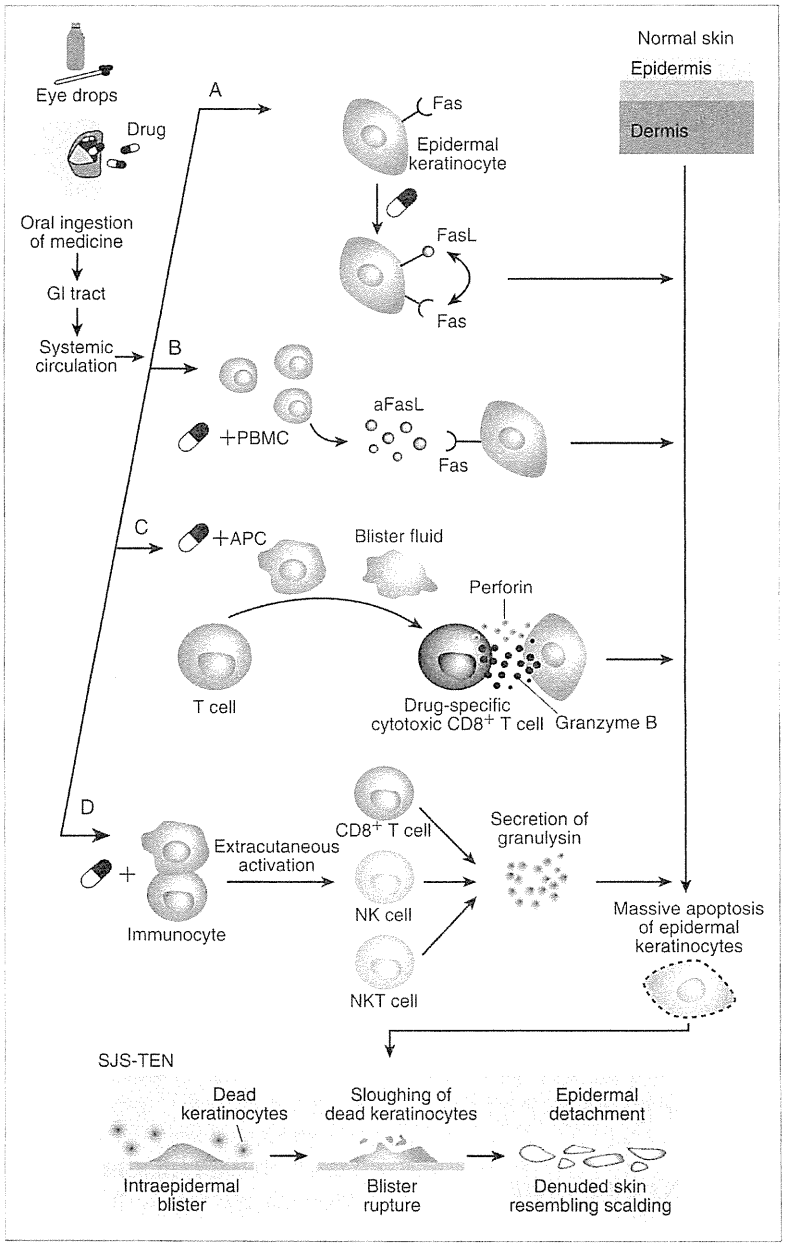

図4　SJS/TENにおける表皮細胞障害のメカニズム

薬剤は表皮細胞(A)や単核細胞(B)にFasLを産生させ，Fasが高発現する表皮細胞をアポトーシスに陥らせたり，$CD8^+$細胞傷害性細胞を活性化させ，パーフォリンやグランザイムを分泌させて表皮細胞を壊死においやる(C)．また，$CD8^-$細胞，NK細胞またはNKT細胞は，グラニュライシンを産生して表皮細胞を殺す(D)．（文献[26]より引用）

表1　DIHSの診断基準

1. Maculopapular rash developing ＞3 weeks after starting with a limited number of drugs
2. Prolonged clinical symptoms 2 weeks after discontinuation of the causative drug
3. Fever（＞38℃）
4. Liver abnormalities（alanine aminotransferase＞100 U/l）*
5. Leucocyte abnormalities（at least one present）
   a. Leucocytosis（$>11\times10^9$/l）
   b. Atypical lymphocytosis（＞5％）
   c. Eosinophilia（$>1.5\times10^9$/l）
6. Lymphademopathy
7. Human herpesvirus 6 reactivation

