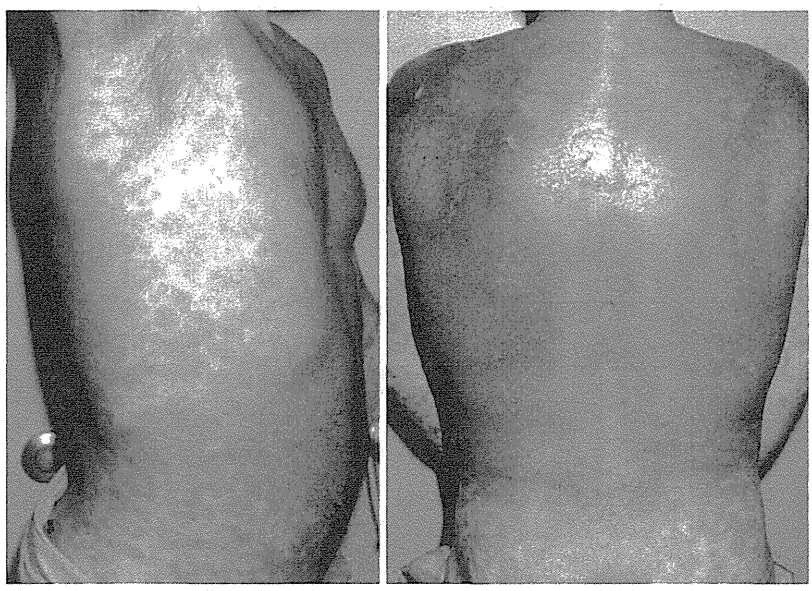
図2 ST合剤によるTEN型薬疹.
HIV感染症では，ST合剤に対して約50%の患者が薬疹を生じ，健常者と比べTEN型の頻度も高い．(Andrew Blauvelt博士提供)



ためと考えられている．また，HIV感染患者では，薬物代謝に関わるグルタチオンの欠乏や薬物のアセチル化の遅延[13]が高頻度にみられるため，薬物の中間代謝産物が毒性を発揮したり，アレルギー反応を引き起こすこともHIV感染患者が薬疹を生じやすい一因ではないかと考えられている．

2. 薬剤によりウイルスの再活性化が引き起こされるタイプ

●DIHSとHHV-6再活性化

DIHSとは，薬剤投与開始から3週間以上たって遅発性に発症し，多臓器障害を伴う重症型薬疹のひとつである．皮疹は，紅斑丘疹型（時に多形紅斑型）に始まって紅皮症となることが多い．皮疹だけでなく，リンパ節腫脹，発熱，異型リンパ球の出現や好酸球増多，肝障害などの症状を認め，しばしば原因薬剤の中止後も，皮疹や臓器障害が遷延する．近年，発症後2〜4週後にHHV-6の再活性化を伴うことが判明し，薬剤アレルギーとウイルス感染症の複合した新たな病態として認識されるようになった[1,2]．

近年，DIHS急性期に血清TARC（thymus and activation-regulated chemokine）値が著しく高値（平均20,000 pg/ml以上）を示すことが明らかとなった（図3a）[14,15]．TARCはTh2細胞を誘導するケモカインの一つで，現在，アトピー性皮膚炎の重症度マーカーとして広く使用されている．TARCの著明な上昇はDIHSに特異的で，一般の紅斑丘疹型薬疹（MPE）やStevens-Johnson症候群（SJS）/TENでは中等度の上昇を示すのみであった（平均約2,000 pg/ml）．さらに，臨床的にDIHSが疑われた薬疹41症例について，HHV-6再活性化を伴った群とHHV-6再活性化を伴わなかった群に分けて，急性期のTARC値を比べたところ，HHV-6再活性化群においてTARCが有意に高いことが判明した（図3b）[15]．このことからTARCの著しい上昇がHHV-6の再活性化に何らかの役割を果たしているのかもしれない．

●DIHSの病態モデルとしてのGVHD

造血幹細胞移植後には，ドナーT細胞がレシピエント細胞上の組織適合抗原を認識し，レシピエントの組織を「非自己」と見なして攻撃するGVHDがしばしばみられる．急性GVHDでは発熱や発疹がみられ，重症型では中毒性表皮壊死症と同様の臨床像を呈する．一方，移植後の免疫抑制状態では，潜伏していたヒトヘルペスウイルスが再活性化しやすい状態になっている．以前から造血幹細胞移植後のGVHDとヒトヘルペスウイルス再活性化との関わりについては様々な議論が行われてきており，特にHHV-6[16〜21]やCMV[22,23]の関与が報告されている．しかし，これらの報告を否定

図3　DIHS急性期における血清TARCの上昇.

(a) DIHS，SJS/TEN，紅斑丘疹型薬疹（MPE）の急性期における血清TARC値の比較．(b) HHV-6の再活性化を伴った典型DIHSと再活性化を伴わなかったDIHS類似薬疹の急性期におけるTARCの比較．（文献15より引用改変）

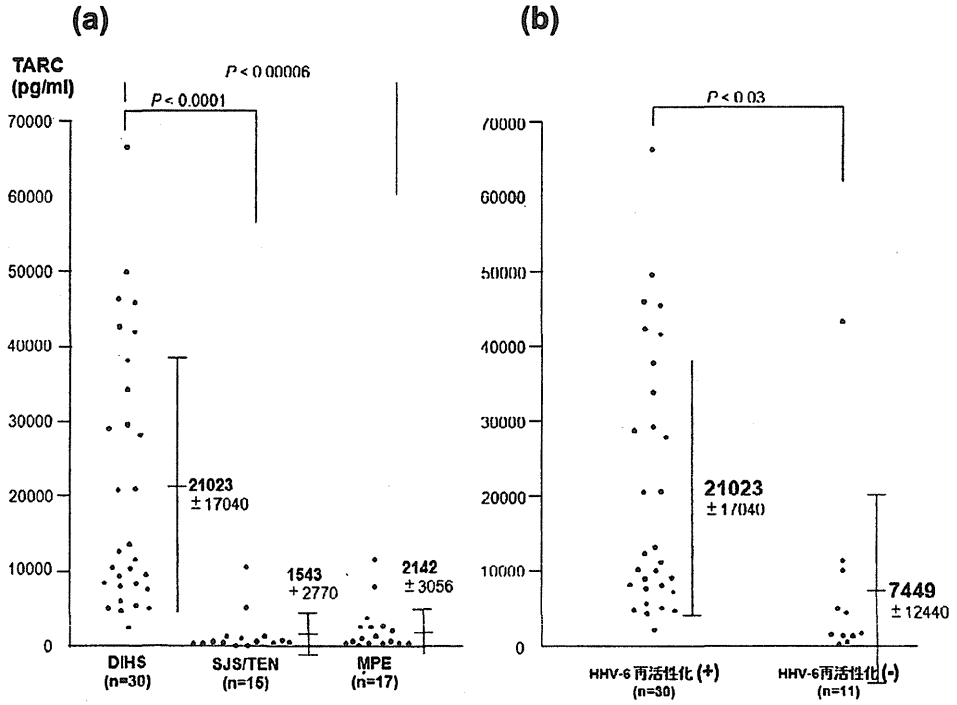
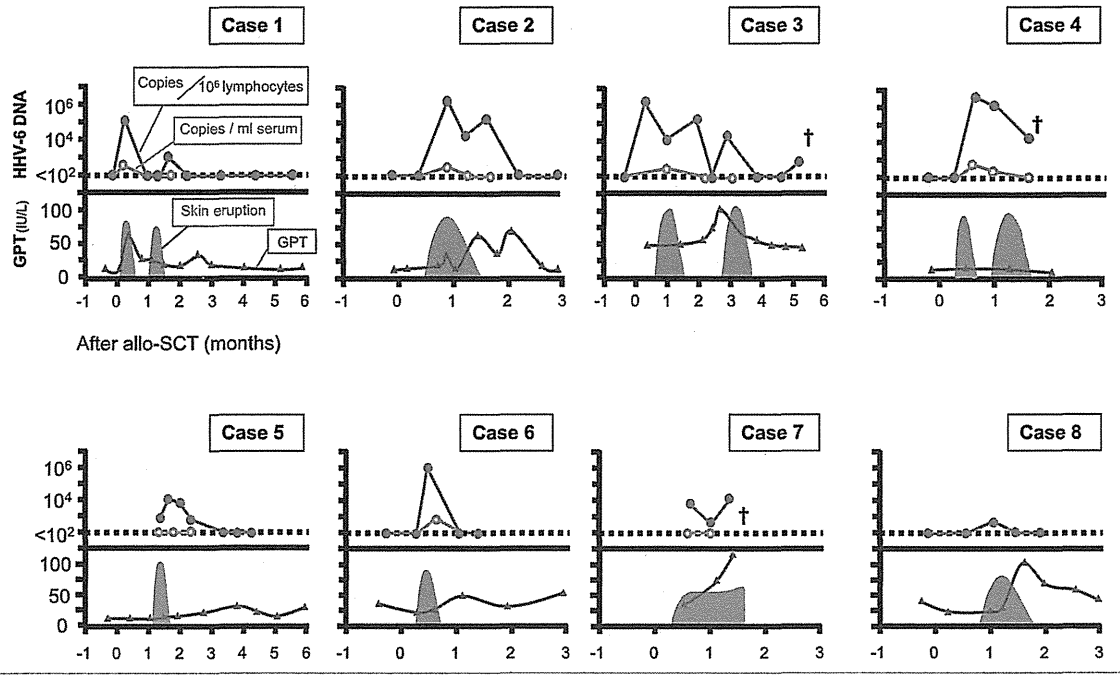


