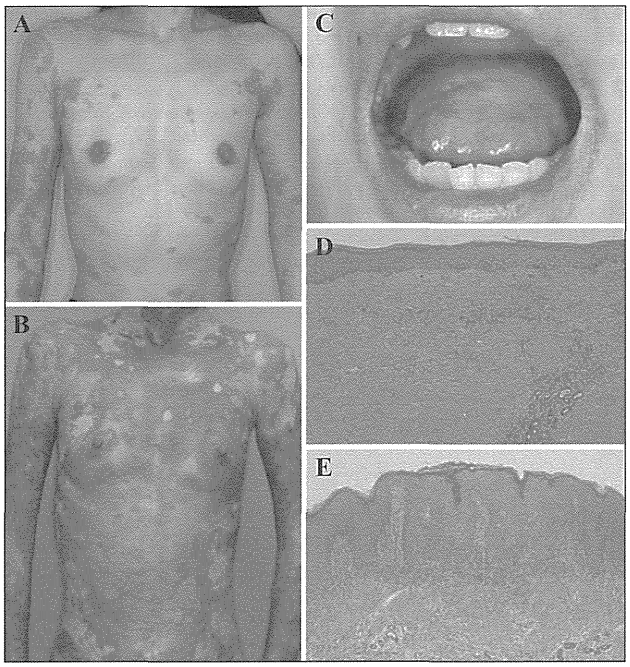

Figure 1. Clinical appearance and histological findings. **A)** Clinical appearance of scaly erythema on the patient's chest and upper arms in March 2011, before adalimumab was administered. The patient presented with scaly erythema of the size of a thumb tip on the trunk and limbs. **B, C)** Clinical appearance of the skin sclerosis, scaly erythema, pigmentation, depigmentation, and erosion **(B),** and oral aphtha **(C)** upon admission in September 2012. This patient exhibited systemic skin sclerosis of the limbs and trunk, sclerodactyly, and microstomia. Alopecia areata was also present. She was unable to join her palms together due to the sclerodactyly. **D)** A skin biopsy of the skin sclerosis from the upper left arm revealed scleroderma-like changes. Hematoxylin and eosin staining indicated acanthosis, dense lymphocytic infiltration around the skin appendages, and thickened collagen bundles (Original magnification: ×100). **E)** A skin biopsy from the scaly erythema on the left knee revealed lichen planus. Hematoxylin and eosin staining revealed parakeratosis, acanthosis, satellite cell necrosis, liquefaction, and band-like lymphocytic infiltrate in the upper dermis (Original magnification: ×40).

planus, respectively *(figures 1D, E)*. Immunohistochemical staining of the biopsy specimens obtained from the lichen planus revealed that $Foxp3^+$ Treg had infiltrated the upper dermis and $CD8^+$ cytotoxic T cells had infiltrated the epidermis and upper dermis of the skin lesion. Direct immunofluorescence staining did not show any significant findings. The biopsy specimen obtained from the site of the oral aphtha also revealed lichen planus. Examination of the inguinal lymph node biopsy did not indicate malignancy but revealed dermatopathic lymphadenopathy without any necrosis; T-cell receptor β chain gene rearrangement was negative in the biopsy specimens whereas soluble interleukin-2 receptor was remarkably elevated at 5445 U/mL (150-505). Bone marrow biopsy did not reveal any malignancy. Thus, we diagnosed the patient as having scleroderma-like changes with lichen planus, possibly induced by anti-TNF-α antibody therapy. Treatment with nbUVB therapy in combination with the application of calcipotriol for 3 months improved the skin sclerosis and lichen planus, resulting in an improvement of the MRSS from 38 to 25. As neutrocytopenia also developed after discharge, 20 mg/day of prednisolone was administered, resulting in an improvement of the neutrocytopenia.

Anti-TNF-α antibody therapy is useful for treating rheumatoid arthritis, Crohn's disease, and psoriasis [1]. However, localized scleroderma and lichen planus can be induced by anti-TNF-α antibody therapy [2, 3]. New onset or exacerbation of psoriasis, cutaneous vasculitis and sarcoidosis caused by anti-TNF-α antibody therapy have also recently been reported [4]. The most widely accepted hypothesis regarding the pathomechanism of these reactions is based on the interaction between increased interferon-alpha (IFN-α) levels and reduced TNF-α levels. TNF-α inhibition using a biological agent may result in uncontrolled IFN-α production and may induce autoimmune skin changes by plasmacytoid dendritic cells [5]. In the present case, the scleroderma-like changes with lichen planus might be either newly developed or be induced or exacerbated by anti-TNF-α antibody therapy. However, it is difficult to distinguish them because the initial psoriasis-like skin lesions had not been definitively diagnosed.

In conclusion, we speculate that anti-TNF-α antibody therapy may possibly induce or exacerbate scleroderma-like changes and lichen planus by increasing IFN-α levels, because type I IFN is also involved in the pathogenesis of scleroderma and lichen planus [6, 7]. ■

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Bullous pemphigoid with IgG autoantibodies to BP180 C-terminal domain and desmocollin 3 associated with transverse colon cancer

An 82-year-old Japanese male presented erythemas and blisters with atrophic scars on the abdomen, lower back, buttocks and legs *(figure 1A,B)*. No mucosal lesion was seen. A skin biopsy revealed subepidermal blisters with infiltration of a few eosinophils, lymphocytes and histiocytes in the dermis. Shrunken keratinocytes with dense nuclei and clear cytoplasm, suggesting apoptotic cells, without apparent acantholysis were also observed in epidermis *(figure 1C,D)*. The results of IgG enzyme-linked immunosorbent assays (ELISAs) were negative for all BP180 NC16a domains, BP230 and desmogleins 1 and 3.

Direct immunofluorescence (DIF) of the skin biopsy showed linear deposits of IgG and C3 at the basement membrane zone (BMZ). Indirect immunofluorescence (IIF) of normal human skin for the patient's serum detected IgG antibodies reactive with cell surfaces in the lower epidermis and BMZ, at a titer of 1:10 *(figure 1E)*. In IIF of 1 mol/L NaCl-split skin, the IgG anti-BMZ antibodies reacted with the epidermal side of the split skin *(figure 1F)*.

In immunoblotting (IB) of normal human epidermal extract, patient IgG antibodies reacted with the 180-kDa BP180 and the110-kDa a-form and the 100-kDa b-form of desmocollin (Dsc) *(figure 1G)*. IgG antibodies did not react with recombinant protein (RP) of the BP 180 NC16a domain *(figure 1G)*, but reacted with RP of the BP180 C-terminal domain *(figure 1G)*. IB did not detect LAD-1 in concentrated culture supernatant of HaCaT cells, laminin-332 in purified human laminin-332, type VII collagen and laminin gamma-1, in normal human dermal extract. We performed novel ELISA using mammalian RPs of Dsc1-3[1], detecting IgG antibodies to Dsc3 (OD 0.435, normal: <0.120), but not to Dsc1 or Dsc2.

Endoscopical examination revealed transverse colon cancer. Oral tetracycline 750mg/day and nicotinamide 1500 mg/day with topical steroids was started and the production of blisters decreased markedly. Two months later, the transverse colon cancer was resected, leading to complete disappearance of the skin lesion, leaving a slight scar. Immunohistochemically, neoplastic cells of the colon cancer did not express Dsc3. Anti-Dsc3 antibody was derived from PROGEN Biotechnik, Heidelberg (clone: Dsc3-U114) and used after microwave pretreatment.

DIF findings of the skin biopsy, IIF of normal human skin and NaCl-split skin for the patient serum were compatible with the results of IB of normal human epidermal extract. Positive reactivity with only the epidermal side of NaCl-split skin was also compatible with negative reactivity with autoantibodies to laminin-332, type VII collagen and laminin gamma-1 in IB.

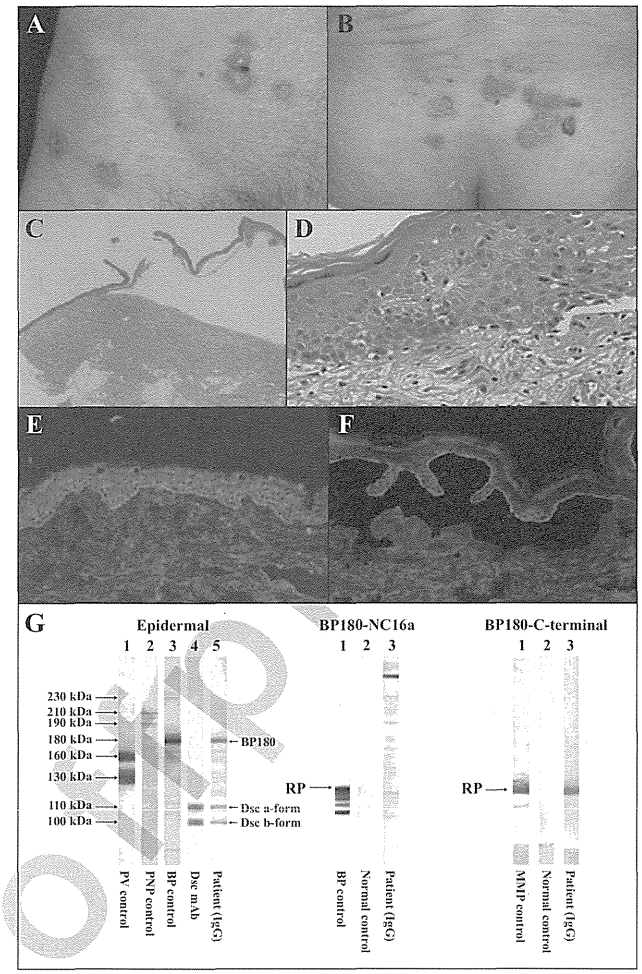

Figure 1. (A,B) Clinical features of our patient on the abdomen **(A)** and the lower back **(B). C,D)** Histopathological features of a skin biopsy (H&E staining). **C)** Lower magnification (original magnification ×20). **D)** Higher magnification (original magnification ×200). **E,F)** The results of indirect IF using normal human skin **(E)** and 1 mol L-1 NaCl-split skin **(F). G-I)** The results of IP IB analyses using normal human epidermal extract and RPs of BP180 NC16a domain and C-terminal domain **(G).**

Autoantibodies to the BP180 C-terminal domain are observed in anti-BP180-type mucous membrane pemphigoid (MMP) [2] and some cases of BP [3], and anti-Dsc3 autoantibodies have been reported in some cases of pemphigus patients with oral mucosal lesions [4]. However, no mucosal lesion was observed in our patient. We considered that IgG antibodies to the BP180 C-terminal domain caused the subepidermal blisters with slight scarring in our case, because no other anti-BMZ autoantibodies were detected. The antibodies might have a different pathogenic role from those found in MMP.