The diagnosis is confirmed by the presence of the seven criteria above（typical DIHS）or of the five（1～5）（atypical DIHS）. *This can be replaced by other organ involvement, such as renal involvement.　（文献[27]より引用）

の淡い紅斑，顔面浮腫，リンパ節腫脹が出現した後，皮疹はゆっくりと紅皮症化し色素沈着を伴いながら消退していく．そのころより異型リンパ球の出現を伴う白血球増多，肝機能障害を含む種々の臓器障害を生じる．本症の制御性T細胞（Treg）に焦点を当てた報告[18]によると，初期に機能亢進し，後期に機能不全に陥っていることが明らかとなっており，これがダイナミックに変化する臨床症状と関連する可能性がある．ヘルペスウイルスの再活性化の一つの可能性として，Tregの機能亢進以外にも，薬剤が直接潜伏感染している細胞を刺激し，ウイルスの増殖を起こすことがin vitroの実験で示されている[19]．われわれは最近，経過中に一過性に出現するmono/myeloid precursorにHHV-6が潜伏感染していることを見出し，これが皮膚に移行して浸潤しているCD4$^+$細胞にHHV-6を感染させることによって，ウイルス増殖を起こしている可能性を示した（図5）[20][21]．なぜmono/myeloid precursorが骨髄より末梢へ動員するのかは明らかではないが，われわれは，少なくとも最近注目されているalarmins, HMGB-1が一つの候補分子であると考えている[21]．

SJS/TENとDIHSの関係

SJS/TENの病態に最も重要なのは，CD8$^+$細胞，NK細胞などの細胞傷害性分子を含む細胞群であることに異論はないが，皮膚浸潤細胞には，この種の細胞以外にも無視できない数のCD4$^+$細胞が存在する．一方，DIHSの皮疹にも，CD8$^+$細胞とCD4$^+$細胞の両者が浸潤している．CD8$^+$細胞の主たる機能は，もっぱら標的細胞に対する直接または間接的な細胞障害である．一方，CD4$^+$細胞は，機能的にいくつかのサブタイプに分類されており，この違いがCD8$^+$細胞による炎症にディバーシティーを与えている可能性がある．

私たちは，SJS/TENおよびDIHSの皮疹浸潤細胞を採取し，CD4$^+$細胞に焦点を当てて，その産生サイトカインを調べてみた．すると，SJS/TENにおいてCD4$^+$細胞はCCR6を発現し，IL-17を産生するTh17が多く，DIHSにおいてはCD25$^+$CD127$^-$で，Foxp3を発現するTregが多いことが明らかとなった（図6：未発表データ）．今までわれわれは，SJS/TENやDIHSの患者末梢血から薬剤反応性T細胞クローンを樹立してきたが，このデータは，これらの細胞でもそれぞれIL-17およびIL-10産生細胞が多かったという結果と一致する[21]．Th17とTregは同じprecursorからTGF-β下で誘導され，それはIL-6の有無によって運命づけられている．すなわち，IL-6が豊富な環境ではTh17へ分化しやすくなり，IL-6が欠如した環境下ではTregが生成されやすい．感染症や急性炎症性疾患など急性炎症サイトカインであるIL-6が高値である環境下で抗生剤または消炎鎮痛剤によってSJS/TENが出現しやすいのは，Th17によってCD8$^+$細胞を中心とした炎症反応が促進されるからであると理解できる．SJS/TENにおいてTh17が関与する可能性は，乾癬治療の手法が応用できる可能性を持つ．台湾のグループは，抗TNF-α抗体療法を用いるとSJS/TENが急速に治癒に向かうことを経