図4　造血幹細胞移植後の発疹と血中HHV-6 DNAレベルの関係.

青線：全血中HHV-6 DNAコピー数をリンパ球10^6個あたりに換算．赤線：血清中HHV-6 DNA濃度．＋：死亡．発疹の出現・消退と血中HHV-6 DNAレベルとの間に相関がみられる．（文献26より引用改変）



する論文もみられ[24][25]，両者の関係については不明な点が多かった．そこで，GVHDとヒトヘルペスウイルス再活性化との関連性を明らかにする目的で，移植前および移植後経時的に，末梢血中のヒトヘルペスウイルス（HHV-6，HHV-7，EBV，CMV）DNAの定量が行われた[26]．その結果，移植患者15例中，GVHDを発症した10例全例にHHV-6の再活性化がみられ，さらに発疹の出現・消退と血中HHV-6 DNAレベルとの間に相関がみられた（図4）．一方，GVHDを発症しなかった5例の内HHV-6 DNAが検出されたのは1例のみで，また，HHV-6以外のヒトヘルペスウイルスとGVHDとの関連はみられなかった．さらに，発疹の出現に一致して，血清中IL-10と可溶性IL-2受容体の上昇も認められた．以上のことから，造血幹細胞移植後のGVHDの発症にはHHV-6の再活性化とIL-10産生T細胞の活性化が密接に関わっているものと考えられた．

以上の様に，GVHDとDIHSとの間には，皮膚症状，発熱，臓器障害，HHV-6再活性化，IL-10産生T細胞の活性化など，多くの類似点があり，GVHDがDIHSの病態モデルになり得るものと考えられる．

おわりに

1980年代後半から1990年代前半にかけて，エイズの出現をきっかけに，免疫不全患者から新たなヒトヘルペスウイルス（HHV-6, -7, -8）が相次いで発見された．この新しく見つかったHHV-6やHHV-7が，DIHSという重症薬疹の病態に関わっていることが，わが国の橋本，塩原らにより見いだされたことは記憶に新しいが，この発見がブレイクスルーとなり，古くから推測されていた「薬疹とウイルスとの関わり」が改めてクローズアップされるようになった．しかし，ウイルスが薬疹の病態形成にいかなる役割を果たしているのかについては，現在のところ，ほとんど分かっておらず，今後解明すべき課題は多い．

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Acta Derm Venereol 2016; 96: 114–115

SHORT COMMUNICATION

Involvement of Human Herpesvirus 6 Infection in Renal Dysfunction Associated with DIHS/DRESS

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Accepted May 27, 2015; Epub ahead of print Jun 3, 2015

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a life-threatening multi-organ hypersensitivity reaction that appears after prolonged exposure to a limited number of drugs. DIHS/DRESS is often related to reactivation of human herpesvirus 6 (HHV-6) (1–3). Renal dysfunction sometimes affects the prognosis (4). However, the pathomechanism of DIHS/DRESS-related renal dysfunction remains unknown. We report here a patient with DIHS/DRESS in whom HHV-6 infection may have been involved in the pathogenesis of renal failure.

CASE REPORT

A 62-year-old Japanese man developed a high fever, oedematous erythema with scale on the face and pallor around the eyes (Fig. 1a), diffuse pruritic maculopapular erythema on the trunk (Fig. 1b), and bilateral cervical lymphadenopathy on day 28 of continuous administration of trimethoprim/sulphamethoxazole (TMP/SMX) 320 mg/day and 1,600 mg/day, respectively, given to treat refractory iliopsoas abscess caused by *methicillin-resistant Staphylococcus aureus* (MRSA), which was resistant to many antibiotics except for TMP/SMX. Laboratory tests on day 9 after onset revealed a white blood cell count of 17.9×10^3/μl with 29% atypical lymphocytes and 1% eosinophils, high concentrations of thymus and activation-regulated chemokine (105,300 pg/ml) (5) and slightly high concentrations of creatinine (1.37 mg/dl). Liver function tests were normal at the initial visit to the dermatologist on day 8 after onset (AST 21 U/l, ALT 18 U/l), but AST and ALT levels were elevated on day 17 after onset (AST 106 U/l, ALT 102 U/l). No HHV-6 DNA was detected by quantitative PCR in the peripheral blood mononuclear cells (PBMC) on day 9 after onset. HHV-6 DNA became positive on day 15 (2×10^3 copies/ml) and continued to be detected thereafter. The patient was diagnosed with DIHS/DRESS due to TMP/SMX. His symptoms disappeared on day 9 after initiation of 30 mg/day oral corticosteroid (prednisolone). However, he suddenly developed renal failure with a creatinine level of 9.13 mg/dl on day 79 after onset. Since renal function did not improve despite intensive therapy, haemodialysis therapy was initiated. The patient subsequently died from an opportunistic infection and multiple organ failure. An autopsy revealed interstitial nephritis with lymphocytic infiltration and necrotized tubular epithelial cells (Fig. 2a). HHV-6 DNA (8×10^2 copies/mg) was detected in an autopsy specimen of the kidney, while HHV-6 DNA was almost undetectable in other organs (8 copies/mg). Immunofluorescence and immunohistochemistry with an anti-HHV-6 monoclonal antibody (OHV-3) (6) revealed that tubular epithelial cells were positive for the HHV-6 antigen (Fig. 2b, 2c). Staining for HHV-6 in the patient's skin did not reveal any viral antigen.

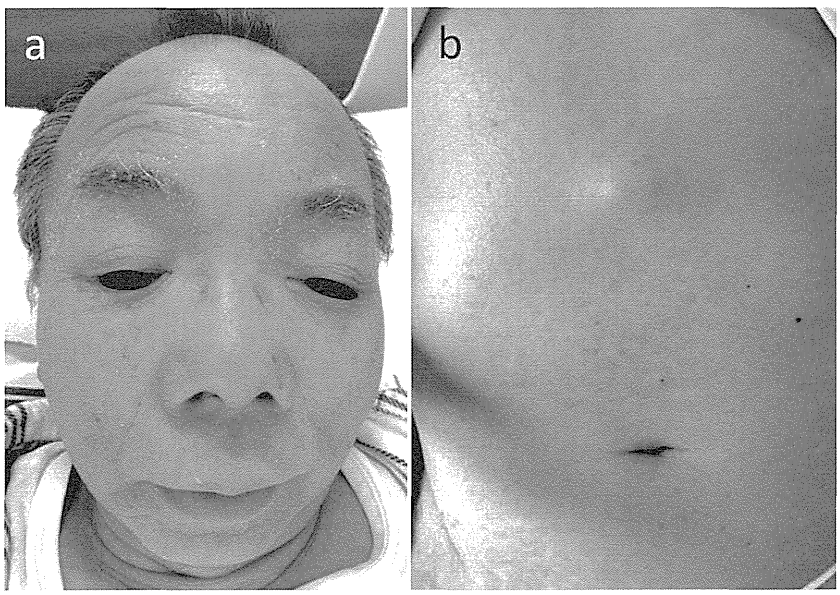

Fig. 1. Clinical appearance at onset. (a) Oedematous erythema with scale on the face and pallor around the eyes. (b) Diffuse pruritic maculopapular erythema on the trunk. The patient provided written permission to publish this photograph.