We recently reported that antibodies to Dscs are frequently detected in the sera of paraneoplastic pemphigus, pemphigus vegetans and pemphigus herpetiformis [1]. Anti-Dsc3

薬疹メカニズム

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要　旨
薬疹のほとんどは，T細胞を介した遅発型アレルギー反応を介する．薬疹発症に関するHLAとの強い関連の発見と，コンピュータの進歩による分子構造解析から，最近，具体的な薬物抗原認識のメカニズムの詳細が明らかになっている．この分野の研究により，高頻度に薬疹を起こす薬物においては，薬疹発症抑制を目的としたオーダーメード医療の時代を迎えようとしている．

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キーワード：薬疹，メカニズム，T細胞

はじめに

正常使用量で生じた有害または意図しない反応に起因する皮疹を薬疹と呼ぶ．濃度依存性にすべての服用者に出現し得るもの，すなわち，上皮成長因子阻害薬などの分子生物学的抗がん薬でほぼ必発する乾燥皮膚や爪囲炎，ざ瘡様皮疹などはType Aに属する．これは濃度依存性であり，休薬で改善する．一方，特定の服用者に限って出現するもの，すなわち，ACE阻害薬やNSAIDsによって出現する非アレルギー性の血管浮腫や，薬物を抗原と認識することによって出現する種々のアレルギー性の皮疹は，Type Bに属する．本稿では，Type Bに属するアレルギー反応によって生じる薬疹に焦点を絞る．

1．アレルギー反応としての薬疹病態の理解

アレルギー性の薬疹の端緒は，薬物投与によって生じる免疫反応の初動である．他の項で論じられる蕁麻疹や血管浮腫などの即時型反応以外，多くの薬疹は数日ないし2週間以内に生じるT細胞を介した遅発型アレルギー反応である．

1）薬疹発症とHLA

ゲノムワイド関連解析によって，特有のHLA保有者に高頻度に薬疹が起こることが明らかとなった．特にカルバマゼピン薬疹は，漢民族ではHLA-B*15：02[1]，本邦ではHLA-B*31：01保有者に[2]，また，アロプリノール薬疹は，洋の東西を問わずHLA-B*58：01保有者に[3]，HIV治療薬のアバカビルでは，HLA-B*57：01保有者に[4]高率に起こることが次々と示

〈Special Article〉 Hypersensitivity/allergic reactions in pain medicine : Reducing the risk of anaphylaxis and adverse events in clinical settings
Mechanisms of drug eruption
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表1　高頻度に薬疹を発症しやすいHLAアロタイプ

原因薬物	HLAタイプ	皮　疹	文　献
carbamazepine	B*15：02	SJS/TEN	Chung et al. Nature, 2004
carbamazepine	B*15：11	SJS/TEN	Kaniwa et al. Epilepsia, 2010
carbamazepine	B*15：08	SJS/TEN	Chung et al. J Dermatol Sci, 2012
carbamazepine	B*15：18	?	Ikeda et al. Epilepsia, 2010
carbamazepine	A*31：01	DRESS	McCormack et al. N Engl J Med, 2011
carbamazepine	A*31：01	SJS/TEN/DIHS	Ozeki et al. Hum Mol Genet, 2011
carbamazepine	A*31：01	DRESS	Genin et al. Pharmacogenomics J, 2013
oxcarbamazepine	B*15：02	SJS/TEN	Hung et al. Pharmacogenomics, 2010
abacavir	B*57：01	ABC HS	Martin et al. PNAS, 2004
alloprinol	B*58：01	SJS/TEN/DRESS	Hunget al. PNAS, 2005
dapson	B*13：01	DRESS	Zhang et al. N Engl J Med, 2013
lamotorigine	B*15：02	SJS/TEN	Cheung et al. Epilepsia, 2013
phenytoin	B*15：02	SJS/TEN	Cheung et al. Epilepsia, 2013

された（**表1**）．そして，特有のアロタイプの患者に薬物を投与しないことによって，薬疹の予防が可能であることが立証されている．本邦でも，カルバマゼピン投与に際し，HLA-B*31：01保有者への代替薬へ変更する臨床治験が施行されている．

2）T細胞受容体を介した薬物の抗原認識機構

多くの薬物は，免疫原性がない分子量数百以下の低分子物質である．薬物がどのように免疫系に異物と認識されるかを知ることは，薬疹のメカニズムを明らかにする上で重要である．

①ハプテン抗原認識モデル（図1A）[5]

低分子は，血清成分や細胞膜上の蛋白質と結合することによって，通常の外来抗原と類似した新たな抗原物質として認識されるというハプテン説がこれまでの定説である．ペニシリンアレルギー患者において，ペニシリン分子はHLAのリジン部位に共有結合して，ハプテン抗原と認識されることが明らかにされた[6]．最近でも，パラフェニレンジアミン，ネビラピン，カルバマゼピン，β-ラクタム系抗生薬，アバカビルがハプテン抗原となることが報告されている．ハプテン抗原による薬物のT細胞認識モデルでは，通常の蛋白質抗原と同様な機序を踏襲する．すなわち，薬物は血清成分や細胞膜上の蛋白質と共有結合したことにより，新規な異物抗原となり[5]，自然免疫系によって活性化した抗原提示細胞は，これらを取り込み，処理して，HLAとともに細胞表面に提示する．これに対して，適度の親和性を持ったT細胞受容体を発現するナイーブT細胞が，感作されてメモリーT細胞となり，再度，抗原提示を受けた時にエフェクターT細胞として炎症反応に寄与する．

②*p-i*コンセプト（図1B，C）[5]

ハプテン抗原認識は，これまでの免疫反応を踏襲するものであり，免疫学の知識をもってすれば，理解しやすい概念である．一方，2000年代に入ってからは，薬疹患者から薬物反応性T細胞クローンを樹立して，より詳細に薬物抗原認識機構を明らかにしようとする研究がPichlerらを中心に盛んに行われた．しかし，

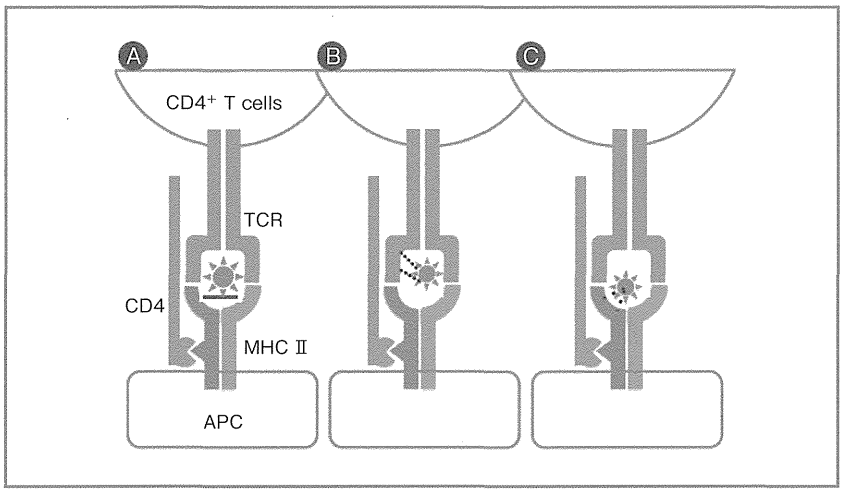

図1　T細胞（CD4+ T cell）と抗原提示細胞（APC）における薬物抗原認識方法
（文献5より引用改変）
ペプチドに共有結合した薬物は，ハプテン抗原としてT細胞に認識される（A）．一方，薬物に親和性のあるT細胞受容体を持つT細胞（B）や抗原提示細胞のHLA（C）は緩やかな結合でもT細胞を活性化する

驚くべきことに，これらのT細胞の薬物との反応は，従来のハプテン抗原認識では説明できないものが多かった．われわれも，2002年にフェノバルビタール薬疹患者から薬物特異的T細胞クローンおよびラインを樹立し，詳細に解析した結果を報告し，抗原提示細胞は，固定された後でも薬物抗原をT細胞へ提示する能力があることを検証した[7]．ハプテン抗原として生じる活性化以外にも，薬物反応性T細胞の解析から，ⅰ）抗原提示細胞が抗原を処理（プロセシング）せずに，直接提示してT細胞活性化を起こすことがある，ⅱ）薬物は抗原提示細胞（のHLA分子）とT細胞受容体とに緩い結合をもたらし，T細胞を活性化させることがある，ⅲ）HLA非拘束性を持たない薬物反応性T細胞が存在するなど，ハプテン抗原のような薬物と蛋白質との強力な共有結合を必要としない緩い結合が，薬物における免疫反応に関連することが示唆された．Pichlerはこれを整理して，pharmacological interaction concept（*p–i*コンセプト）を提唱している[8]（**図1 B, C**）[5]．薬物によるT細胞の活性化には，薬物が抗原提示細胞上に発現するHLA分子とT細胞上のT細胞受容体とに結合することが必要であるが，電気的結合やファンデルワールス結合のように，極めて弱い力による緩やかな結合によって，十分反応する．ハプテン抗原では，提示される抗原エピトープに対し1つのT細胞受容体が認識され，その抗原に対する感作と惹起が免疫反応に必須であり，その抗原に対する抗体産生も起こり得る．しかし，このような*p–i*コンセプトにおける免疫反応では，非特異的にT細胞は活性化されやすく，抗原に対する感作の必要はないし，抗原に対する抗体産生も起こらない．