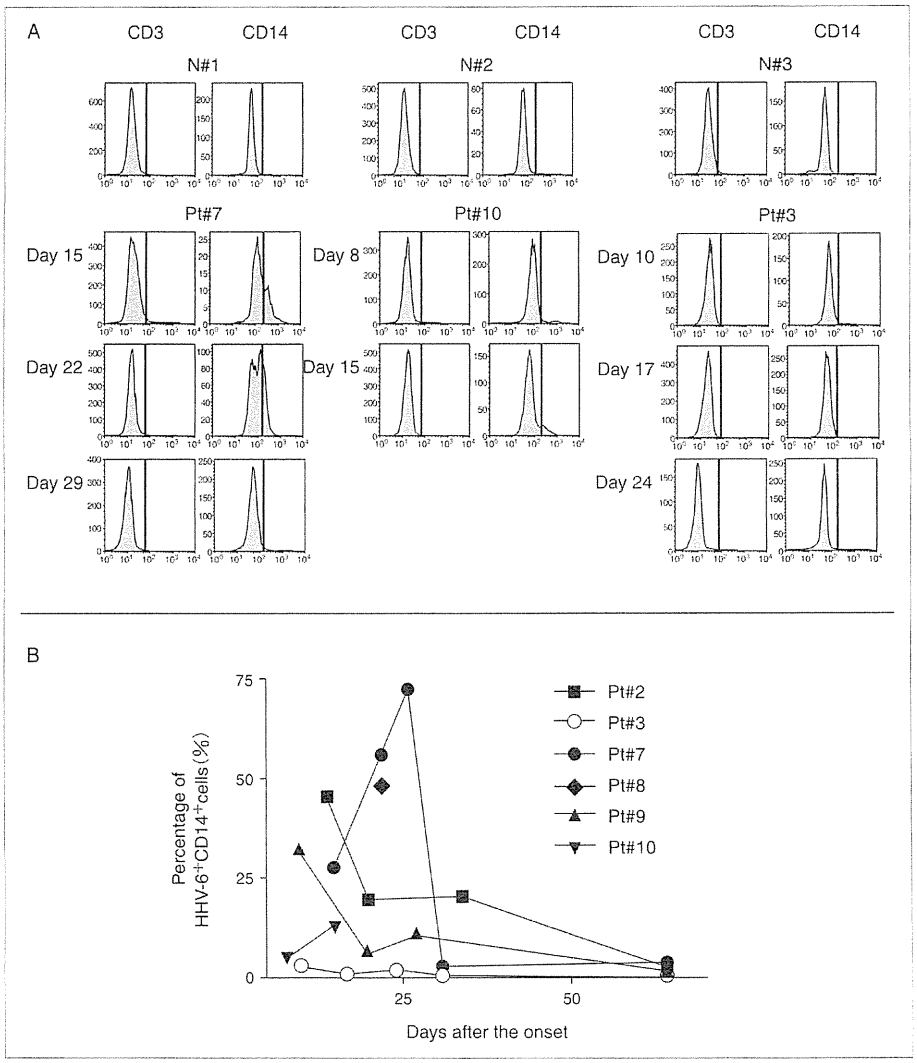

図5　DIHS発症経過中の患者(Pt)および健常人(N)の末梢$CD3^+$細胞および$CD14^+$細胞におけるHHV-6抗原の発現(A)と$CD14^+$細胞中のHHV-6抗原陽性細胞の割合の経時的変化(B)
Pt#3はHHV-6に未感染の患者.

験しているという(Cung WH, et al. personal communication). 一方, 精神神経疾患で抗けいれん剤によって突然発症するDIHSでは, 薬剤反応性の$CD8^+$細胞による炎症はなんらかの誘因によってTregの割合が多くなり, これによって抑制されているために, 皮疹発現までの期間が長いと想像できる.

最近, SJS/TENで発症し, 後にDIHSと診断された症例の報告がある. このような症例ではTh17とTregとのバランスの時間的変化によって説明可能なのかもしれない.

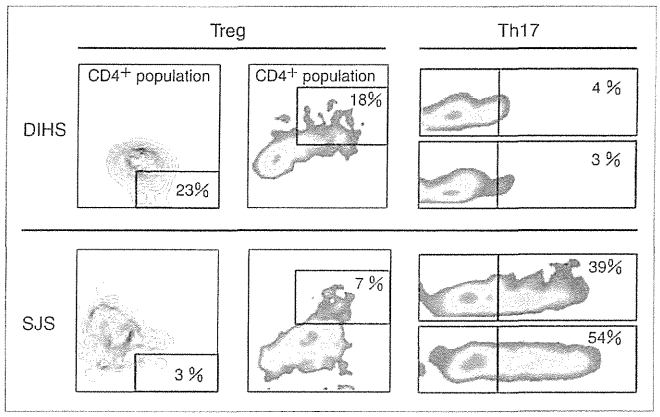

図6　DIHS（上段）およびSJS（下段）における皮疹部浸潤細胞のTregとTh17
皮疹部浸潤細胞を抗CD3/CD28抗体ビーズとIL-2で増幅してから，常法に従い発現分子（左）とIL-17産生（右）をフローサイトメーター解析した．Tregに関する発現分子はY軸，左：CD127，右：Foxp3でX軸は両者ともCD25．それぞれ一名の患者について示す．IL-17産生はPMA刺激後24時間後にintracytoplasmic cytokine stainingを行った．図はそれぞれ患者2名のデータを示す．

