0	5	0.400	0.043–3.760	0.650	0.107
5502	2	2	2.385	0.303–18.788	0.579	0.077
5601	1	1	2.286	0.133–39.203	0.532	0.019
5801	0	2	0.103	0.004–2.418	1.000	0.005
5901	0	1	0.699	0.027–18.157	1.000	0.004
6701	1	2	1.107	0.093–13.248	1.000	0.018

*Fisher's exact test. ADR, adverse drug reactions; CBZ, carbamazepine; CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio (determined using Haldone's modification, which adds 0.5 to all cells to accommodate possible zero counts); ND, no data.

Table 6. Comparison of DLST between subjects with or without HLA-A31 allele in CBZ-induced ADR patients and CBZ-tolerant patients

Subjects	HLA-A31	n	DLST (c.p.m.)	P-value	Measurement (day)*	P-value	Total systemic steroid (mg)†	P-value
CBZ-induced ADR patients	(+)	10	302.8 ± 140.5	0.147	72 ± 70	0.111	3128 ± 2440	0.212
	(−)	5	540.6 ± 461.7		25 ± 16		1827 ± 3577	
CBZ-tolerant patients	(+)	4	103.3 ± 27.8	0.167				
	(−)	28	128.5 ± 28.8					

*Measurement day from onset. †Hydrocortisone titer conversion administrated until DLST measurement day. ADR, adverse drug reactions; CBZ, carbamazepine; c.p.m., counts per minute; DLST, drug-induced lymphocyte stimulation test; HLA, human leukocyte antigen.

Table 7. Amino acid sequence of α1-helix structure in human leukocyte antigen

Allele	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
B*1502	D	R	N	T	Q	I	S	K	T	N	T	Q	T	Y	R	E	S	L	R	N
A*3101	D	Q	E	T	R	N	V	K	A	H	S	Q	I	D	R	V	D	L	G	T
A*240201	D	E	E	T	G	K	V	K	A	H	S	Q	T	D	R	E	N	L	R	I

Near the HLA gene, several inflammatory cytokine genes are mapped, such as g-interferon and tumor necrosis factor-β.[39] The genes located in these areas are highly polymorphic, some involving single-nucleotide polymorphism. If a disease-sensitivity gene exists in close association with a HLA gene, the disease seems to be caused by the HLA type. An association between HLA-B51 and Behcet's disease is such an example.[40] Thus, a pathogenic gene for CBZ-induced ADR might be strongly connected to HLA-B*1502 in Han Chinese and Asians, but the gene might be connected to HLA-A*3101 in Europeans and Japanese. However, a recent

detailed genome-wide association study concerning CBZ-induced ADR indicated that the CBZ-induced ADR gene is located at the HLA locus area; thus, it is not likely that another gene with polymorphisms causes CBZ-induced ADR.

A second possible reason is that HLA-B*1502 is associated with SJS/TEN, but not with HSS/DIHS or MPS, whereas HLA-A*3101 is associated with HSS/DIHS, but not with SJS/TEN. HLA-B*1502 was found to be specific to CBZ-induced SJS/TEN, and no association was seen in patients with CBZ-induced HSS or MPE in Han Chinese residing in Taiwan.[2] In addition, no association with HLA-B*1502 was confirmed in Caucasian patients with HSS.[16] Recently, an association between HLA-A*3101 and CBZ-induced ADR was reported in both the Japanese and Europeans by genome-wide approaches.[15,16] In the present study, we found an association between HLA-A31 and DIHS, but only one of three patients with SJS/TEN had HLA-A31, supporting this hypothesis.

Human leukocyte antigen is well documented to be associated with some chronic inflammatory diseases and autoimmune diseases; for instance, HLA-B27 is strongly associated with Reiter syndrome and ankylosing spondylitis. Thereby, the HLA molecule plays some role in the pathogenesis by modulating the immune system. In the present study, we also tested the association between HLA-A31 and SI of DLST. We failed to demonstrate the HLA-A31-associated enhancement of lymphocyte proliferation (Table 6), although we were able to confirm strong lymphocyte activation with CBZ in the patient group.

We found that HLA-A11 and HLA-A51 are weakly associated with CBZ-induced ADR patients. Because the number of cases was small in the present study, we cannot confirm the presence of an association. We need to evaluate more cases to ascertain an association between other HLA alleles and CBZ-induced ADR patients.