最近，ある薬物による薬疹の発症が，特定のHLAアレル保有者に高頻度に起こることが明らかになり，分子構造解析から，薬物，T細胞受容体，HLAとの相互関係に関する研究が飛躍的に進んでいる．カルバマゼピンはHLA-B*

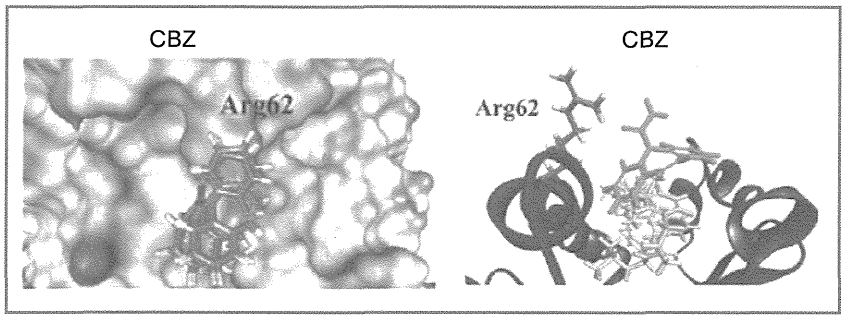

図2　カルバマゼピン（CBZ）のHLA-B＊15：02との結合様式
（文献9より引用改変）
CBZは抗原提示細胞に取り込まれ，処理されることなく，電気的な緩い結合によってT細胞を活性化させる．中央がCBZ

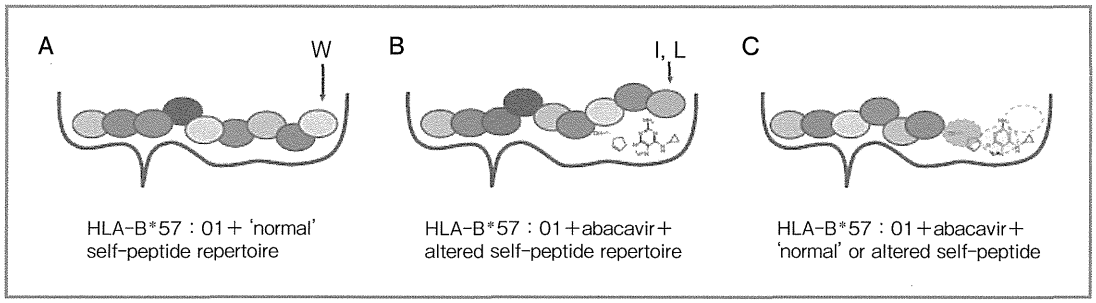

図3　アバカビル（ABV）のHLA-B*57：01との結合様式（文献10，14より引用改変）
ABVは，HLAのFポケットに結合することによって内在自己ペプチドに構造変化を引き起こし（B），非自己ペプチドとして認識してしまう．また，非自己ペプチドを自己ペプチドと誤認識する可能性（C）もある

15：02との緩やかな結合によって薬物抗原のT細胞認識が行われることが明らかになり（**図2**），*p-i*コンセプトが実際の薬物抗原認識様式として存在することが立証された[9]．

③ Altered peptide repertoire説

抗HIV薬として用いられているアバカビルは，HLA-B*57：01の保有者に高率に重症薬疹を引き起こす．アバカビル反応性T細胞の樹立により，HLAと薬物との相互関係が詳細に検討され，新しい機序が提唱された．すなわち，Altered peptide repertoire説である[10]．これはアバカビルがHLAのFポケットという窪みに入り込み，通常の自己ペプチドがHLAに嵌まり込む際に，構造的な変化をもたらすことによって，異物抗原エピトープとしてT細胞に認識されてしまうというものである（**図3**）．したがって，反応するT細胞はハプテン抗原のように特定なT細胞受容体を持つT細胞ではなく，様々なT細胞受容体を持つものが一斉に活性化し，激烈な炎症をきたす．最近，抗水痘帯状疱疹ウィルス薬であるアシクロビルも，構造類似性からアバカビルと同様にHLAに結合することによって，自己ペプチドの構造的変化がもたらされる可能性が指摘されている[11]．

④ 薬物によるT細胞受容体の構築の変化

Altered peptide repertoire説と逆に，薬物が直接T細胞受容体に構造変化を起こす場合があることも最近，明らかになっている．ある

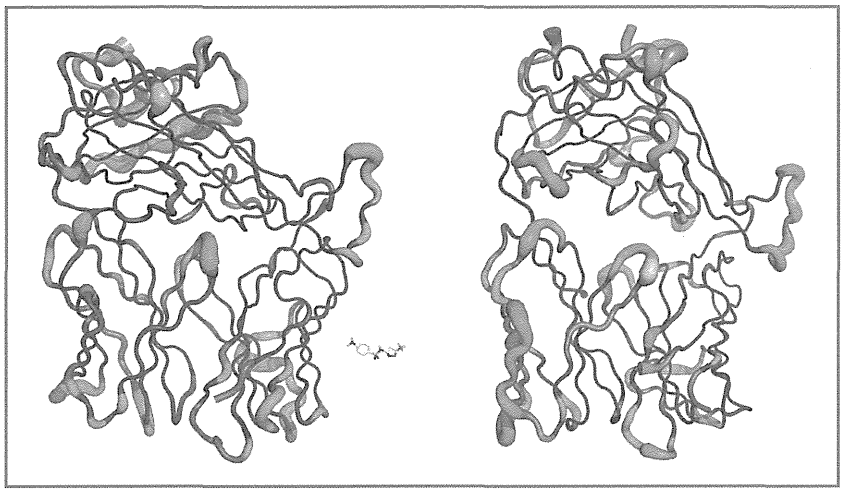

図4　サルファメトキサゾール（SMZ）の結合による薬物特異的T細胞上に発現するT細胞受容体Vβ20-1の構造変化（Bファクターによる色分け表示）
この受容体のCDR2βにSMZが結合すると，結合前（右）と比較して，構造的な変化を認める（左）とともに，原子のゆらぎが結合後に安定している

サルファメトキサゾール反応性T細胞のT細胞受容体Vβ20-1鎖は，薬物結合によってT細胞受容体そのものの構造変化をきたし，その結果，ペプチド-HLA認識に変化が起こる（**図4**）[12]．本薬以外にも，T細胞受容体に結合することによって，T細胞受容体自身の構造変化をもたらす薬物がある可能性がある．

⑤ その他の因子について

薬疹を引き起こしやすくする因子としては，薬物代謝に関わる酵素の遺伝子多型[13]，ウィルス感染などの有無，膠原病の合併などもいわれている．

3）薬疹の臨床型

薬疹は皮疹の臨床像によって分類されるが，最も多いのは播種状紅斑丘疹型（maculo-papular eruption：MPE）であり，全体の薬疹の半分近くを占める．発疹学的な特徴は体幹四肢に散在する丘疹および紅斑であり，ほぼ左右対称性である（**図5A**）．個疹に大きな特徴がなければ，すべてこの範疇に分類されているが，個疹に水疱形成がある場合は，水疱型，紅斑がやや隆起性で中央に暗紫色の水疱を持ち，標的のような形状（target lesion）を示し，融合傾向のあるものは，多形（滲出性）紅斑型（erythema exudative multiforme：EEM）（**図5B**）と呼び，その臨床上の特徴を捉えて分類する．しかし，実際には両者の鑑別が難しい臨床もある．特にEEMの中で口唇にびらんを伴うものをEEM majorと呼び，臨床的に後述する重症薬疹であるStevens-Johnson症候群と鑑別が難しい．近年，高血圧に対する薬物にサイアザイド系薬物を含む合剤が頻用されるようになってから，サイアザイド系薬物に起因する光線過敏型薬疹が増加している．これは，露光部（顔面，特に鼻背，前腕，頸部のV領域）に紅斑を呈するもので，紫外線の強さによって発症時期が異なることから，投薬時期と皮疹の出現時期が必ずしも一致しない点で本症に気づかないことがある．その機序については，まだ不明な点が多い．固定薬疹は，セフェム系抗生薬，サリチル酸製剤などの投与によって，同一部位に

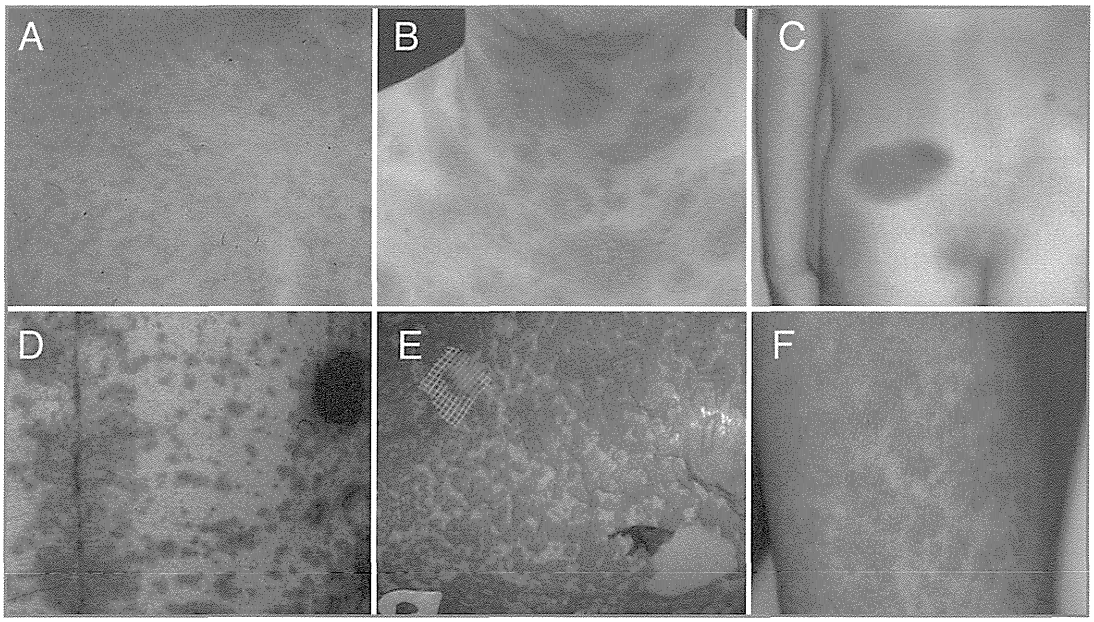

図5　種々の薬疹の臨床像
A：播種状紅斑丘疹型（MPE），B：多形滲出性紅斑型（EEM），C：固定薬疹型（FDE），D：Stevens-Johnson 症候群（SJS），E：中毒性表皮壊死症（TEN），F：薬剤性過敏症症候群（DIHS/DRESS）