自然免疫系と薬疹

定常状態では細胞代謝にかかわるが，いったん細胞が障害されたときに細胞外へ放出されると，樹状細胞や単球に作用し，炎症を惹起し，組織修復反応を促進する分子があることが判明し，これをalarminsと呼んでいる．Alarminsはこれらの細胞膜表面に発現する自然免疫に重要なToll-like receptorやpattern recognition moleculesに結合し，単球や樹状細胞の活性化や遊走を促進して炎症反応を促進し，組織修復に関与している[22]．これらは膠原病や熱傷など感染が関与しない炎症反応に深くかかわっていることが明らかになっている．

最近，SJS/TENなどの薬疹の末梢血においてalarminsの分子群が高発現することが，DNAアレイを用いた網羅的解析によって明らかになった[23]．さらに，本疾患の血中HMGB-1濃度が高いことも確認されている[24]．われわれは，最近DIHSの皮疹や末梢血中に特にHMGB-1が高発現していることを見出し，これが骨髄からHHV-6を潜伏感染するmono/myeloid precursorsの末梢への放出を促している可能性を想定している．また，SJS/TENにおいて直接表皮細胞を障害する分子として注目されてきたグラニュライシンのうち15kDaアイソフォームは，TLR4に結合して，樹状細胞の活性化と遊走をもたらすalarminsであることが確認された[25]．

DIHS患者の多くは，すでに脳疾患や高尿酸血症，膠原病などを合併している患者が多く，障害細胞から放出されるalarmins値は健常人より高い可能性がある．すなわち，このような疾病を持つ患者は，骨髄からの樹状細胞や単球系前駆細胞が末梢へと動員されやすい状況がある．HHV-6を含む種々のヘルペスウイルスは，骨髄内の単球や樹状細胞をreservoirとしていることから，これらの患者の血中内には，HHV-6潜伏感染細胞が循環している可能性がある．HHV-6の爆発的な増殖には，$CD4^+$細胞への感染が必須であるから，この状況下ではHHV-6の再活性化は起こらない．しかしながら，薬疹などによって皮膚に対するT細胞の攻撃が始まると，皮膚由来のalarminsによってHHV-6感染細胞が皮膚内へ動員され，皮膚浸潤$CD4^+$細胞にウイルスを伝播させてしまうのだろうとわれわれは推測している．皮膚は再活性化をもたらすためのHHV-6増殖の場と考えられる．

将来への展望

病気を治療するための薬剤副作用によって，後遺症を起こしたり，命を脅かすことはあってはならない．商業化と結びつきやすい新規薬剤の開発において多額の研究費が投じられるのに対し，残念ながら薬剤の副作用に関する研究に対する助成は，なかなか得られにくいのが現状である．しかし，目の前には多くの薬疹患者が存在する．この現実を私たち医療者は直視し，薬疹を減らす努力をしなければならない．ゲノムワイド関連解析の進歩は，薬疹発症危険因子を明らかにし，薬疹を起こさないためのテーラーメイド薬物療法が現実となる日も近い．今後，薬疹の治療や予防，さらには薬疹を起こさない創薬に繋げるためにも，薬疹の発症機序解明の研究は進めなければならない現代医療の課題だともいえる．