DISCUSSION

Renal dysfunction is occasionally observed in patients with DIHS/DRESS (10%) and sometimes affects the prognosis of these patients (4). In the case

 doi: 10.2340/00015555-2149
 ISSN 0001-5555

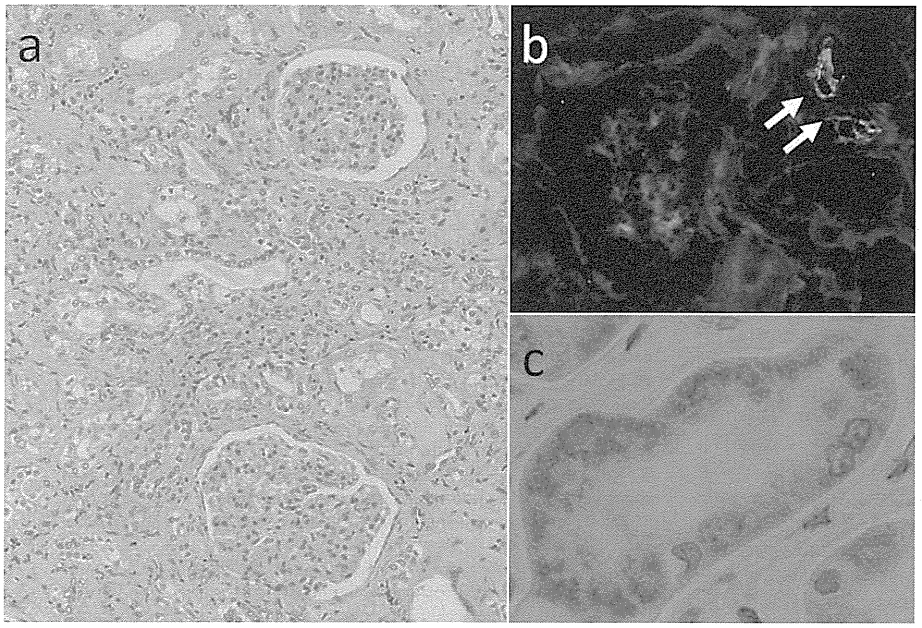

Fig. 2. Histopathological findings of the renal specimen. (a) Degenerated tubular epithelial cells, with lymphocytic infiltration in the renal interstitial region and minor abnormalities in the glomeruli. (H&E × 200) (b) Expression of human herpesvirus 6 (HHV-6) antigen in tubular epithelial cells by immunofluorescence with anti-HHV-6 monoclonal antibody (OHV-3, × 200) (*arrows*). (c) Expression of HHV-6 antigen in the cytoplasm of tubular epithelial cells by immunohistochemistry with OHV-3 (× 400).

described here, interstitial nephritis with lymphocytic infiltration was observed in a renal specimen from a patient with DIHS/DRESS, persistent HHV-6 infection was detected in the PBMC, significantly high HHV-6 viral load in renal specimens, and HHV-6 antigen expression in tubular epithelial cells. As no other cause of renal dysfunction was suspected in this case, these results suggest that HHV-6 may infect tubular epithelial cells and cause acute renal dysfunction in the course of DIHS/DRESS. The role of HHV-6 infection in allograft rejection has been examined in renal transplant recipients. Helanterä et al. (7) and Hoshino et al. (8) showed that HHV-6 antigen was expressed in tubular cells in renal transplant recipients, but they described no clear association of HHV-6 infection with renal dysfunction. On the other hand, some researchers have suggested that reactivation of HHV-6 may induce rejection of renal transplants (9). HHV-6 expression in affected organs of patients with DIHS/DRESS has not been reported previously. Although the involvement of HHV-6 infection in rejection of renal transplant is controversial, we conclude that HHV-6 may be associated with the pathogenesis of renal failure in this DIHS/DRESS case.

ACKNOWLEDGEMENTS

This study was supported in part by Health and Labor Sciences Research Grants (Research on Intractable Diseases) from the Ministry of Health, Labor, and Welfare of Japan and KAKENHI (to HA).

The authors declare no conflicts of interest.

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patterns, pustular development is rare. In our review of the literature we found only one reported case showing generalized pustular dermatosis with high-grade fever caused by isoniazid in a tubercular patient [2], which was introduced as acute generalized exanthematous pustulosis (AGEP) in a recent study [3].

AGEP is often induced by drugs and takes a self-limiting course [3]. Generalized pustular psoriasis (GPP) is characterized by an acute generalized pustular eruption lasting for weeks and becoming recurrent [4]. Several drugs, including antibiotics [4, 5] and terbinafine [6], have been reported to provoke GPP. These two pustular dermatoses share similar clinical features and this occasionally makes distinguishing between the two difficult, both clinically and histologically [3, 7]. Kardaun *et al.* [8] described histological features pointing at AGEP instead of GPP as including the presence of eosinophils in the pustules or dermis, necrotic keratinocytes, a mixed neutrophil-rich interstitial and mid-dermal infiltrate. In addition, erythrocyte extravasation occurred in 54% of AGEP cases [7]. Although the distribution of the pustular eruption in AGEP was mostly of a non-follicular pattern, follicular pustules were also seen in 23% of cases [3, 7]. In our case, however, both the presence of follicular pustules and the absence of keratinocyte necrosis, extravasated erythrocytes and eosinophil infiltration did not necessarily exclude the diagnosis of AGEP. The distribution of pustules and rapid improvement of clinical symptoms favored AGEP. Moreover, the AGEP scoring system [3] was applied to our case and the score sum of 8 points fulfilled the criteria for definite AGEP. Since AGEP can develop in patients with a history of psoriasis [7], the diagnosis should be made comprehensively based on the clinical course and histological findings.

Mutations of IL36RN, which encodes the anti-inflammatory IL36 receptor antagonist (IL36Ra), have recently been found in GPP, especially the majority of cases with GPP not accompanied by psoriais vulgaris [9]. Sugiura *et al.* [5] reported GPP in identical twins with compound heterozygous IL36RN mutations. They speculated that amoxicillin might have induced GPP although the initial symptoms of the patients suggested amoxicillin-induced AGEP. On the other hand, Navarini *et al.* [10] analyzed 96 cases with AGEP, detected IL36RN mutations in 4 patients and suggested that a small subset of AGEP might be related to IL36Ra dysfunction. To elucidate the relationship between AGEP and IL36RN mutation, further analysis and accumulation of cases are required.

In summary, isoniazid is a drug that can cause AGEP, which is difficult to differentiate from an initial episode of GPP. As preventive administration of isoniazid has increased with increased use of biologics for treating severe psoriasis, the onset of AGEP due to isoniazid may increase. ■

Disclosure. *Financial support: none. Conflict of interest: none.*

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doi:10.1684/ejd.2014.2464

Differential expression profile of Th1/Th2-associated chemokines characterizes Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) as distinct entities

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) and Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) are two of a triad of severe cutaneous adverse reactions (SCAR) to drugs [1, 2]. The rapid recognition of DIHS/DRESS and SJS/TEN is essential because they are potentially life-threatening syndromes. Thus, diagnostic markers or predictive factors need to be defined.