繰り返して紅斑を生じる薬疹のタイプで，投与中止で炎症後色素沈着を繰り返すことから，臨床的にはむしろある部位に存在する類円形の色素沈着を主訴として来院する（**図5C**）．重症薬疹として，Stevens-Johnson 症候群（SJS），中毒性表皮壊死症（toxic epidermolytic necrosis：TEN）および薬物性過敏症症候群（drug-induced hypersensitivity syndrome / drug rash with eosinophilia and systemic symptoms：DIHS/DRESS）が知られている（**図5E, F, G**）．それぞれ特徴的な臨床像を呈する．これらの薬疹は，生命を脅かす可能性があるため，その病因解明のために研究者の情熱が注がれているが，未だ不明な点も多い．

4）活性化するT細胞の機能との関係

T細胞を介して起こるアレルギー性の薬疹は，様々な臨床像を呈する．その重症度や特徴的な皮疹の性状は，活性化するT細胞の機能を反映していると考えられている．従来から有名なGellとCoombsによるアレルギー反応分類から考えると，T細胞を介する反応はIV型と考えられるが，サイトカイン産生の違いによる機能的なT細胞サブセットの発見から，近年，このIV型反応をa～dの4つの亜型に細分類することが提唱されている（**表2**）[14]．

IVa型は，いわゆるTh1型免疫反応である．Th1細胞によってIFN-γが産生され，マクロファージを活性化させると同時に，補体結合性抗体の産生とCD8陽性細胞の活性化をもたらす．ツベルクリン反応はこのタイプの反応である．IVb型はTh2型免疫反応で，Th2細胞から産生されるIL-4，IL-5，IL-13によってB細胞活性化によるIgEとIgG4産生，肥満細胞や好酸球の活性化が起こる．薬疹の多くは血中および組織中の好酸球増加を伴い，血中や浸潤

表2　Pichlerの提唱する新Gell-Coombs分類（文献4より引用改変）

タイプ	免疫反応	病態生理	臨床所見	典型的な時間経過
I	IgE	肥満細胞と好塩基球の脱顆粒	アナフィラキシー，血管浮腫，蕁麻疹，気道攣縮	薬剤投与から1～6時間
II	IgGと補体	IgGと補体依存性の細胞傷害	血球減少	薬剤投与から5～15日
III	IgG/IgMと補体，Fc受容体	免疫複合体の沈着	血清病，蕁麻疹，血管炎	7～8日後：血清病など 7～21日後：血管炎
IVa	Th1細胞	単球の関与する炎症	湿疹性病変，肉芽腫	1～21日後
IVb	Th2細胞	好酸球性炎症	MPE/DIHS/DRESS	1～数日後：MPE 1～6週後：DIHS/DRESS
IVc	細胞障害性T細胞	CD4/CD8細胞による表皮細胞壊死	FDE/MPE/SJS/TEN/膿疱型薬疹	1～2日後：FDE 2～28日後：SJS/TEN
IVd	T細胞（IL-8産生）	好中球性炎症	AGEP	1～2日後が多い（長い場合もある）

リンパ球からは高濃度のIL-5が検出されることから，このタイプに属する反応を伴うものが多い．寄生虫感染やアトピー性皮膚炎などもこのタイプである．IVc型は，細胞傷害性T細胞の直接的な組織傷害による反応である．グランザイムB，パーフォリン，Fas-FasLなどの細胞傷害分子を介して表皮細胞や肝細胞を直接攻撃する[15]．Stevens-Johnson症候群や中毒性表皮壊死症の反応の多くはこのタイプに基づいていると考えられている．IVd型は，抗原によって活性化したIL-8（CXCL-8）産生細胞，おそらく多くはTh17細胞によってもたらされる無菌性の好中球性炎症である．急性汎発性発疹性膿疱症（acute generalized exanthematous pustulosis：AGEP）はこのタイプに属すると考えられる．このように，活性化T細胞のサブセットの違いが炎症の質を決定していると考えられるが，その因子が何であるかは依然不明である．

① 播種状紅斑丘疹型薬疹

最も遭遇することが多い臨床型であるが，活性化するT細胞の特性についての検討は少なく，炎症の主軸はTh1細胞であるという報告とTh2細胞である報告とがあって，一定していない[16,17]．おそらく，MPEはある程度広いスペクトラムを持つ軽症の薬疹を含んでいるため，解析する症例によって異なるのだろうと想像される．われわれの検討では，同一の薬物に起因した薬疹でも細胞障害性CD8陽性細胞の浸潤の程度がMPEかSJS/TENかを規定していた[7]．Th1細胞とそれに拮抗するTh2細胞の浸潤のバランスによって，IVa型またはIVb型の範疇に属する反応であると考えられる．

② 固定薬疹

T細胞が抗原感作を受けた場合，セントラルメモリー細胞（T_{CM}）としてリンパ節や末梢に循環するもののほかに，レジデントメモリー細胞（T_{RM}）として組織に固着して，長期間免疫維持のために機能するものがあることが明らかになり，これが連続的に供給されていることが判明した．さらに，この細胞が種々の疾患に関与していることが明らかになっている．固定薬疹は，長期間皮膚に存在するCD8陽性T_{RM}が薬物投与によってすみやかに活性化し，IFN-γなどの炎症性サイトカインを産生すること

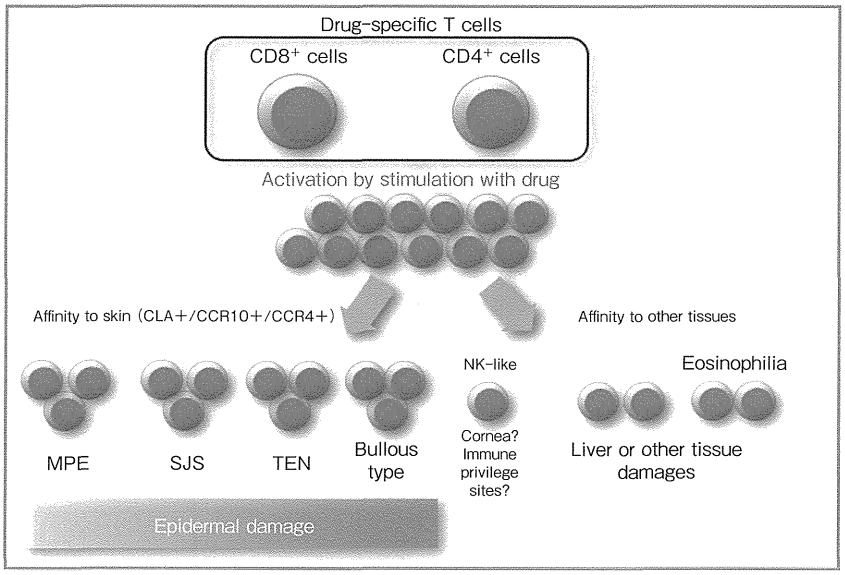

図6　CD4陽性およびCD8陽性薬物反応性T細胞（drug-specific T cell）と皮疹との関連
CD8陽性細胞の皮膚浸潤の程度により，表皮細胞の障害が強くなる傾向がある

が原因であることが知られている[18]．

③ SJS/TEN型薬疹

活性化するT細胞は細胞障害性CD8陽性細胞であり，IVc型に属する反応が主体となり，これが細胞障害性分子を介して表皮細胞を殺傷する（**図6**）．しかし，実際には病変部や末梢血中には活性化したCD4陽性細胞も存在し，IL-5を高濃度で産生するものも少なくない．末梢循環IL-17産生細胞をみると，MPE型に比べ多く，本細胞の病変への関与が示唆されているが[19]，われわれは，病変浸潤細胞にはTh17細胞が多く含まれることを見い出している[20]．IL-17は，Th1型炎症を促進させて炎症反応を遷延させるのと同時に，組織修復や細菌感染にも重要な役割を演じていることから，本症の病態形成に重要な役割を演じている可能性がある．また，制御性T細胞（Treg）の減少も指摘されており[21]，本疾患の炎症の遷延は，Treg機能不全による可能性もある．

表皮細胞を障害する分子に関しては，前述したグラニュライシンB，パーフォリン，Fas-FasLなどが有名である（**図7**）[15]．T細胞から産生される高濃度TNF-α，IFN-γによってinducible nitric oxide synthetaseを介して表皮細胞のFasL発現を促し，Fas-FasLメカニズムによる表皮壊死が起こる．また，グラニュライシンは，直接，表皮障害をもたらすほか[22]に，alarminsの一つとして，炎症細胞の遊走に関与することが判明しており，本疾患の炎症に大きな影響を与えていると考えられる．われわれは，最近，細胞障害性CD8陽性細胞のほかに，浸潤するCD4陽性細胞からもこの分子が産生されることを見い出している．また，新規の表皮傷害性因子として，単球などから産生されるアネキシン-1がその候補として挙がっている[23]．また，内在性アポトーシス分子であるB-cell lymphoma/leukemia-2-like protein 10を抑制するmiroRNAが，TENなどで高発現していることも判明している[24]．表皮障害因子と同様に内在する分子制御に関してからのアプ