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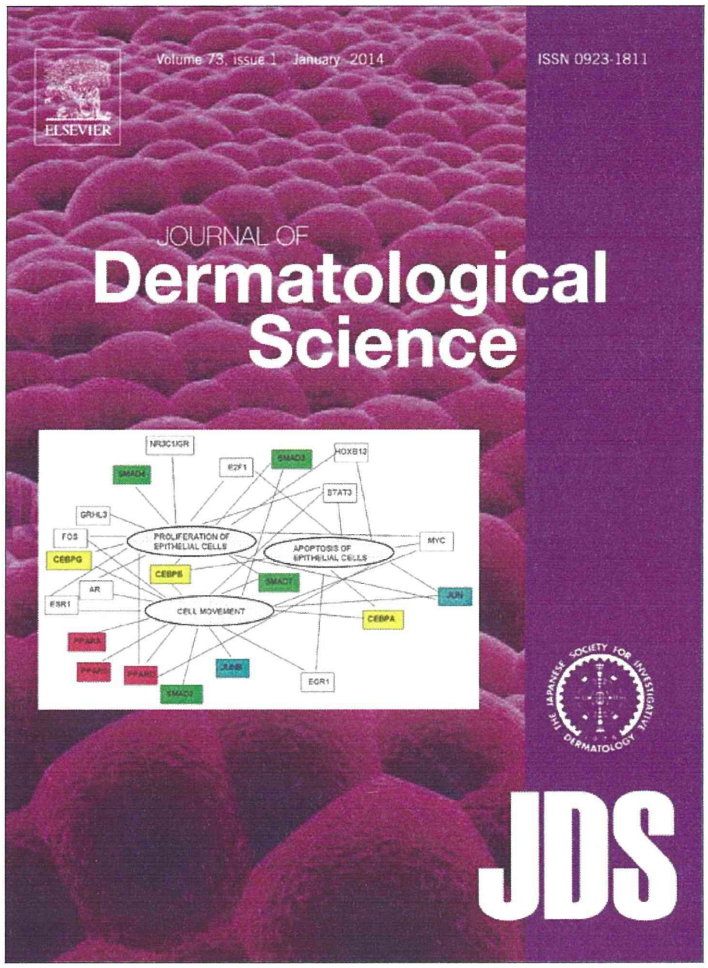

Table 1
Result of immunohistochemical analysis for immune competent cells infiltration. Data represented as mean number of cells and SD. Statistical analysis for comparison was performed by using unpaired *t*-test.

	MelanA	CD8	Foxp3	CD4	CD4+ IL17A+	Epi. CD1a	CD11c	AHR	HLA-DR	CD123	Der. CD1a+ CD207−	CD56
LS ($n=6$)	1.7±3.5**	39.5±22.7*	10.2±3.4	16.5±3.8	15.6±6.3*	33.5±10.0*	3.7±3.2	15.8±11.5	31.1±21.1	11.6±5.5	14.4*	4.5
LE ($n=6$)	8.5±3.6**	19.9±15.4*	9.16±3.4	14.5±4.2	10.3±2.8	22.6±7.1*	17.3±8.3*	12.0±9.2	26.5±15.1	13.2±4.0	10.2	4.5
NL ($n=6$)	19.2±7.8	14.3±10.5	9.1±3.3	10.7±3.3	5.2±1.4	19.2±3.9	5.8±3.7	7.8±2.3	18.1±8.6	10.3±3.3	7.6	1.75
Psoriasis ($n=1$)	41.3	37.7*	18.3	32.3*	28.3**	46.7**	24.7**	13	28	11.3	ND	2
Normal ($n=3$)	17.8±2.2	7.8±2.4	7.9±3.2	12.3±2.1	8.5±1.0	5.9±0.9	5.88±1.1	7.8±3.3	16.3±9.2	7.9±2.5	0	0

ND represent not done.
* *p*-Value < 0.01.
** *p*-Value < 0.05.

TNF-α and IL-6 [8]. Since LCs are commonly activated under IL-1α and TNF-α condition in case of contact hypersensitivity [9], it is conceivable that activated LCs may be an important factor on the occurrence of vitiligo as the interface of melanocyte-specific adoptive immunity cooperating with cytotoxic T cells and may also induce innate immunity in participation with Th17 cells. Following increased infiltration of CD11c+ myeloid dendritic cells and dermal CD1a+ dendritic cells in vitiligo skin can act as antigen trafficking to draining lymph nodes and can produce proinflammatory cytokines such as IL-6 and TNF-α leading to determine helper T cells polarization [10]. Taken together with the effect of Th17 cell-related cytokines on surrounding keratinocyte and fibroblast [6], this positive feedback linage of local cytokines is possibly important for transient appearance of indeterminate dendritic cells and subsequent mature melanocyte disappearance. Given this idea on the underlying immunogenic mechanism, early therapeutic intervention of molecular targeting biologics is considerable for the treatment with progressive nonsegmental vitiligo.