We previously reported that thymus and activation-regulated chemokine (TARC/CCL17) serum levels were markedly higher in the acute stage of DIHS/DRESS than in other forms of drug eruption [3, 4]. We also demonstrated that TARC levels in the acute stage of DIHS/DRESS correlated with disease activity [3]. In this study, we attempted to identify chemokine patterns that would allow us to

distinguish between the different forms of drug eruptions and gain insight into the pathomechanisms involved.

We first examined the expression of macrophage-derived chemokine (MDC/CCL22), a chemokine related to TARC, in patients with DIHS/DRESS, SJS/TEN and maculopapular exanthema (MPE). A previous study showed that the CC chemokines, TARC and MDC, were T helper (Th) 2-associated chemokines that bind to CC chemokine receptor 4 (CCR4) on Th2 cells [5]. We subsequently compared the expression of the Th1-associated chemokines, monokine induced by IFN-γ (MIG/CXCL9) and IFN-inducible protein 10 (IP-10/CXCL10). MIG and IP-10 are related chemokines of the CXC subfamily that are known to share the receptor, CXCR3, on Th1 cells [5]. Blood samples were obtained from 11 patients with DIHS/DRESS, 18 patients with SJS/TEN and 15 patients with MPE in the acute stage and after recovery; serum concentrations of TARC, MDC, IP-10, and MIG were measured by ELISA. Diagnosis of SJS/TEN and DIHS was made on the basis of the criteria proposed by Auquier-Dunant *et al* [6] and Shiohara *et al*.[7], respectively. The results obtained revealed marked differences between the different types of SCAR. In addition to an increase in TARC (*figure 1A*), which is consistent with the findings of our previous study [3, 4], the expression of MDC was markedly higher in DIHS/DRESS than in the other forms of drug eruption (*figure 1A*). In contrast to DIHS/DRESS, the expression of the Th1-associated chemokines (MIG and IP-10) was higher in SJS/TEN than in MPE (*figure 1A*). These results were consistent with the

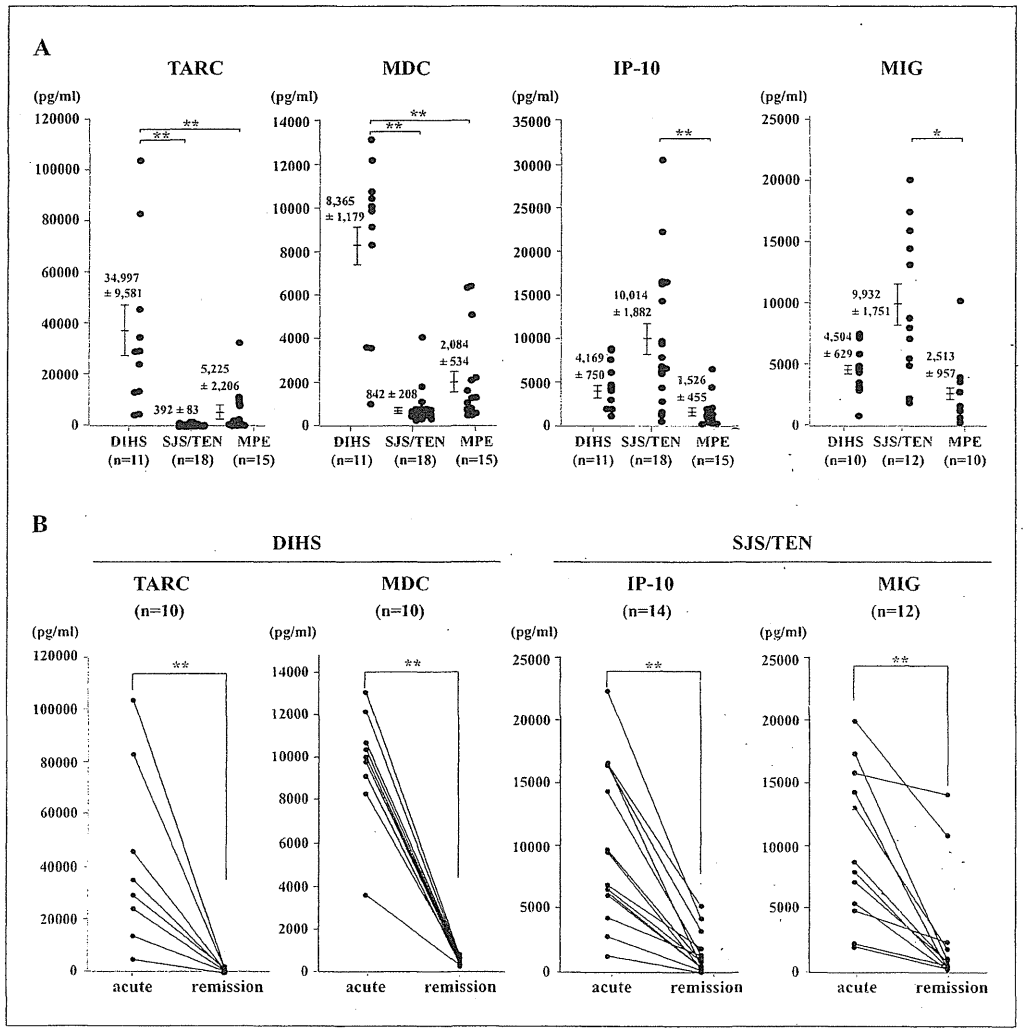

Figure 1. A) Distinct expression of chemokines in SCAR in the acute stage. Th2 chemokines (TARC and MDC) were highly elevated in DIHS/DRESS, while Th1 chemokines (IP-10 and MIG) predominated in SJS/TEN. The average TARC and MDC levels in patients with DIHS/DRESS were 34,997 ± 9,581 pg/mL (average ± SEM) and 8,365 ± 1,179 pg/mL, respectively. The average levels of IP-10 and MIG in patients with SJS/TEN were 10,014 ± 1,882 pg/mL and 9,932 ± 1,751 pg/mL, respectively. Blood samples were obtained on days 0-31 (average day 8.8) after the onset of DIHS/DRESS, days 0-25 (average day 5.6) for SJS/TEN, and days 1-24 (average day 6.5) for MPE. * $p<0.05$, ** $p<0.01$, Kruskal-Wallis test. **B)** Serum chemokine levels in the acute and remission stages in SCAR. The levels of chemokines that were upregulated during the acute stage (TARC and MDC in patients with DIHS, and IP-10 and MIG in patients with SJS/TEN) declined upon remission. ** $p<0.01$, Student's *t*-test.

findings of previous studies in which SJS/TEN was characterized by a predominantly Th1 pattern of activation [8, 9]. Our results clearly showed slightly higher MIG and IP-10 values in SJS/TEN than in DIHS/DRESS but statistical significance was not reached between these two groups, which indicated that DIHS/DRESS also exhibited a Th1 pattern of activation to some extent. The relatively small number of patients and the overlap in the range distribution in DIHS/DRESS *vs* SJS/TEN and DIHS/DRESS *vs* MPE imply that further extended studies are needed to reach a concrete conclusion.

We then investigated whether the levels of the upregulated chemokines described above declined upon remission. Serum levels of MDC in DIHS/DRESS and MIG and IP-10 in SJS/TEN decreased in the remission stage (*figure 1B*), which was also observed in TARC in DIHS/DRESS (*figure 1B*) [3].

A recent study reported that biopsy specimens from SJS/TEN cutaneous lesions exhibited a mixed Th1/Th2 pattern [10]. The same group subsequently demonstrated that TARC levels were significantly higher in the sera of SJS/TEN patients than in the sera of healthy donors (HD) [9]. The median TARC levels reported were 580 pg/mL in SJS/TEN and 205.2 pg/mL in HD [9]. Our previous studies also showed elevated levels of TARC in SJS/TEN patients; average TARC levels were 2,198 pg/mL in one study [3] and 1,543 pg/mL in the other [4] (normal value of TARC: <450 pg/mL). However, our present study did not confirm this finding. Although further evidence is needed for a clear conclusion, the markedly lower TARC values observed in SJS/TEN than in DIHS/DRESS suggest that a Th1 response may play a major role in SJS/TEN with the minimal coexistence of a Th2 response, if any.