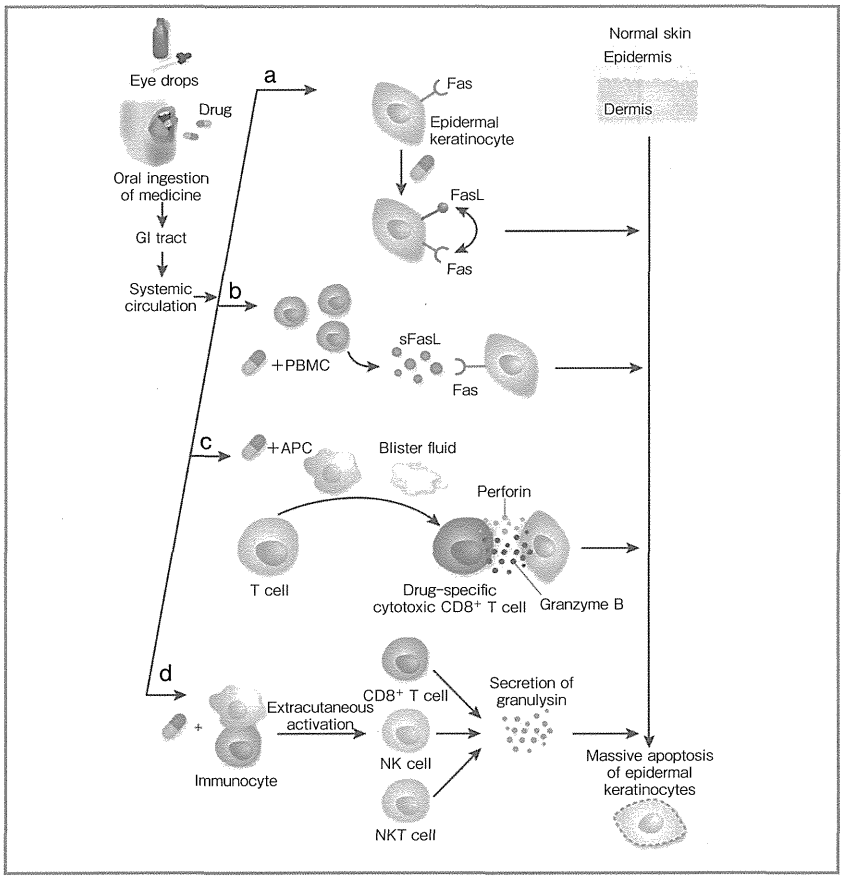

図7　SJS/TEN の発症にかかわる種々の細胞障害性因子（文献 15 より引用改変）

ローチは，今後の本症のメカニズム解明に寄与するであろう．

④ DIHS

DIHS は，重症薬疹の中でも謎が多い．本症の経過は極めてユニークである．抗痙攣薬やアロプリノールなどの特定の薬物投与によって，通常より長期間（数週から数カ月）を経てから発症し，多くは紅皮症様の皮疹，発熱，好酸球増多や異型リンパ球出現を伴う白血球増多と肝機能障害，リンパ節腫脹を伴う．経過中，およそ発症から 3 週間後くらいからヒトヘルペスウイルス（HHV）-6 やサイトメガロウィルス，EB ウィルスなどの内在性潜伏ウィルスの再活性化が生じ，それによって様々な臓器障害が起こる[25]．皮疹には CD4 陽性および CD8 陽性リンパ球がともに浸潤するが，両者は経時的にダイナミックに変化すると考えられる[25]．Treg の優位な皮膚浸潤によって免疫抑制状態が誘導され[26,27]，HHV-6 をはじめとするヘルペスウィルス群の活性化に関与している可能性がある．われわれは，病初期の末梢血中に増加する未熟な単球に内在する HHV-6 の存在を確認した[28]．この細胞は，障害皮膚から分泌される alarmins，HMGB-1 によって骨髄から皮膚へ遊走されて，皮膚に浸潤する CD4 陽性細胞に HHV-6 感染をもたらすと推測される[29]．また，本疾患

ではケモカインの一つであるTARCが著増しており，これがHHV-6再活性化と関連することから[30]，この分子も内在する単球のリクルートに関与しているのかもしれない．解決されるべき謎はまだまだある．

おわりに

薬疹発症に関するHLAとの強い関連の発見と，コンピュータ解析による分子構造解析から，この10年で薬疹のメカニズム解明に関する研究は飛躍的に進展した．これは，台湾によるカルバマゼピン薬疹患者の発生率抑制の成功をはじめとするオーダーメード医療につながり，文字どおり“bench to bedside”を実現する成果を生み出している．しかしながら，まだ，研究は一部の薬物に関するものだけであり，われわれがやるべきことはまだ多い．医原性の中で最も多い薬疹を撲滅することは，患者や医師にとっても，また，医療経済学的にも重要な課題である．今後の更なる躍進を願ってやまない．

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Reciprocal contribution of Th17 and regulatory T cells in severe drug allergy

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Toxic epidermal necrolysis
Th17
Treg
Drug-induced hypersensitivity syndrome

Letter to the Editor

Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) are pathogenetically classified into the same entity characterized by epidermal necrosis with diversity of the affected area. Drug-induced hypersensitivity syndrome (DIHS), also called as drug rash with eosinophilia and systemic symptoms, is clinically distinct from SJS/TEN by a delayed onset after taking the inducing drug, severe cutaneous and extracutaneous organ involvement and

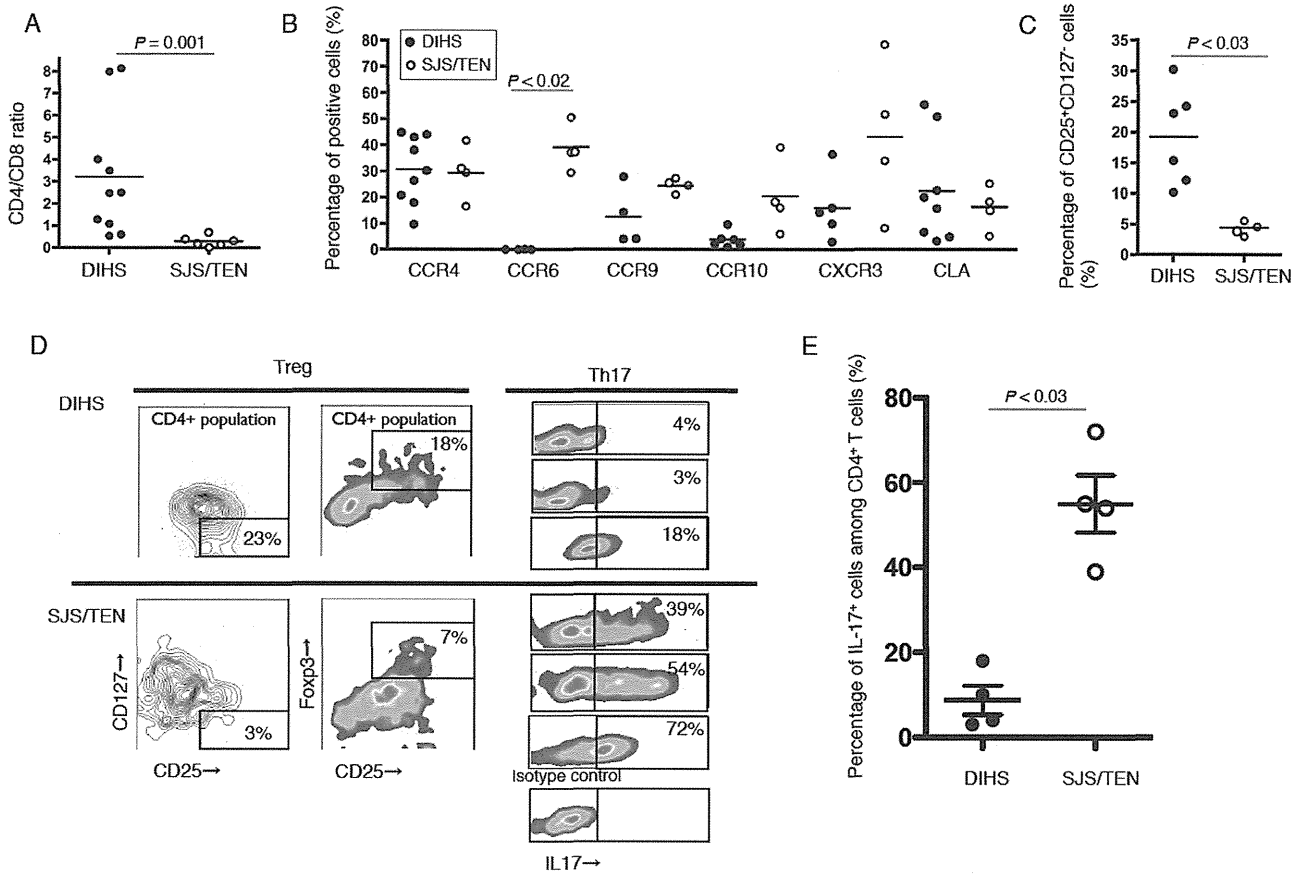

Fig. 1. Different characteristics of $CD4^+$ T cells from skin lesions between SJS/TEN and DIHS. (A) CD4/CD8 ratio. (B) Chemokine receptor expressions. (C) Percentage of T cells indicating Treg phenotype ($CD25^+CD127^-$) in $CD4^+$ cells. (D) Foxp3 and IL-17 expression. IL-17 expression was measured after stimulation with phorbol myristate acetate (10 ng/ml) for 3 h. Numbers indicate percentage of gated cells among the $CD4^+$ cells. Representative data of $CD4^+$ $CD25^+$ CD127- cells/$CD4^+$ $CD25^+$ $Foxp3^+$ cells from a DIHS and a SJS patients (left), and IL-17 expression of 3 DIHS patients (Pt#1-3) and 3 SJS/TEN patients (Pt#4-6) (right). (E) Percentage of T cells expressing IL-17 in $CD4^+$ cells.