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Letter to the Editor

Increased frequencies of Th17 cells in drug eruptions

T cell-mediated hypersensitivity is the most frequent type of drug eruptions [1] and includes maculopapular eruption (MPE), erythema multiforme (EM), Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-induced hypersensitivity syndrome (DIHS). SJS and TEN, and some of EM are characterized by cytotoxic T (Tc) cell attack toward epidermal keratinocytes with granulysin and Fas–Fas ligand [2], and DIHS is associated with

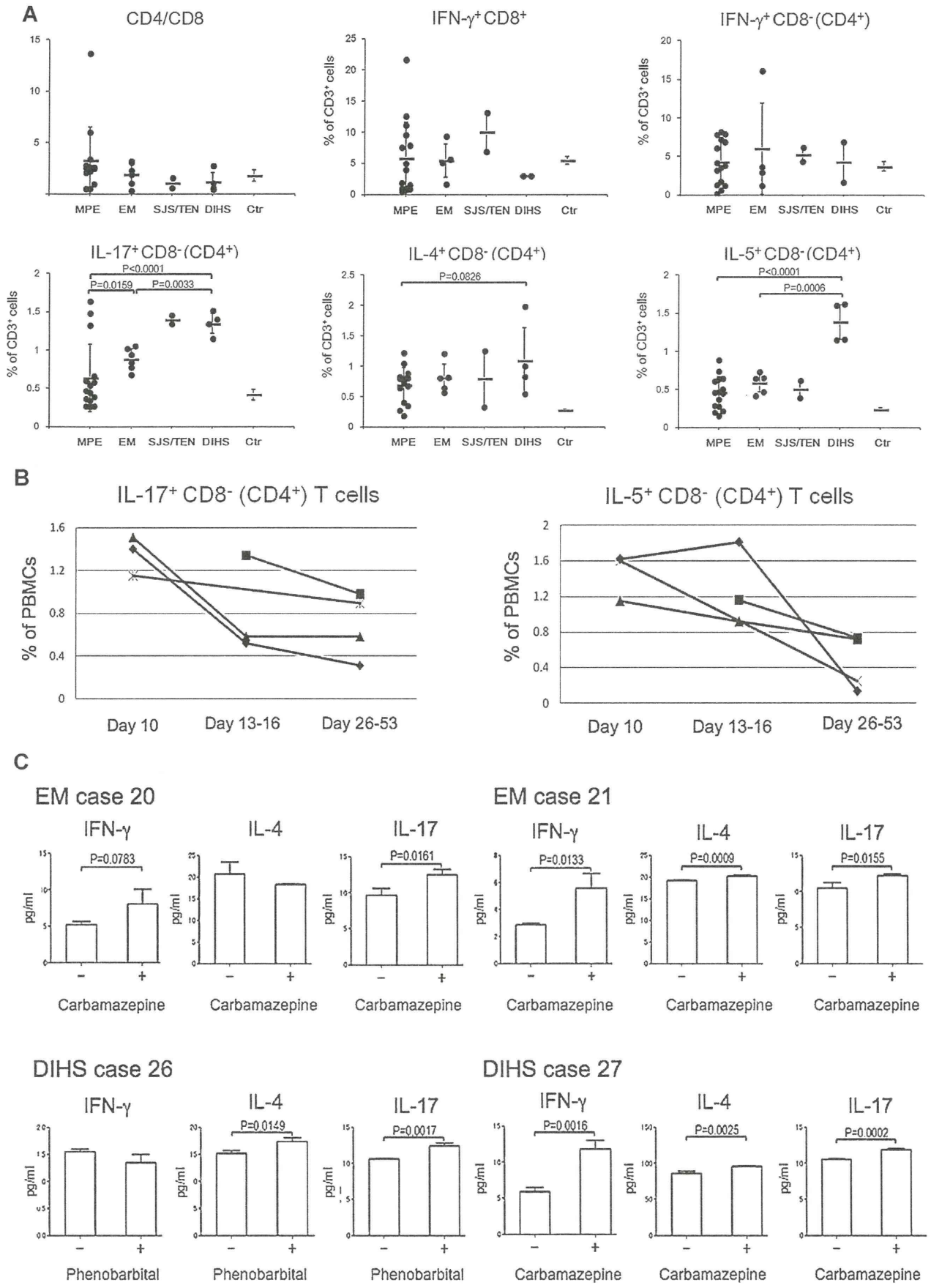

Fig. 1. (A) Percentages of IL-17, IFN-γ, IL-4, or IL-5-producing T cells in the peripheral blood. Blood samples were collected on 2–6 days after onset in EM and SJS/TEN and on 10 days after onset in DIHS and analyzed on a FACSCanto (BD Bioscience, San Diego, CA). In MPE, EM and SJS/TEN, the mean percentages of $CD3^+$, $CD4^+$, and $CD8^+$ cells were within normal ranges ($CD3^+$ cells, 58–84%; $CD4^+$ cells, 25–56%; $CD8^+$ cells, 17–44%). Intracellular cytokines of PBMCs were stained according to the protocol of Cytostain with a few

Table 1
Cytokine conncentrations in the culture supernatants from skin-infiltrating T cells. Cytokine concentration (pg/ml).