Taken together, our results showed that Th2-associated chemokines were markedly upregulated in DIHS/DRESS, while Th1-associated chemokines predominated in SJS/TEN. This result indicates that the mechanisms underlying DIHS/DRESS and SJS/TEN are distinct. We further suggest that a prompt differentiation of SCAR may be achieved using TARC/MDC and IP-10/MIG chemokine sets. ■

Disclosure. *Financial support: This study was supported in part by Health and Labor Sciences Research Grants (Research on Intractable Diseases) from the Ministry of Health, Labor, and Welfare of Japan and KAKENHI (to H.A.). Conflict of interest: none.*

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doi:10.1684/ejd.2014.2477

Extramammary Paget's disease occurring in the context of Cowden syndrome: true association or mere coincidence?

Cowden syndrome (CS) is a rare inherited disorder characterized by multiple hamartomas and an increased risk of carcinomas, such as thyroid and breast cancers [1]. Characteristic mucocutaneous manifestations include multiple facial trichilemmomas, oral mucosal papillomatoses and acral keratosis. A mutation in the phosphatase and tensin homolog (*PTEN*) gene has been identified as the cause of CS [2]. The present report describes a case of extramammary Paget's disease (EMPD) which developed in a Japanese patient with CS.

A 64-year-old Japanese male initially presented with a three-month history of an erythematous lesion involving his scrotum. On presentation, an erythematous lesion without itching, measuring 3.0 cm × 4.0 cm in size, was observed on the scrotum and right inguinal area (*figure 1A*). In addition, there were many small keratotic papules on the patient's dorsal hands and feet (*figure 1B*). He had two daughters and they were under treatment for breast and thyroid cancers, respectively. These findings suggested that the

LETTERS TO THE EDITOR

Drug Eruption Following High-calorie Infusion: A Possible Systemic Type IV Allergic Reaction to Sulphites

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Accepted Mar 17, 2015; Epub ahead of print Mar 18, 2015

Sulphites, such as sodium bisulphite and sodium metabisulphite, are widely used as antioxidants or preservatives in a wide range of substances, such as food, cosmetics and medications (1). It has been reported that sulphite intake can cause systemic type I allergic reactions, such as urticaria (2) and anaphylaxis (3, 4). In addition, external application of sulphites can cause local type IV allergic reactions, such as contact dermatitis (1, 5). However, it is not known whether sulphite intake can cause systemic type IV allergic reactions. We describe here a case of drug eruption, thought to be a systemic type IV allergic reaction to sulphites, following a high-calorie infusion.

CASE REPORT

A 50-year-old woman was referred to our dermatology department because of a systemic skin eruption. She had been diagnosed with myasthenia gravis 10 years earlier. Because she had difficulty with food intake due to decreased bowel movement resulting from the myasthenia gravis, she had started a high-calorie infusion (Aminotripa 2® given 1,800 ml every 3 days [Otsuka Pharmaceutical Co. Ltd, Tokyo, Japan]) 10 days previously. Three days after the start of infusion, small red pruritic papules developed over most of the patient's body (Fig. 1a, b). It was suspected that this symptom was indicative of a drug eruption. Because she had not changed any medications before the eruption, we suspected that Aminotripa 2® was the cause. As topical steroid treatment did not improve the eruption, Aminotripa 2® was discontinued and changed to a sugar electrolyte maintenance transfusion. After stopping Aminotripa 2®, the eruption gradually disappeared. Aminotripa 2® is composed of amino acids, electrolytes, sugar, and some additives (sulphite, glacial acetic acid, and citric acid hydrate) available from: http://www.kegg.jp/medicus-bin/japic_med_product?id=00055508. Among the ingredients, we focused on the sulphite (sodium bisulphite) in the additives, because it has been reported that sodium bisulphites can cause allergic reactions, such as contact dermatitis (1) and anaphylaxis (3, 4). A 48-h closed patch test was performed with Finn Chambers® (Epitest Oy, Tuusula, Finland) on Scanpor tape® (Alpharma AS, Vennesla, Norway) for sodium bisulphite (0.1% and 1% in pet) (provided by Otsuka Pharmaceutical Co. Ltd), Aminotripa 2®, and Neoparen 2® (Otsuka Pharmaceutical Co. Ltd), another high-calorie infusion that contains a much lower concentration of sodium bisulifite (0.002%). The reactions were determined at 48 h and 72 h after application of the patch test, in accordance with the recommendations of the International Contact Dermatitis Research Group (ICDRG). Although the patient was under treatment with predonisolone 10 mg/day as treatment for myasthenia gravis, she exhibited a positive reaction to 0.1% and 1% sodium bisulphite (Fig. 1b). She also reported pruritus at the Aminotripa 2® (containg 0.04% of sodium bisulphite) test site. She did not exhibit any positive reaction or report pruritus to Neoparen 2®. Based on these findings, we diagnosed the patient with a drug eruption due to sodium bisulphite in Aminotripa 2®. She resumed high-calorie infusion therapy using Neoparen 2®, and the skin eruption has not re-appeared.

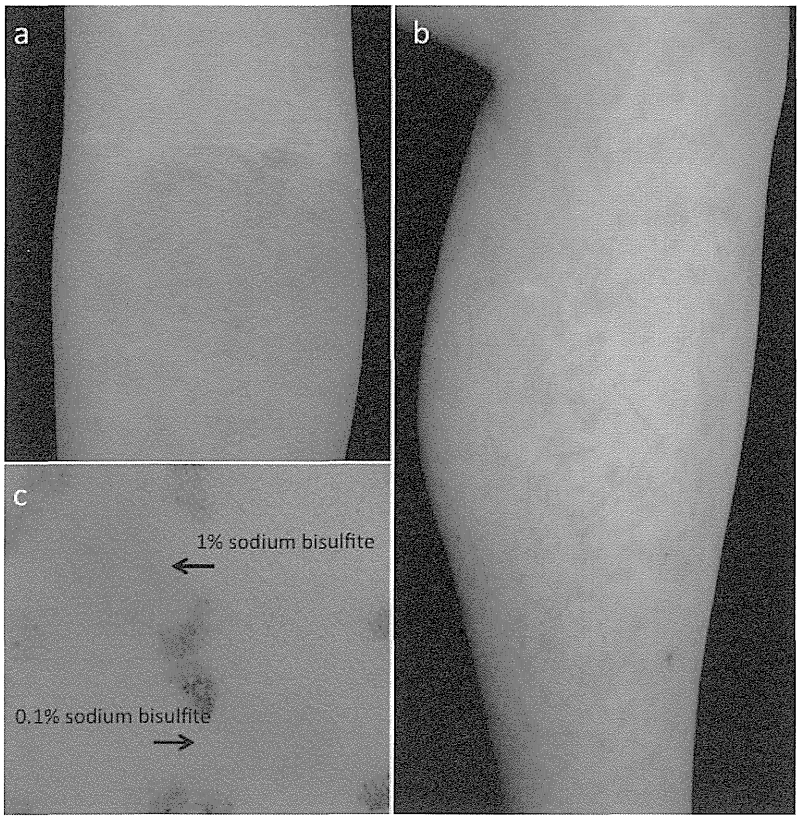

Fig. 1. Clinical presentation. (a, b) Accumulation of red papules on (a) the right forearm and (b) the leg. (c) Seventy-two hours after the patch test. The reactions were classified according to International Contact Dermatitis Research Group (ICDRG) guidelines. Positive reactions (+) were detected for sodium bisulphite 1% and 0.1% pet.

DISCUSSION

To date, type IV allergic reations to sulphites have been reported only as contact dermatitis following the use of sulphite-containing ointments or cosme-

tics (1). However, the case described here suggests that sulphite intake could also cause a type IV allergic reaction leading to systemic eruption (systemic type IV allergic reaction).

Sulphites are frequently used as additives in a wide range of foods and medications. The amount of sulphites in food is kept below a certain level (for example, in Japan wine contains less than 0.35 g/kg of sulphites). However, the intake of various types of food containing sulphites would increase the total amount of sulphites consumed, and may cause a systemic type IV allergic reaction, as in the current case. In this context, sulphite allergy should be considered as a differential diagnosis of systemic eruption.