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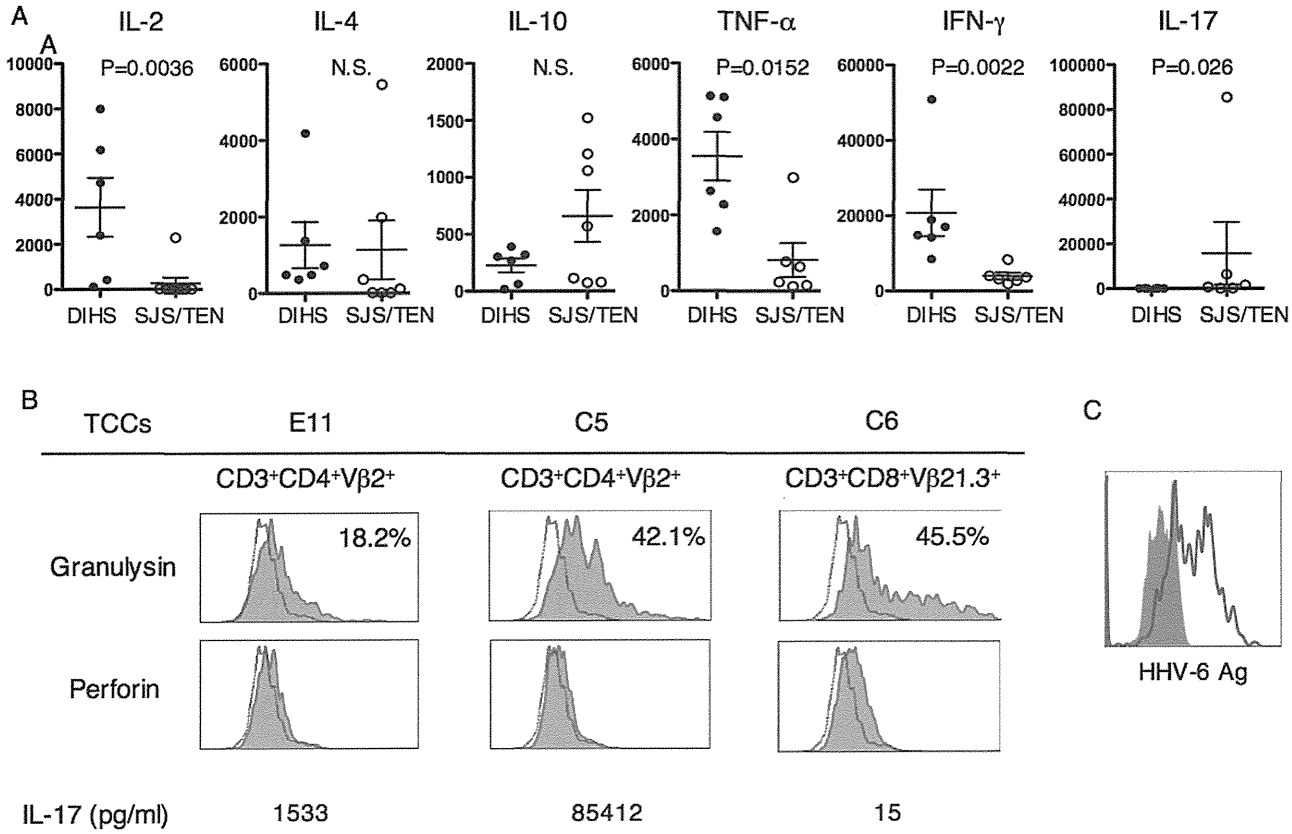

Fig. 2. Different characteristics of drug-specific TCCs from skin lesions/blood between SJS/TEN and DIHS. Drug specificity of TCCs was confirmed as previously described (Hashizume et al., J. Immunol. 2002; Hashizume et al., J. Imunnol. 2005). (A) Cytokine production (pg/ml) between SJS/TEN and DIHS. Vertical bars – mean value; horizontal bars – standard deviation. (B) Granulysin and perforin expression of 2CD4$^+$ TCCs (E11, C5) and a CD8$^+$ TCC (C6) from the skin lesions of SJS. Blue dots—isotype controls. C: HHV-6 antigen expression of a TCC from the skin lesion of DIHS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the reactivation of human herpesviruses during disease course. Although cytotoxic CD8$^+$ T cells play a crucial role in the pathogenesis of both disease spectrums, the roles of CD4$^+$ T cells that collocate with CD8$^+$ T cells in skin lesions remain poorly understood. To clarify this issue, we immunologically investigated pathogenetic CD4$^+$ T cells from the skin lesions of these diseases and delineated their characteristics.

All patients (SJS/TEN, $n=6$; DIHS, $n=10$, supplementary Table E1) enrolled in this study were informed and agreed to participate. The skin-infiltrating T cell analysis was approved by the ethical committee of Hamamatsu Univirsity School of Medicine. We first expanded infiltrating (for SJS and DIHS) and blister-containing (for TEN) T cells from skin lesions using our previously established method [1] and generated drug-specific CD4$^+$ T cell clones (TCCs) from these cells. After stimulation with phorbol 12-myristate 13-acetate and ionomycin an intracellular interleukin (IL)-17 was stained with fluorescent-tagged antibody (R&D Systems, Minneapolis, MN, USA) in skin-infiltrating cells, and after stimulation with immobilized anti-CD3 mAb for 72 h, concentrations of IL-2, IL-4, IL5, IL-6, IL-8, IL-10, IL-17, interferon (IFN)-γ, and tissue necrosis factor (TNF)-α were measured in culture supernatants of CD4$^+$ TCCs by cytometric bead array assay (Th1/Th2/Th17 cytokine CBA and inflammation cytokine CBA kits; BD Biosciences).

The ratio of CD4$^+$ T cells/CD8$^+$ T cells in SJS/TEN skin lesions ($n=6$) was significantly lower than in DIHS/DRESS skin lesions ($n=10$) (Fig. 1A, $P<0.001$, Mann–Whitney test). The percentage of CCR6$^+$ cells among total CD4$^+$ T cells was significantly greater in SJS/TEN ($n=4$) than DIHS ($n=4$) (Fig. 1B, $P<0.02$, Mann–Whitney test). Higher levels of IL-17 production were observed in CD4$^+$ T cells from SJS/TEN skin lesions ($n=4$) than in DIHS skin lesions ($n=4$) after phorbol myristate acetate stimulation (Fig. 1D, right and Fig. 1E). Consistent with this, immunofluorescence analysis revealed that IL-17$^+$ CD4$^+$ cells infiltrated in the SJS/TEN skin lesions but not in the DIHS skin lesions (Supplementary Fig. E1). On the other hand, the percentage of CD4$^+$CD25$^+$CD127$^-$ cells, likely Treg cells, increased in T cells from DIHS skin lesions compared with those from SJS/TEN (Fig. 1C, $P<0.03$, Mann–Whitney test, and Fig. 1D, left in Treg). We also confirm an increase of Treg cells by comparison of CD4$^+$CD25$^+$Foxp3$^+$ cells in expanded T cells between DIHS and SJS/TEN skin lesions after a more 4 days' culture with low dosage of IL-2 in several cases (Fig. 1D, right in Treg). We also found higher production of IL-17 in CD4$^+$ T cells expanded from SJS/TEN lesions than those from DIHS/DRESS lesions (Fig. 1D). These observations suggest that Th17 and Treg cells dominated in skin lesions of SJS/TEN and DIHS, respectively, although we could not convince that these cells were pathogenetic. To confirm these findings, we further investigated cytokine production of drug-reactive CD4$^+$ T cells; generated 15 drug-reactive CD4$^+$ TCCs from skin lesions including acetaminophen-induced TEN (4 clones), ibuprofen- and phenobarbital-induced SJS (3 clones and 2 clones), and carbamazepine-induced hypersensitivity syndrome (DIHS) (6 clones). TCCs of SJS/TEN released significantly higher IL-17 (mean ± SD, 15,680 pg/ml ± 13,990 pg/ml; $p=0.026$, Mann–Whitney test) compared with DIHS, in which TCCs produced marginal levels (58.7 pg/ml ± 21.2 pg/ml) (Fig. 2A). Furthermore, IL-17 concentration had tendency to be greater in a severer clinical type, TEN than SJS. High amounts of IFN-γ and TNF-α were detected in SJS/TEN and DIHS groups, however, production levels were significantly higher in the latter than the former ($p=0.008$,

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Mann–Whitney test). Interestingly, we found that two drug-reactive IL-17$^+$ CD4$^+$ TCCs, E11 and C5 from SJS skin lesions had significant expression of granulysin as well as a drug-reactive IL-17$^-$CD8$^+$ TCC, C6 (Fig. 2B). Furthermore, we established one TCC that expressed the molecules specific to Tregs and showed an inhibitory effect on autologous lymphocyte proliferation form DIHS skin lesions (supplementary Fig. E2) despite hyporesponsiveness of Tregs to IL-2. Surprisingly, this clone expressed the human herpes virus (HHV)-6 antigen (Fig. 2C).

Th17/Treg cells are reciprocally generated from naïve CD4$^+$ T cells depending on their surrounding cytokine milieu. IL-17-producing CD4$^+$ T cells, designated Th17, express CCR6 and high levels of transcription factor RORγt, and contribute to defense against microorganisms and enhance inflammation and regeneration. On the other hand, Treg cells are CD4$^+$CD25^{high}CD127$^-$ and the forkhead family transcription factor Foxp3$^+$ and have regulatory function against inflammatory responses.

Our observation suggests involvement of Th17 cells in the pathogenesis of SJS/TEN as reported previously [2,3] although their role remains unclear. Moreover, we firstly found that granulysin-expressing drug-reactive Th17 cells infiltrated in the skin lesions, suggesting that they originally infiltrate the skin in response to drug. Granulysin is a member of the saposin-like protein family that forms cytotoxic molecules with proinflammatory activity for the induction of keratinocyte apoptosis in SJS/TEN [4] and the activation of monocytes and dendritic cells by binding to Toll-like receptor-4/Myd88 as an alarmin [5]. On the other hand, the pathogenesis of DIHS/DRESS is complicated: CD4$^+$ T cells that respond to drug antigen initially infiltrate the skin following emergence of CD8$^+$ T cells that target virus-infected cells [6]. Emergence of great numbers of functional Treg cells in skin and blood in DIHS/DRESS [7] would modify antivirus immune response, resulting in the replication of HHV-6 [8], contrasting numerical and functional loss of Treg cells in SJS/TEN [7,9]. Furthermore, HHV-6 can infect Tregs resided in the skin and may play a special role in the pathogenesis [10]. Our observations demonstrate that the Th17/Treg axis contributes to the pathogenesis of SCARs, and may provide a perspective on therapeutic options in severe cutaneous adverse reactions by targeting the Th17/Treg axis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2015.11.002.