Case	Type	Biopsy day	IL-2	IL-4	IL-6	IL-10	TNF-α	IFN-γ	IL-17A	CD4/CD8
28	EM	2	892	14,191	12	32	1495	1565	25	0.10
29	EM	2	4330	3640	9	878	4300	1145	32	0.60
30	EM	7	8	7332	25	519	395	10,115	3497	0.63
31	EM	1	242	5177	92	2988	1587	18,525	41	0.21
32	SJS	7	23	4959	1011	2	1264	5840	15	0.50
33	DIHS	21	20	8175	782	333	465	16,060	3700	6.1
34	DIHS	12	432	3452	264	545	3944	22,770	1659	0.77
35	DIHS	2	8	10,245	436	2684	637	4725	27	0.73
36	DIHS	7	63	7380	338	37	467	317	35	30
37	DIHS	12	19	11,965	89	1035	1423	4210	176	8.7
38	DIHS	24	21	11,130	636	1119	2976	3053	24	15
39	DIHS	6	19,650	19,395	118	3279	12,300	20,115	25	1.4

Biopsy day: the day of biopsy after skin eruption onset. For expansion of T cells, 4-mm skin samples were immersed in complete RPMI supplemented with 20–50 U/ml human recombinant IL-2 and anti-CD3/CD28 mAb-conjugated microbeads (T-cell Expander; Dynal, Copenhagen, Denmark) as previously reported [8]. We obtained $>10^7$ cells/specimen by this method. After phenotyped by flow cytometry, the cells (2×10^5/well) were cultured in an immobilized anti-CD3 mAb-coated 96-well plate for 48 h. The culture supernatants were then harvested to measure the levels of IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ and TNF-α with CBA.

reactivation of human herpesvirus 6 (HHV6) [3]. Upon occurrence of these eruptions, the populations of circulating T cells may be polarized [4]. Interleukin (IL)-17A-producing Th17 cell is a $CD4^+$ T helper subset, and dysregulated Th17 responses mediate psoriasis, allergic diseases, and others [5]. Drug eruption merits Th17 investigation to see the biased relationship between Th17 cells and the other T cell subsets [6] and the host-defensive role of Th17 cells in DIHS. We investigated circulating and skin-infiltrating Th17 cells in drug eruptions.

This study consisted of two types of experiments using different samples: peripheral blood mononuclear cells (PBMCs) and biopsied skin specimens. Totally 39 cases of drug eruption were enrolled in this study (Supplemental Table 1). Cases 1–27 (MPE, 15; EM, 6; SJS, 1; TEN, 1; and DIHS, 4) were seen in University of Occupational and Environmental Health for the PBMC study, and cases 28–39 (EM, 4; SJS, 1; and DIHS, 7) were in Hamamatsu University School of Medicine for the skin-infiltrating T cell study. In 14 patients, the lymphocyte transformation test (LTT) was assessed by ^{3}H-thymidine uptake after 3-day culture with drugs [1], and 9 patients showed positive results. The diagnoses of SJS, TEN [7], and DIHS (seroconversion of anti-HHV6 IgG) [3] were reported. All patients were informed and agreed to participate. The skin-infiltrating T cell analysis was approved by the ethical committee of Hamamatsu University.

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PBMCs were taken from the patients with MPE, EM, SJS or TEN 2–6 days after the onset of eruption and from those with DIHS2–3 times (on day 10, 13–16, and 26–53), and analyzed by flow cytometry. There was no significant difference in CD4/CD8 ratio between the eruption types (Fig. 1a). However, $CD8^+$ cell percentage in DIHS (43%, day 13–16) was significantly higher ($P = 0.0054$) than that in MPE (18%).