ACKNOWLEDGEMENT

Written consent to publish was obtained from the patient.

The authors declare no conflicts of interest.

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Review

Novel concept of iSALT (inducible skin-associated lymphoid tissue) in the elicitation of allergic contact dermatitis

By Tetsuya HONDA[*1,†] and Kenji KABASHIMA[*1,†]

(Communicated by Shizuo AKIRA, M.J.A.)

Abstract: Allergic contact dermatitis (ACD) is one of the most common inflammatory skin diseases, which is classified as a delayed-type hypersensitivity immune response. The development of ACD is divided into two phases: sensitization and elicitation. In the sensitization phase, antigen-specific effector T cells are induced in the draining lymph nodes by antigen-captured cutaneous dendritic cells (DCs) that migrate from the skin. In the elicitation phase, the effector T cells are activated in the skin by antigen-captured cutaneous DCs and produce various chemical mediators, which create antigen-specific inflammation. In this review, we discuss the recent advancements in the immunological mechanisms of ACD, focusing on the mechanisms in the elicitation phase. The observations of elicitation of CHS lead to the emerging novel concept of iSALT (inducible skin-associated lymphoid tissue).

Keywords: allergic contact dermatitis, contact hypersensitivity, dendritic cells, T cells, iSALT

Introduction

Allergic contact dermatitis (ACD), such as metal allergy and plant allergy, is a major occupational skin disease that affects approximately 15 to 20% of the general population all over the world.[1] ACD is generally induced by small compounds called haptens (less than 500 daltons of molecular weight). Although haptens do not possess antigenicity, they bind to self-carrier protein in the skin, and the hapten-self complex works as an antigen.[2)–4)] This complex is captured by cutaneous dendritic cells (DCs), which migrate to the draining lymph nodes (dLNs) and present the antigen to naïve T cells. Then, the naïve T cells proliferate and differentiate to several T cell subsets, such as $CD4^+$ helper T (Th)1 cells and $CD8^+$ cytotoxic T (Tc)1 cells. This priming phase is called the sensitization phase (Fig. 1). When the same hapten enters the skin, the antigen-specific Th1/Tc1 cells are activated by the antigen-captured cutaneous DCs. The activated Th1/Tc1 cells produce cytokines such as IFN-γ, which stimulates neighboring cells such as keratinocytes, and provokes inflammation that peaks around 24 to 48 h after the hapten exposure. This phase is called the elicitation phase (Fig. 1).

Since ACD is a prototype of the cutaneous immune response, and the murine model of ACD, which is called contact hypersensitivity (CHS),[5)] is easily induced, a number of studies have been performed to determine its mechanisms, especially in the sensitization phase. For example, extensive studies have been performed for the cutaneous DC subset analysis that induces Th1/Tc1 differentiation in the sensitization phase. In contrast, relatively few studies have been performed on the DC subset responsible in the elicitation phase. This may be partly because of the complex cell-cell interactions in the elicitation phase. However, with the introduction of novel techniques such as multi-photon microscopy for live imaging, the complex mechanisms of the elicitation phase have recently been gradually revealed.

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Abbreviations: DC: dendritic cell; ACD: allergic contact dermatitis; CHS: contact hypersensitivity; iSALT: inducible skin-associated lymphoid tissue; LC: Langerhans cell.

doi: 10.2183/pjab.92.20

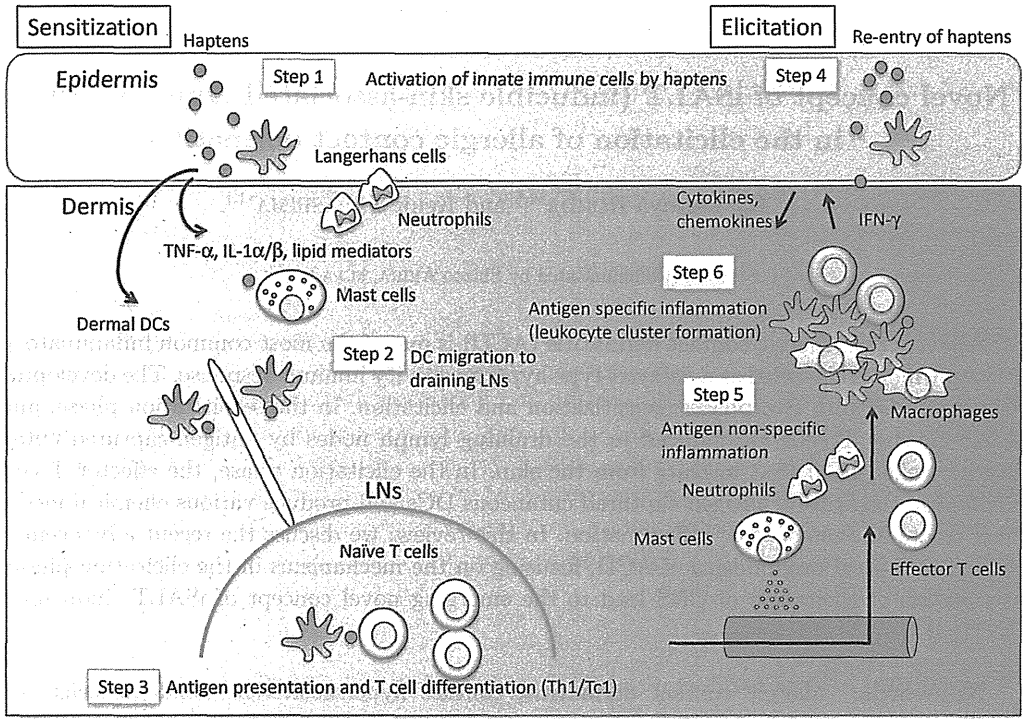

Fig. 1. Overview of the immunological mechanisms of CHS. Step 1. Haptens activate innate immune cells (*e.g.*, keratinocytes, mast cells) and induce the production of various chemical mediators. Step 2. Antigen-captured activated DCs migrate to the dLNs. Step 3. Migrated DCs present the antigen to naïve T cells, which mainly differentiate to Th1 and Tc1 cells. Step 4. Haptens cause subtle inflammation by activating innate immune cells, and recruit neutrophils. Step 5. Leukocytes, including antigen-specific effector T cells, are recruited. Step 6. The antigen-specific effector T cells are then activated in the skin by antigen-captured dermal DCs, which create antigen specific inflammation. Activation of effector T cells mainly occurs in leukocyte clusters.

In this review, we will introduce the updated mechanisms of ACD, as revealed by using mouse CHS, mainly focusing on the mechanisms in the elicitation phase.

1. Induction of antigen-specific T cells

Although various kinds of cells are involved in the development of CHS, the most important cells for antigen specific inflammation are T cells. Both Th cells and Tc cells play important roles in the development of CHS.[6)–10)] Th/Tc cells are further divided into several kinds of Th/Tc cell subsets, such as Th1/Tc1, Th2, and Th17/Tc17 cells, depending on the pattern of its cytokine production. Among the Th/Tc cell subsets, Th1/Tc1 cells, which produce IFN-γ, are the most important cell subsets that induce the elicitation reaction,[5),11)] although the involvement of other Th/Tc cell subsets such as Th2 or Th17 have been reported.[9),10)] For many years, the cutaneous DC subsets responsible for inducing Th1/Tc1 have been a matter of debate. However, recent developments in the DC-subset-specific depletion system have gradually answered these questions. First, we summarize the recent findings on the role of each cutaneous DC subset in the sensitization phase.