In the present study, we confirmed a strong association between HLA-A31 and CBZ-induced ADR in a Japanese population. However, HLA-A31 does not determine CBZ-induced lymphocyte proliferation.

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Policies promoting use of fewer variations of precautionary statements and use only when the risk of contamination is unavoidable should be promoted. Furthermore, all subjects with food allergies, particularly those who are not members of allergy advocacy groups, must be made aware of the importance of meticulous avoidance of the offending allergen.

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CD203c expression–based basophil activation test for diagnosis of wheat-dependent exercise-induced anaphylaxis

To the Editor:

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a special form of wheat allergy induced by the combination of wheat ingestion and physical exercise. We previously identified wheat ω-5 gliadin, a component of the water/salt-insoluble protein (gluten), as a major allergen in patients with WDEIA.[1] Recently, increased incidence of a new WDEIA subtype caused by hydrolyzed wheat protein (HWP) has been observed.[2,3] We have encountered several patients with WDEIA who were sensitized to HWP primarily through percutaneous routes, rhinoconjunctival routes, or both by using HWP-containing facial soaps.[3] Patients with this type of WDEIA showed HWP-positive results in a skin prick test (SPT) and had serum HWP-specific IgE. In addition, these patients had characteristic features of facial angioedema distinct from those seen in patients with conventional WDEIA (CO-WDEIA). Thus they were designated as having WDEIA sensitized by HWP (HWP-WDEIA). We examined the sera of several patients with HWP-WDEIA using the CAP-FEIA (Phadia, Uppsala, Sweden; detection range, 0.35-100 kU_A/L) and found that these patients have no or only low levels of ω-5 gliadin–specific IgE.

To establish a predictive *in vitro* test for differentiating these 2 subtypes of WDEIA (HWP-WDEIA and CO-WDEIA), we measured basophil CD203c expression induced by different types of wheat proteins and evaluated the diagnostic efficiency of the reactions in the patients. CD203c is an ectoenzyme belonging to a family of ectonucleotide pyrophosphatases and phosphodiesterases. It is expressed on the cell membrane of human peripheral basophils and mast cells, and cross-linking of the high-affinity IgE receptor upregulates CD203c expression on the cell membrane.

Ten patients with WDEIA were enrolled in this study: 5 with HWP-WDEIA and 5 with CO-WDEIA. The clinical features and the results of immunologic studies of these patients are summarized in Table I. All of the patients with HWP-WDEIA had been using the same brand of soap, which included HWP-A (Katayama Chemical, Osaka, Japan). None of the patients with CO-WDEIA had a prior history of using soap or other cosmetic products supplemented with HWP. Sensitization to wheat proteins was determined by means of SPT responses, specific IgE levels (CAP-FEIA), and challenge test results with wheat combined with exercise/aspirin.[4] None of the patients had atopic diathesis.

Wheat fractionation and purification of ω-5 gliadin were performed according to a previously described method.[5] SDS-PAGE and IgE immunoblotting were also performed as described previously.[5] All IgE from patients with HWP-WDEIA reacted with HWP-A, and the molecular size ranged from 15 to 250 kDa. The IgE of all patients with HWP-WDEIA also reacted to both water-soluble and water-insoluble wheat proteins but not to ω-5 gliadin. The IgE of the patients with CO-WDEIA did not react to HWP-A but reacted to

TABLE I. Clinical features and results of immunologic studies

Patient no.	Age (y/sex)	Food	Triggers	Symptoms	SPT				Specific IgE (kU_A/L)			
					Wheat	Bread	Soap 0.1%	HWP-A 0.1%	Wheat	Gluten	-5 Gliadin	CT
HWP-WDEIA												
1	51/F	Spaghetti	Walking	A, U, S, NS, ND, P	3+	3+	3+	3+	4.70	4.76	<0.34	NT
2	49/F	Gyoza dumplings	Table tennis	A, U, S, NS, C, D, AP, V	1+	1+	1+	2+	2.27	4.56	<0.34	+
3	52/F	Steamed bread	Jogging	A, U	−	−	3+	2+	0.46	0.65	<0.34	NT
4	44/F	Bread, Udon noodles	Tennis	A, U, AP, anaphylaxis	2+	2+	2+	2+	9.26	15.8	1.16	+
5	54/F	Udon noodles, Gyoza dumplings	Walking	A, U, S, ND, AP, L	2+	3+	3+	3+	5.17	7.37	<0.34	+
CO-WDEIA												
6	55/F	Pan-fried noodles, Chinese noodles, bread, spaghetti	Working	U, anaphylaxis	2+	2+	−	1+	<0.34	<0.34	2.14	+
7	41/F	Bread, cake, pudding	Working	U, D, anaphylaxis	3+	2+	−	−	0.76	0.90	14.1	+
8	73/M	Bread, Chinese noodles	Driving, internal use of analgesics	U, anaphylaxis	2+	2+	−	1+	<0.34	<0.34	2.36	+
9	39/F	Bread, cookie	Tennis	U, P	3+	3+	−	−	<0.34	1.90	3.82	+
10	60/M	Fried chicken, Chinese noodles, curry and rice	Football, jogging, internal use of analgesics	U, C, anaphylaxis	2+	2+	−	1+	1.82	5.39	11.0	+