Intracellular cytokines were stained after stimulation of freshly isolated PBMCs with phorbol 12-myristate 13-acetate (PMA) and ionomycin [5]. Since CD4 expression on T cells is downregulated with the stimulants, Th17 cells were expressed as $IL\text{-}17A^+CD3^+$ and $IL\text{-}17A^+CD8^-$ T cells, and the major source of IL-17A, IL-4, and IL-5 was $CD8^-(CD4^+)$ cells [5]. The percentages of $IL\text{-}17A^+CD8^-(CD4^+)$ were dramatically increased in DIHS (10 days after onset) and tended to be high in SJS/TEN (2–6 days after onset) as compared to normal subjects (NS, $n = 9$) and MPE patients (2–6 days after onset) (Fig. 1a). In Tc1 ($IFN\text{-}\gamma^+CD8^+$) and Th1 ($IFN\text{-}\gamma^+CD8^-$) cells, no significant differences were found among the groups. $IL\text{-}5^+$ cells were significantly higher and $IL\text{-}4^+$ cells tended to be higher in DIHS. During the clinical course of DIHS, $IL\text{-}17A^+CD8^-$ cells (normal, 0.4%) and $IL\text{-}5^+CD8^-$ cells (normal, 0.3%) were declined in percentage (Fig. 1b).

To investigate the *in vitro* cytokine production, LTT-positive PBMCs from 2 patients with EM (cases 20 and 21) and 2 patients with DIHS (cases 26 and 27) were cultured with the causative drugs. The drug concentration was determined by the highest LTT proliferation, and the culture supernatants were measured for cytokines. In all four cases, the causative drug significantly stimulated the PBMCs taken 26–53 days after onset to produce IL-17A (Fig. 1c). The produced IL-17A amounts were not high, however, considering the relatively low percentages (0.8–1.3% of PBMCs) of Th17 cells and the inability of IL-17A to stimulate bystander T cells, the stimulation degrees seem to be significant. The productions of IFN-γand IL-4 were also significantly increased in 2 EM and 1 DIHS cases, and 2 EM and 1 DIHS cases, respectively.

The biopsied skin specimens from 4 EM (cases 28–31), 1 SJS (case 32), and 7 DIHS patients (cases 33–39) were recruited for the production of cytokines by skin-infiltrating T cells. T cells were expanded with IL-2 and anti-CD3/CD28 mAb-conjugated microbeads [8] and further cultured in an immobilized anti-CD3 mAb-coated 96-well plate (2×10^5/well) for 48 h. IL-17A variously produced in these cases (Table 1). In the 7 patients with DIHS, however, the IL-17A levels appeared to fluctuate in parallel with the clinical courses. The timing of skin biopsy after eruption onset and the IL-17 values are as follows: day 2, 27 pg/ml (case 35); day

modifications. Briefly, freshly isolated PBMCs (2×10^6 cells/ml) were incubated in complete RPMI in a 24-well plate with 10 ng/ml of PMA (Sigma Chemical Co.), 10^{-6} M of ionomycin (Wako, Osaka, Japan) and 0.7 μl of Golgistop (BD Biosciences) for 8 h [5]. The cells were washed and directly stained with PerCP-conjugated anti-CD8 mAb and subsequently with APC-conjugated anti-CD3 mAb. After washing, 100 μl of Cytofix/Cytoperm buffer was added to each well and incubated for 20 min at room temperature, and washed with Perm/Wash solution as manufacture's protocol. They were stained with PE-labeled anti-IL-17, IL-4, or IL-5 and FITC-labeled anti-IFN-γ mAb. Fluorescence profiles were analyzed by flow cytometry in FACSCanto. The $IL\text{-}17^+$, $IL\text{-}4^+$, and $IL\text{-}5^+$cell population was found exclusively in $CD3^+CD8^-$ T cells. Vertical bars represent the mean ± SD.Ctr: healthy control. (B) Changes in the percentages of IL-17-or IL-5-producting cells in patients with DIHS. PBMCs were taken from the patients at the indicated time points, stimulated with PMA and ionomycin, and intracytoplasmically stained with antibodies to IL-17 and IL-5. (C) Cytokine production by PBMCs from patients with EM or DIHS in response to causative drugs. PBMCs were taken from patients 26–53 days after onset. Patients' PBMCs were cultured in triplicate with a causative drug in complete RPMI in 24-well plates (2×10^6 cells/1 ml/well). The final concentration of drugs was determined on the basis of known C_{max} of each drug. In LTT, the drugs were added to the culture at 1/10, 1, and 10 times of C_{max}. For cytokine production, PBMCs were cultured with the causative drug at the concentration that yielded the highest proliferation, and the culture supernatants were subjected to cytokine measurement. Three-day culture supernatants were measured for IFN-γ, IL-4, and IL-17 with the Human Th1/Th2/Th17 Cytokine Beads Array kit (CBA, BD). Vertical bars represent the mean ± SD. Wilcoxon test was employed to evaluate statistical significance between the means. $P < 0.05$ was considered significant.