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REVIEW ARTICLE

Immunological response in Stevens–Johnson syndrome and toxic epidermal necrolysis

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ABSTRACT

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening cutaneous adverse drug reactions that induce widespread epidermal necrosis. Recent advances in pharmacogenomic studies have provided evidence of genetic predispositions to SJS/TEN. Several concepts have been proposed to explain the pathogenesis of severe cutaneous adverse drug reactions. In the hapten concept, small molecules called haptens elicit an immune response only when attached to proteins. The "p-i" concept postulates that the causative drugs can stimulate cells by binding directly and reversibly to immune receptors. In addition, there is the idea that drugs alter the antigen by binding to the human leukocyte antigen pocket. With regard to keratinocyte death, several cell death mediators, such as FasL, granulysin and annexin A1, have been proposed as playing a role in SJS/TEN pathogenesis. A subset of T lymphocytes, including regulatory T cells, also may play a role in SJS/TEN.

Key words: drug, hapten, p-i concept, Stevens–Johnson syndrome, toxic epidermal necrolysis.

INTRODUCTION

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare, life-threatening, mucocutaneous reactions characterized by extensive detachment of the epidermis.[1] They exist at different points on the same spectrum, with SJS having skin involvement of less than 10% and TEN having body surface area detachment of more than 30%.[2] The incidences of SJS and TEN are rare (TEN, 0.4–1.2 cases per million).[3] The eruptions can spread rapidly to the whole body within a day. Mucous membrane involvement is observed in approximately 90% of cases. Approximately 85% of patients have conjunctival lesions including chronic conjunctivitis, conjunctival scarring, corneal vascularization and corneal damage, which can lead to blindness.[4] Although high-dose corticosteroids, i.v. immunoglobulin and plasmapheresis have been attempted for treatment of SJS/TEN, the mortality rate is still high (TEN, 25%).[3]

Genetic hypersensitivity or a type IV allergic reaction has been suggested. In addition, several mediators have been proposed, such as Fas/Fas ligand (FasL),[5] soluble FasL (sFasL),[6] perforin, granzyme B[7] and granulysin.[8,9]

The binding of human leukocyte antigen (HLA) with T-cell receptor (TCR), cytotoxic molecules and subsets of immunocytes is involved in SJS/TEN. Briefly, SJS/TEN is initiated by covalent binding of a drug to a cellular peptide (hapten), by non-covalent, direct interaction of a drug with a specific major histocompatibility complex (MHC) I allotype (p-i concept), or by the action of a self-peptide on the drug-incorporated MHC I altered-self repertoire, forming an immunogenic molecule. Drug-specific lymphocytes, activated by antigen-presenting cells (APC) expressing specific MHC I and the antigen, release perforin/granzyme B, sFasL and granulysin. These cytotoxic molecules diffuse through the epidermis, acting as major inducers of cell death. Regulatory T cells (Treg) influence the intensity of the immune reaction.

This paper focuses on immunological phenomena, including antigen (causative drug) presentation and cytotoxic signaling, as well as focusing on immune molecule and T-lymphocyte subtypes.

INTERACTION OF HLA, DRUG ANTIGENS AND T-CELL RECEPTORS

Human leukocyte antigen alleles are divided into classes I and II, and they are involved in antigen presentation to T cells and subsequent activation of the immune response. It has been proposed that the drug/metabolite is presented by HLA molecules with HLA restriction predisposed to an inappropriate immune response and subsequent adverse drug reaction.[10,11] Recent advances in pharmacogenomic studies have provided evidence for genetic predispositions to SJS/TEN. Evidence for a pathomechanism in which there is HLA-restricted presentation of a drug or its metabolites for T-cell activation comes from the findings of strong genetic associations with HLA alleles (e.g. HLA-B*15:02 and carbamazepine [CBZ] SJS/TEN,[12] and HLA-B*58:01 and allopurinol SJS/TEN[13]). Furthermore, the strong genetic association between HLA and

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specific-drug-induced SJS/TEN[12] makes screening tests prior to drug intake practicable in preventing SJS/TEN.[14] Such pharmacogenetic risks vary among ethnicities. For instance, the risk of HLA-B*15:02 is much higher in Han Chinese than in Caucasians and Japanese (Table 1).

Most HLA alleles associated with SJS/TEN are class I. In contrast, several HLA class II alleles have been reported to be associated with drug-induced liver injury.[11] It is also suggested that $CD8^+$ cytotoxic T cells activated by antigen-presenting HLA class I are fundamental to keratinocyte death, a phenomenon that is characteristic of SJS/TEN.

THE HAPTEN/PRO-HAPTEN CONCEPT

Common skin reaction-inducing drugs tend to be small molecules; thus, they are not antigenic on their own. Instead, it is thought that their immunogenicity may result from their binding to proteins.[15] Stable covalent binding by a chemically reactive drug to a protein allows the formation of a neoantigen that can be recognized by T cells after antigen processing of the hapten–carrier complex (Fig. 1). The chemical properties of hapten-like drugs are crucial for the generation of antigenic epitopes and the activation of the innate immune system. Haptens have been shown to bind to particular amino acids, for example, penicillin has a tendency to bind to lysine residues.[16] Drug hypersensitivity to a hapten–peptide complex is less likely to be HLA-restricted, as multiple binding sites in a protein suggest that, after processing, a number of potential drug-bound peptides are available for loading onto different types of HLA alleles. Indeed, there are no proven examples of hapten-restricted immune responses that are strictly associated with HLA alleles. The pro-hapten concept proposes that a chemically inert drug may become reactive after undergoing metabolism, when it is then able to form a hapten and stimulate an immune response.[17] A classic example of this is sulfamethoxazole, which forms a hapten after being metabolized to a reactive compound. It is important to note that neither the hapten nor the pro-hapten necessarily needs to undergo processing to become antigenic. This was illustrated by Horton *et al.*,[18] who showed that amoxicillin, which forms a hapten by means of a covalent bond, is able to stimulate T cells in a processing-independent manner by binding directly to peptide–MHC complexes.

Table 1. Presence of HLA-B*15:02 in patients with CBZ-induced SJS/TEN (%)

	Allele frequency of HLA-B*15:02 (%)	Presence of HLA-B*15:02 in patients with CBZ-induced SJS/TEN (%)	References
Taiwan	8	93.1	50
Japan	0.1	0	69
Thailand	6.1	88.1	70
India	6	75	71
Korea	0.4	14.2	72
Europe	0.4	0	73

CBZ, carbamazepine; HLA, human leukocyte antigen; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

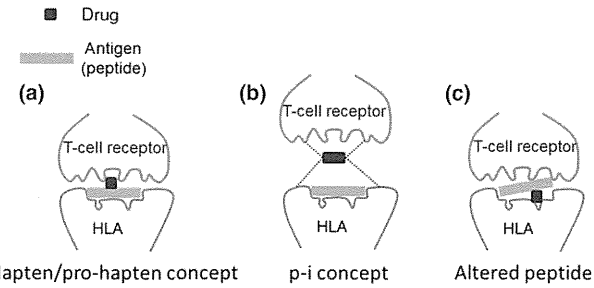

Figure 1. (a) The hapten-modified peptide is recognized, and it stimulates T cells. The hapten also may have the ability to activate the innate immune system. (b) The drug binds to the T-cell receptor and provides some initial signal. That signal is strengthened by the additional interaction with human leukocyte antigen (HLA) molecules. Drug binding and HLA interactions stimulate T cells in the same way as a normal peptide/HLA complex. (c) The drug can bind directly to the pocket of a specific HLA while not binding to a closely related HLA molecule. HLA and the drug form a complex before the HLA molecules are loaded with peptides inside the cell.

THE p-i CONCEPT

As an alternative to the hapten and pro-hapten concepts, the p-i concept proposes that a drug is able to stimulate T cells directly without forming a hapten, in an HLA-dependent manner.[19] This may occur if a drug that cannot form a covalent bond with a larger carrier interacts directly with TCR or MHC molecules with sufficient affinity.

According to the hapten and pro-hapten concepts, drugs and other substances that are not chemically active and that are therefore incapable of coupling to a protein would not be antigens and could not induce hypersensitivity reactions. However, this hypothesis has been challenged by clinical and immunological evidence that cannot be explained by hapten or pro-hapten models.[15]

The p-i concept of "pharmacological interactions of drugs with immune receptors" represents a non-conventional presentation pathway that contradicts the initial thinking that the immune stimulatory capacity of most chemicals and drugs can be predicted by their protein reactivity.[20,21] Consistent with the p-i concept, chemically inert drugs, which are unable to bind covalently to peptides or proteins, can nevertheless activate certain T cells, if they fit with sufficient affinity into some of the various TCR or MHC molecules (Fig. 1). Evidence for the p-i mechanism lies in observations in which even fixed APC, which are unable to process antigens, are still able to activate specific T-cell clones.[20] Such immune processes are associated with high cytokine levels and increased expression of MHC and co-stimulatory molecules. Consequently, T cells more readily react to a minor signal, such as the binding of a drug to its TCR. This would explain the several specific drugs, such as

CBZ, that mainly cause SJS/TEN. On the other hand, in some cases, metabolites simultaneously react as well as the parent compound. This suggests that the hapten characteristic of a drug is required for p-i stimulation to occur.

ALTERED-SELF REPERTOIRE

In general, peptides associate with HLA molecules by inserting parts of their amino-acid residues into a set of six binding pockets in the HLA.[22] The structure of these pockets is highly allele-specific, thereby dictating peptide-binding preferences for each HLA molecule.

A recent paper showed that a drug can bind directly to the pocket of a specific HLA while not binding to a closely related HLA molecule.[23] These data suggest that HLA and the drug form a complex before the HLA molecules are loaded with peptides inside the cell, thereby altering the pool of self-peptides that are bound to the HLA and are displayed on the cell surface for T-cell recognition. This shift in the specific HLA-associated cell-surface-peptide display leads to the activation of different T cells. Indeed, the activation of a wide range of $CD8^+$ T cells occurs as the cellular basis of abacavir hypersensitivity reactions.[24]

Part of the drug protrudes into the HLA molecule's pocket, reducing that pocket's size, which accounts for its preferential binding of smaller amino acids following drug exposure. From the HLA structure, the drug was also shown to bind to amino-acid residues that are unique to the HLA molecule, which would explain the drug's allele specificity. This shift in the bound-peptide repertoire is a plausible explanation for drug-induced hypersensitivity.[25] T cells lack self-reactivity but have the potential to recognize foreign antigens.