Wheat was a commercial wheat flour extract (1:20 wt/vol; Torii Pharmaceutical Co, Tokyo, Japan). Bread was commercial bread (1:20 wt/vol, Torii Pharmaceutical Co). Soap 0.1% was 0.1% diluted solution of soap supplemented with HWP-A in saline. SPT responses were read at 15 minutes, and responses were compared with those after positive histamine controls (10 mg/mL): *1+*, 25%; *2+*, 50%; *3+*, 100%; and *4+*, 200% of the area of the wheal induced by the positive histamine control. The SPT responses were negative for all 5 nonallergic control subjects.

A, Angioedema; *AP*, abdominal pain; *C*, conjunctivitis; *CT*, challenge test; *D*, dyspnea; *L*, lacrimation; *ND*, nasal discharge; *NS*, nasal stuffiness; *NT*, not tested; *P*, pharyngalgia; *S*, sneeze; *U*, urticaria; *V*, vomiting.

water-insoluble wheat proteins and ω-5 gliadin. One hundred microliters of serum was incubated with HWP-A and serially diluted from 100 to 5 μg/mL at 37°C for 2 hours during constant stirring to perform immunoblotting inhibition assays for determining specific binding of IgEs to HWP-A on a polyvinylidene difluoride membrane. The serum was then diluted 1:10 in 5% skim milk/Tris-buffered saline with Tween 20 for immunodetection of IgEs by means of Western blotting. The reaction of the IgE with water-soluble and water-insoluble wheat proteins was also inhibited by HWP-A in a dose-dependent manner (data not shown).

A commercial kit (Allergenicity Kit; Beckman Coulter, Fullerton, Calif) was used for quantifying basophil CD203c expression, as described previously.[6] HWP-A was found to enhance CD203c expression in a concentration-dependent manner in the patients with HWP-WDEIA (Fig 1). No significant enhancement of CD203c was observed with purified ω-5 gliadin. Interestingly, native ω-5 gliadin did not enhance CD203c expression in patients with HWP-WDEIA, including patient 4, who had serum ω-5 gliadin–specific IgE, as determined by using the CAP-FEIA. This indicates that IgE produced against HWP in patients with HWP-WDEIA does not react to ω-5 gliadin but to other undetermined protein components with specific epitopes. Instead, purified ω-5 gliadin enhanced CD203c expression in a concentration-dependent manner in the patients with CO-WDEIA (Fig 1). No significant enhancement of CD203c was observed in the presence of HWP. To study whether the basophil activation was mediated by IgE, we removed the cell-surface IgE in the leukocyte mixture with lactic acid treatment, as described previously.[7] We found that basophil activation was abolished by this removal of cell-surface IgE (data not shown).

In the present study we showed that the *in vitro* wheat protein–induced basophil activation test for quantifying CD203c expression is highly useful for diagnosing the subtypes of WDEIA: HWP-WDEIA and CO-WDEIA. Specifically, the determination of CD203c expression clearly differentiated the sensitization conditions of both types of WDEIA, which is consistent with SPT responses, determination of serum allergen-specific IgE levels, and results of immunoblotting. CD203c was previously proposed to be a useful marker in the diagnosis of wheat-induced pediatric allergies.[8] The present study extends the use of the CD203c test to the determination of major allergens in adult patients with wheat allergies. The fact that the basophil activation test requires only small amounts of blood and allergen is an additional advantage of the test because it can be used to simultaneously identify a series of allergens.