In the skin in the steady state, there are at least three DC subsets; Langerhans cells (LCs), $CD103^+$ dermal DCs, and $CD103^-$ dermal DCs.[12)–14)] LCs exist in the epidermal layer, and are the most abundant DCs in the skin. $CD103^-$ dermal DCs occupy most of the dermal DCs (around 80% of dermal DCs) while $CD103^+$ dermal DCs occupy around 10% of dermal DCs.[12)–14)]

LCs have long been assumed to be the DCs responsible for inducing Th1/Tc1 cells in CHS, because of their abundance in the skin, the easy accessibility to haptens, and the strong antigen-presentation ability *in vitro*. However, recent studies have revealed that depletion of LCs during the sensitization phase does not impair CHS responses, while depletion of $CD103^+$ dermal DCs causes

significantly impaired CHS responses.[12),15),16)] Therefore, the general current understanding is that CD103$^+$ dermal DCs are the most important DC subset that mediates sensitization in CHS. However, CD103$^+$ dermal DCs may not be the essential DCs for sensitization, because the impairment in CHS responses caused by the depletion of CD103$^+$ dermal DCs is partial, and Batf$^{-/-}$ mouse, which lacks CD103$^+$ dermal DCs constitutively, exhibits normal CHS responses.[17)] In addition, the redundant roles of CD103$^+$ dermal DCs and LCs in sensitization[18),19)] and the importance of CD103$^-$ dermal DCs in sensitization have been reported.[18),20)] Therefore, although CD103$^+$ dermal DCs are proposed as the DCs responsible for mediating sensitization in CHS, the current data suggest that each DC subset has the ability to mediate sensitization in a context dependent manner.

In humans, two dermal DC subsets are also identified, depending on the expression pattern of CD1a, CD1c, and CD141 (CD1a$^+$CD1c$^+$ dermal DCs and CD1a$^+$CD141$^+$ dermal DCs).[21)] CD1a$^+$CD141$^+$ dermal DCs are proposed to be identical to mouse CD103$^+$ dermal DCs.[21)] These DCs may mediate sensitization in human ACD.

2. Mechanisms of the effector T cell activation in the elicitation phase

Effector T cells are recruited to and retained in inflammatory skin with limited dependency on their antigen specificity.[22)] Therefore, effector Th1/Tc1 cells infiltrate the skin following the subtle inflammation induced by haptens. Indeed, initial neutrophil infiltration is necessary for subsequent effector T cell infiltration in the elicitation phase.[23)–26)] When the concentration of haptens is not high enough to provoke this antigen non-specific inflammation, no CHS response occurs.[27)] Haptens induce skin inflammation directly by causing cellular stress and indirectly by activating toll-like receptors (TLRs) and NOD-like receptors (NLRs). The infiltrated effector T cells are then activated by antigen-captured cutaneous DCs, and produce cytokines, which provoke antigen-specific inflammation.

Although the detailed mechanisms by which haptens induce skin inflammation remain unknown, recent reports indicate that activation of TLRs and NLRs in innate immune cells is an important mechanism of hapten-induced inflammation.[11),28),29)] Haptens induce cellular stresses and damages that lead to the production of reactive oxygen species (ROS).[30)–35)] ROS degrade the extracellular matrix (such as hyaluronic acid), and generate low-molecular-weight hyaluronic acid,[28)] which stimulates TLR2 and TLR4 on the surrounding cells, such as DCs, keratinocytes, and mast cells, all of which express TLR2 and TLR4.[36)–40)] Stimulation of TLR2 and TLR4 eventually leads to the activation of nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs), and induces the expression of various pro-inflammatory cytokines and chemokines, which drive both DC migration to dLNs and the inflammatory cell infiltration into the skin. Haptens also induce the release of adenosine triphosphate (ATP).[29),30)] ATP stimulates purinergic receptor P2X7, which activates NOD-, leucine rich region repeat (LRR)-, and pyrin domain-containing 3 (NLRP3).[41)] Activation of NLRP3 in keratinocytes leads to the release of IL-1β and IL-18, which also drives skin inflammation.[42)–44)] Haptens also increase vascular permeability by inducing histamine release from mast cells.[45)] Overall, haptens cause innate immune cell activation and induce the initial neutrophil infiltration to recruit effector T cells in the skin (Fig. 2).

3. Antigen-specific inflammation: Leukocyte cluster formation as an essential structure for effector T cell activation in the skin

Following the hapten-induced antigen non-specific inflammation, T cell-mediated antigen-specific inflammation is initiated. When T cells infiltrate into the skin, they induce a stable interaction with antigen-bearing cutaneous DCs and produce cytokines.[22),46)] Dermal DCs play essential roles in the effector T cell activation,[47)] although the dermal DC subset responsible for the antigen presentation in the elicitation phase remains unclear. Cytokines produced by activated T cells then stimulate skin-resident cells, which leads to further recruitment of T cells and amplification of the inflammation.

We recently discovered that dermal leukocytes form a cluster structure after hapten application, and that this structure is essential for efficient effector T cell activation in the skin.[47)] In skin in the steady state, cutaneous DCs are distributed randomly and exhibit active motility. However, after hapten application, DCs exhibit cluster formation around postcapillary venules. Effector T cells also accumulate in the cluster. Depletion of macrophages completely abrogates the DC cluster formation and is accompanied by impaired effector T cell activation in the skin. Blockade of IL-1α or CXCL2 impair the leukocyte cluster formation and effector T cell

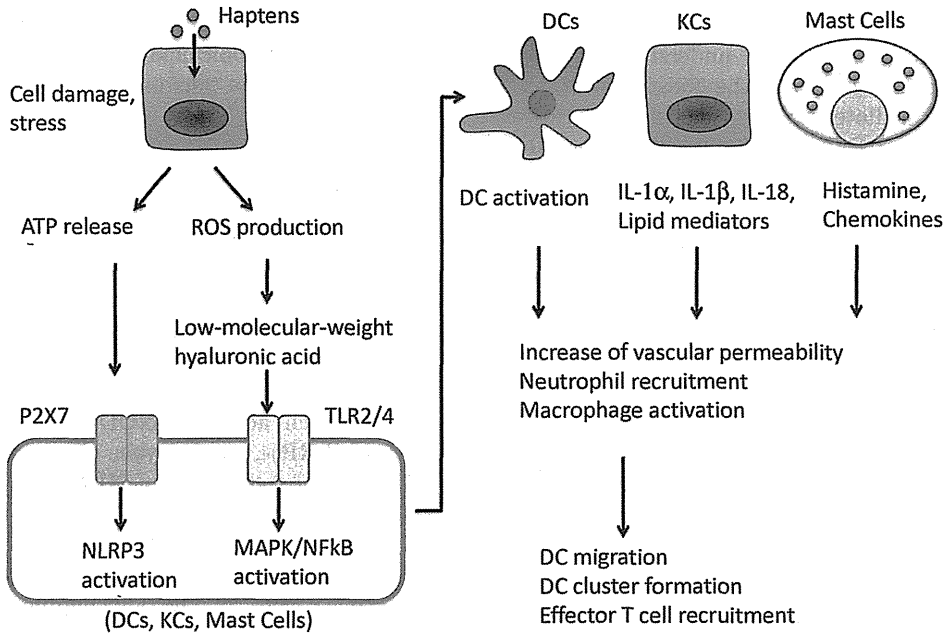

Fig. 2. A schematic view of hapten-induced inflammation. Haptens induce ATP release and ROS production from several skin cells, such as keratinocytes. NLRP3 activation and/or MAPK/NF-κB activation are induced via P2X7 signaling and TLR2/4 signaling, which are stimulated by ATP and low-molecular-weight hyaluronic acid, respectively. KCs and mast cells produce various chemical mediators, and drive skin inflammation. This first round of inflammation is essential for the subsequent DC migration in the sensitization phase and effector T cell infiltration in the elicitation phase.