Some self-peptides are never encountered during T-cell development, but exposure can occur under pathological conditions.[24] When this occurs, a case of "mistaken identity" can arise, in which self-peptides are perceived as foreign by the immune system.

Complexes that consist of immunogenic HLA, the drug and a peptide may be generated via incorporation of the drug with peptides of the constitutive repertoire, or this may occur within the endoplasmic reticulum, such that the peptides are presented with an altered conformation, or the stabilization of "novel self" ligands may be absent from the constitutive repertoire but favored in the presence of the drug.

T-CELL RECEPTOR CLONOTYPES

Despite the strong HLA predisposition to drug hypersensitivities, it remains unknown whether particular TCR participate in the recognition of small drug/peptide-HLA complexes. Chung *et al.*[26] globally investigated the expression levels and third complementarity-determining region length distribution of the TCR profile. The specific clonotype (VB-11-ISGSY) was identified as the predominant clonotype in CBZ-induced SJS/TEN, and it was found to be shared among different subjects. This clonotype was present in most patients with SJS/TEN but absent in all other patients. CBZ-specific cytotoxicity could be primed *in vitro* in the peripheral blood mononuclear cells (PBMC) of healthy subjects who are carriers of the susceptible HLA allele (HLA-B*1502) and VB-11-ISGSY. This study suggests a key role for TCR in the pathogenic mechanism of SJS/TEN.

CYTOTOXIC SIGNALS AND IMMUNE MOLECULES IN SJS/TEN

The phenomenon of antigen (causative drug) presentation seems to be shared by SJS/TEN and non-severe adverse drug reactions. Indeed, several HLA haplotypes that have been reported to be associated with adverse drug reactions do correlate with severity.[27] With regard to histological findings, an observation of keratinocyte death differentiates SJS/TEN from non-severe adverse drug reactions. Therefore, keratinocyte death may be implicated in the SJS/TEN pathomechanism. Several cytotoxic signals and immune molecules have been reported to contribute to keratinocyte death in SJS/TEN. In particular, granulysin is strongly expressed by blister cells in skin lesions and plays a crucial role in the widespread keratinocyte apoptosis of SJS/TEN.

Fas-FasL INTERACTION

Fas is a cell surface receptor that is involved in apoptotic signaling and that interacts with its ligand FasL to initiate the death signal cascade that leads to apoptotic cell death.[28,29]

In 1998, Viard *et al.*[5] reported that the activation of the Fas apoptosis receptor through the FasL is an important initial step in keratinocyte apoptosis in TEN. They assumed that both Fas and FasL derive from keratinocytes and that FasL leads to the apoptosis of neighbor keratinocytes in TEN.[5] The current author previously showed the following three things: (i) the levels of sFasL in SJS/TEN patient serum are elevated; (ii) sFasL is secreted by causal-drug-stimulated PBMC; and (iii) TEN patient serum with high levels of sFasL induces apoptosis in cultured keratinocytes.[6]

Before disease onset (day −4 to day −2), seven samples were available, and we detected high concentrations of sFasL in five out of the seven cases (71.4%). The elevated sFasL level declined rapidly within 5 days after disease onset. In all 32 patients with non-severe adverse drug eruptions and in the 33 normal controls, no elevation of sFasL was detected. Other soluble factor concentrations showed no significant differences between SJS/TEN before disease onset and non-severe adverse drug reactions.[30] Lan[31] also reported a diagnostic role for sFasL secretion by PBMC from patients who had recovered from SJS/TEN. However, other groups reported that elevated levels of FasL were detected not only in TEN patients but also in the sera and lesional skin of patients with maculopapular adverse drug reactions.[32] A dispute remains as to whether lytically active forms of FasL are expressed by TEN keratinocytes. Viard-Leveugle *et al.*[33] reported that the FasL expression in keratinocytes is quite low; therefore, they concluded that keratinocyte FasL is not cytolytic under basal conditions. However, keratinocyte FasL has been reported to have lytic potential

upon appropriate stimulation.[34] In addition, Sayama *et al.* have shown that FasL signaling is functional in human keratinocytes.[35,36] Drug-induced hypersensitivity syndrome (DIHS), another severe adverse drug reaction, also shows high serum levels of sFasL.[37] The question remains as to whether the increased levels of sFasL in the sera of TEN patients derive from the shedding of membrane-bound FasL on keratinocytes or on PBMC.[5,6]

PERFORIN AND GRANZYME B

Perforin is a pore-forming protein that can assemble into barrel-shaped pores within the cell membrane.[38] Granzyme B belongs to a member of the serine proteinase family and proteolytically cleaves proteins after aspartate residues.[39]

Perforin/granzyme B have been reported to play a key role in keratinocyte death in SJS/TEN.[40] Nassif *et al.*[40] showed that the cytotoxic effect of TEN blister lymphocytes on keratinocytes could be attenuated by the inhibition of perforin/granzyme B expression, but not by the anti-Fas monoclonal antibody. The activated cytotoxic T lymphocytes (CTL) and natural killer (NK) cells produce perforin, which can bind and punch a channel to the target cell membrane and promote the entrance of granzyme B into keratinocytes. Once granzyme B enters the target cells, it activates the caspase cascade and the succeeding apoptosis.[41] Levels of perforin, granzyme B, tumor necrosis factor (TNF)-α and FasL have been observed to relate to the disease severity of drug hypersensitivity, from mild maculopapular rashes to severe TEN.[7]

GRANULYSIN

Granulysin is a cytotoxic molecule that is produced against virus-infected cells, tumor cells, transplant cells, bacteria, fungi and parasites.[42] It plays an important role in the host defense against pathogens. The 15-kDa granulysin, a cationic cytolytic protein, is secreted extracellularly by CTL and NK cells via a non-granule exocytotic pathway.[43] The expression level of granulysin rises upon T- and NK-cell activation. Granulysin has been reported as a serum marker for cell-mediated immunity. Chung *et al.*[8] reported that granulysin is strongly expressed by blister cells in skin lesions and plays a crucial role in the widespread keratinocyte apoptosis of SJS/TEN. Granulysin has a direct cytotoxic effect on keratinocytes at concentrations detected in blister fluids. The cytotoxic effect of SJS/TEN blister fluids on keratinocytes can be reduced by granulysin depletion. In addition, injections of granulysin into mouse skin were found to result in blistering and epidermal necrosis mimicking SJS/TEN.[8] They concluded that high levels of secretory granulysin in blistering skin lesions could explain the histopathology observed in SJS/TEN, in which the infiltration of sparse dermal mononuclear cells results in extensive epidermal necrosis. In addition, the serum levels of granulysin were found to increase during the early stage of SJS/TEN, but not in patients with drug-induced maculopapular exanthema (MPE),[9] suggesting granulysin as an early diagnostic marker of SJS/TEN. Indeed an immunochromatographic test for granulysin (with a procedure time of <15 min) showed positive results for four out of five patients with SJS/TEN but only one patient out of 24 with non-severe adverse drug reactions.[44] These results correlate closely with those of enzyme-linked immunoassay (ELISA). DIHS also shows high serum levels of granulysin.[45]

ANNEXIN A1/FORMYL PEPTIDE RECEPTOR 1

Annexin A1 has roles in many diverse cellular functions, such as membrane aggregation, inflammation, phagocytosis and proliferation.[46] Annexin A1 binds to formyl peptide receptor 1 (FPR1) and acts via that receptor.[47] FPR1 is in the family of G protein-coupled receptors and is associated with tissue damage.[48]

We recently showed that keratinocyte death in SJS/TEN can be triggered by the interaction of annexin A1 and FPR1 and may contribute to the pathogenesis of SJS/TEN.[49] When exposed to the drug, susceptible patients secrete the immune regulatory protein annexin A1 from immune cells, with deadly effect on keratinocytes, the main skin cell. Annexin acts on FPR1, located on the surface of the skin cells, to cause necroptosis, a programmed form of cell death. Inhibition of necroptosis completely prevented the occurrence of SJS/TEN-like responses in a mouse model of SJS/TEN. Annexin A1/FPR1 are candidate markers of disease occurrence and may be promising therapeutic targets. In addition, necroptosis is a potential drug target for SJS/TEN treatment.[49]

OTHER MOLECULES

Several reports have shown that several cytokines/chemokines are involved in the SJS/TEN pathomechanism, such as TNF-α, interleukin (IL)-2, IL-5, IL-6, IL-10[50] and CCL27.[51] The expression of these cytokines/chemokines is elevated in skin lesions and plasma blister fluids in SJS/TEN. These cytokines/chemokines may be responsible for the trafficking, proliferation, regulation or activation of T cells and other leukocytes involved in SJS/TEN. In addition, the α-defensin gene was recently found to be expressed in PBMC from patients with cutaneous adverse drug reactions.[52] Expression of α-defensin was confirmed by intracellular flow cytometry in mononuclear cells from the patients, including monocytes, NK cells and T cells from peripheral blood and blister fluid. Levels of α-defensin were estimated by ELISA to be higher in blister fluid when compared with simultaneously drawn plasma samples.

SUBTYPES OF T LYMPHOCYTES

$CD8^+$ lymphocytes are necessary to SJS/TEN pathogenesis. Reports have shown that $CD4^+$ T cells are the predominant population that infiltrates into "maculopapular rash" skin lesions, and reports have shown also that most drug-specific T cells are $CD4^+$ T cells.[53] However, in severe cutaneous adverse drug reactions, $CD8^+$ T cells were found to be the predominant population that infiltrated into the epidermis of skin lesions of SJS/TEN patients,[8] and HLA-B*1502 was found to be associated with CBZ-induced SJS in all cases.[12] In addition,