The mechanism of IgE cross-reactivity to natural wheat in patients with HWP-WDEIA remains unclear. Leduc et al[9] have suggested that acidic hydrolysis induces a conformational change in HWP and converts a glutamine residue to glutamic acid and an asparagine residue to aspartic acid. Thus new epitopes that differ from the epitopes of natural wheat proteins might be produced. A tolerance to wheat proteins can develop essentially during the infantile stage through recognition of wheat allergen epitopes. It is conceivable that human subjects do not have sufficient tolerance to HWPs that are not natural proteins. Thus human subjects appear to be sensitized easily to HWPs. Because wheat proteins contain repetitive amino acid structures highly rich in glutamine

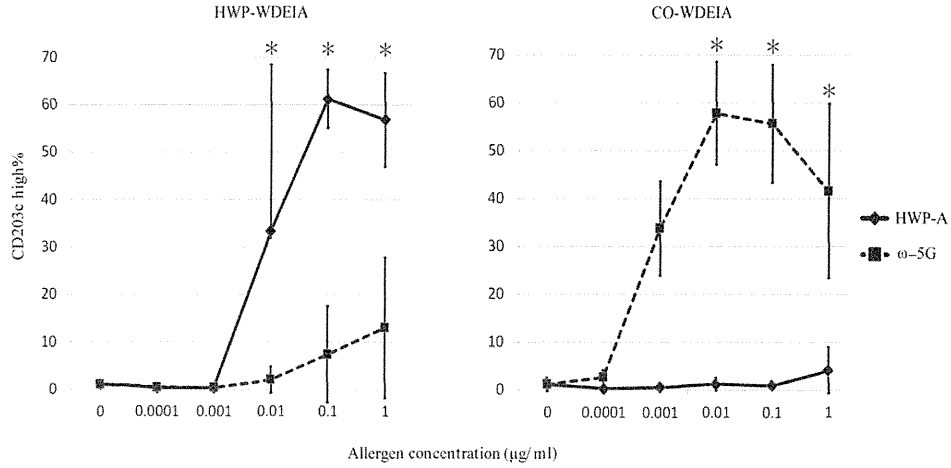

FIG 1. Expression of CD203c on basophils induced by HWP-A and ω-5 gliadin *(ω-5G).* Mean levels of CD203c expression in 5 patients with HWP-WDEIA and 5 patients with CO-WDEIA are presented. Data are expressed as means ± SEMs. $*P < .01$ when comparing HMP-A and ω-5 gliadin, as determined by using the Student *t* test.

and proline, IgE produced against HWPs probably cross-reacts with natural wheat proteins. In fact, preincubation of sera with HWP-A clearly revealed a decreased binding of IgE to natural wheat proteins.

In conclusion, measurement of basophil CD203c expression induced by various preparations of wheat proteins is highly useful in predicting causative allergens in patients with WDEIA. Furthermore, the basophil activation test based on the expression of CD203c might help determine causative allergens for a wide variety of food allergies.

This study was approved by the Ethics Committee of the Shimane University Faculty of Medicine (approval nos. 469 and 703). All participants provided written informed consent.

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High sensitivity of CAP-FEIA rVes v 5 and rVes v 1 for diagnosis of *Vespula* venom allergy

To the Editor:

Ves v 5 (antigen 5) is a 23-kDa protein from *Vespula* venom, and it is recognized as the most potent allergen in venoms of the *Vespidae* family.[1] There is a high sequence similarity of Ves v 5 within species of the same genus, such as yellow jacket, that is, *Vespula* (>95%); however, when it is compared with other genera such as *Dolichovespula* or *Polistes*, the sequence identity is much lower (about 60%).[2] Another potential *Vespula* allergen is a 37-kDa phospholipase A1, known as Ves v 1.[1] Neither Ves v 5 nor Ves v 1 is found in honeybee venom. Ves v 5 and Ves v 1 recombinant allergen components, both expressed in insect cells, became available in 2010 and 2011, respectively, for analyses on the ImmunoCAP solid-phase IgE assay (CAP-FEIA; Phadia, Uppsala, Sweden).

Very recently we demonstrated that the current CAP-FEIA recombinant major honeybee venom allergen rApi m 1 (i208) has a limited clinical usefulness for the detection of honeybee venom allergy because of its low diagnostic sensitivity, which