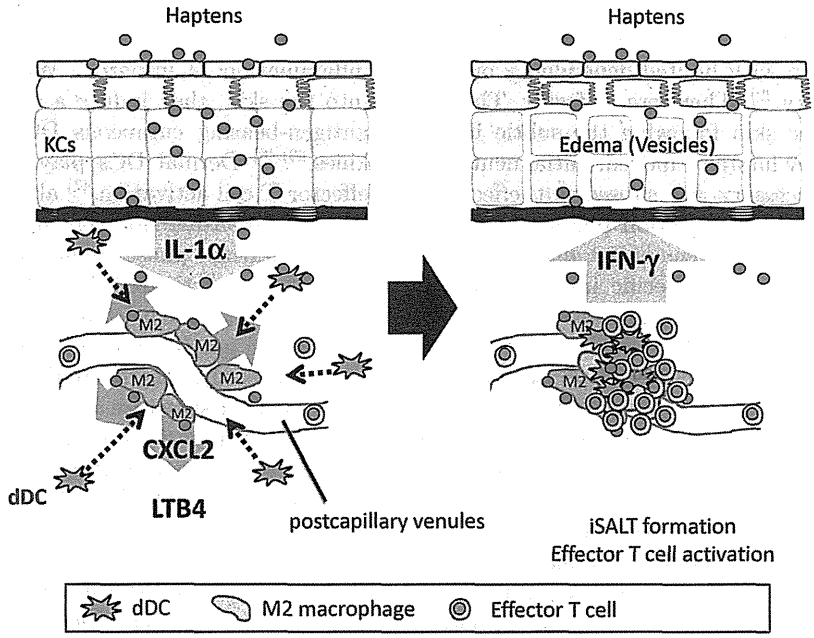

Fig. 3. A schematic view of iSALT formation. Haptens induce IL-1α production from keratinocytes (KCs), which stimulates M2-type macrophages located around postcapillary venules. The stimulated macrophages then produce CXCL2, which accumulate dermal DCs. LTB4 also plays an important role in DC accumulation by increasing DC motility. Effector T cells are activated within the DC clusters (iSALT), and produce cytokines.

activation. Keratinocytes are the main producers of IL-1α. Among the two types of macrophage subsets (classically activated (M1)- and alternatively activated (M2)-type macrophages), M2-type macrophages have significantly higher expression of IL-1 receptor, and produce CXCL2 upon IL-1α stimulation.[47] These results indicate that the leukocyte cluster is an essential structure for efficient T cell activation in the skin, and that the IL-1α-CXCL2 axis via M2 macrophages mediates the cluster formation[47] (Fig. 3). In addition to CXCL2, leukotriene B4 (LTB4), a lipid mediator, mediates the leukocyte cluster formation by promoting DC motility through Cdc42 and Rac activation.[48]

This leukocyte cluster formation may also be important for the development of human ACD, because the leukocyte clusters are observed in human ACD, and edema of the epidermal layers (which is an indicator of effector T cell activation) occurs above the leukocyte clusters.[47] These findings suggest the importance of the leukocyte cluster in human ACD.

Since the 1980's, the concept of skin-associated lymphoid tissues (SALT) was proposed as the structure for T cell activation in the skin.[49] However, the existence and significance of SALT has never been proved. The leukocyte clusters we observed may correspond with the old concept of SALT. However, we propose that the leukocyte clusters are inducible SALT (iSALT), since they are induced in the inflammatory state and do not exist in the steady state.[50]

4. Regulation of elicitation

4-1. Regulatory T cells. Regulatory T cells (Tregs) are a T cell subset that exerts a potent immunosuppressive effect to inhibit auto-reactive T cell activation.[51] Tregs also exert regulatory roles in various inflammatory diseases, including CHS.[52] The number of Tregs in the skin significantly increases during the skin inflammation process,[53] and depletion of Tregs in the elicitation phase as well as the sensitization phase causes enhanced and prolonged inflammatory responses,[53),54] indicating that Tregs play crucial roles in the regulation of elicitation. Although the detailed mechanisms of the suppression remain unknown, the inhibition of leukocyte influx into the skin via IL-10 or CD39/73 in Tregs is proposed as one of the regulatory mechanisms.[55)–57] Inhibition of stable DC-T cell interaction may be another possible regulatory mechanism by Treg in CHS.[58] In addition, skin Tregs may exert a suppressive function by inhibiting antigen-specific T cell proliferation in the dLNs by recirculation.[53]

Using a new cell labeling system with the photoconvertible protein Kaede, we succeeded in tracking T cell migration after their infiltration to the skin. Interestingly, the skin-derived Tregs exhibited an activated phenotype with high expression of cytotoxic T lymphocytes antigen-4 and IL-10, and had much more potent suppressive activities than resident Tregs in the dLNs, suggesting that skin Tregs exert their potent immunosuppressive activity not only in the skin but also in the dLNs by circulating in the body.[53]

4-2. Langerhans cell as a possible regulator of sensitization and elicitation. As mentioned earlier, the theory that LCs work as initiators of CHS is now challenged. Rather, recent reports suggest that LCs work as regulators of sensitization and elicitation. In a constitutive or inducible LC-depletion system, LC depletion in the sensitization phase causes enhanced CHS responses.[59),60] In that system, LCs exert regulatory functions via cognate CD4 interaction and the production of IL-10.[59] In a dinitrothiocyanobenzene-induced cutaneous immune tolerance model, LCs induce tolerance by activating Tregs.[61] It is also reported that LCs induce Tregs in an ultraviolet (UV)-induced immunosuppression model as well as a skin graft-induced immunosuppression model.[62),63]

Thus, LCs may play regulatory roles in the sensitization of CHS, at least under certain conditions. In the elicitation phase, the role of LCs is less clear. However, several reports also suggest the regulatory role of LCs in the elicitation phase. In an old study that depleted skin DCs during the elicitation phase by topical steroid, mice exhibited enhanced CHS responses,[64] suggesting the existence of DC subsets that play a regulatory function in the elicitation phase. Considering the stimulatory roles of dermal DCs in the elicitation phase,[47] the regulatory roles may be executed by LCs. In human studies, it is reported that LCs play essential roles in the maintenance of Tregs in skin in the steady state.[65] LCs may inhibit effector CD4 T cell activation in patients with nickel allergy via expression of programmed-death ligand-1.[66] Overall, although the function of LCs in CHS remains controversial, LCs may work as regulators of CHS in both sensitization and elicitation phases. Clarification of the mechanisms would be of great interest and benefit from both basic science and clinical points of view.

Conclusion and discussion

By introducing new technologies for the analysis of CHS, the immunological mechanisms of ACD have been getting clearer. One of the remaining points to be clarified is whether findings in mouse CHS are relevant to human ACD. Another interesting question is the role of iSALT in inflammatory skin diseases other than ACD. In fact, in psoriasis, a common inflammatory skin disease, an iSALT-like structure has been reported in the skin lesions.[67] iSALT may serve as an important structure to provoke inflammation in psoriasis as well. In addition, although T cells and DCs are the major players in the development of ACD, the roles of innate immune cells, such as mast cells[45),68] and macrophages, neutrophils,[69] and natural killer cells[70] in acquired immunity are also attracting attention. The crosstalk between innate immune cells and acquired immune cells would be an important mechanism to create antigen-specific immune responses. Evaluation of these points may lead to a breakthrough in the understanding of the immunological mechanisms of various cutaneous immune responses, including ACD.

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(Received Aug. 25, 2015; accepted Oct. 13, 2015)

Profile

Kenji Kabashima was born in Takayama City in 1970, and brought up in northern part of Kyushu in Japan. He graduated from Kyoto University in 1996. He trained in Medicine/Dermatology at the United Naval Hospital, Kyoto University Hospital, and University of Washington Medical Center. He started research on lipid mediators in immunology at Kyoto University, which led to a Ph.D. (Prof. Shuh Narumiya) in 2003. That year, he was appointed as assistant professor in Dermatology at Kyoto University (Prof. Yoshiki Miyachi), and researched on dendritic cell homeostasis and plasma cell mobilization at University of California San Francisco (Prof. Jason Cyster). In 2005, he moved to University of Occupational and Environmental Health as an associate professor (Prof. Yoshiki Tokura). He was assigned as an associate professor in 2008 and became a professor and chairman in Dermatology at Kyoto University in 2015. He has been researching on the translational medicine and mechanism of inflammatory skin diseases by gene-targeted mice and visualization of the skin. For his accomplishment, he received the JSPS prize and PhARF award (from Europe). He is currently an executive board member of the Japanese Dermatological Association, International League of Dermatological Societies, and International Eczema Council.