MB Derma, **275**：93-97，2018.

◆特集／外来でてこずる皮膚疾患の治療の極意—患者の心をつかむための診療術—

多種多様な薬剤を服用して現れる薬疹患者への対応—診療の極意：被疑薬を絞るには—

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Key words：薬疹(drug eruption)，薬剤添加リンパ球刺激試験(drug-induced lymphocyte stimulation test；DLST)，パッチテスト(patch test)，内服テスト(oral challenge)

Abstract　薬疹を疑われる症例は通常，複数の薬剤を服用していることが多い．感冒時には，NSAIDs，去痰剤，胃薬や抗生剤を服用し，高齢者では血圧，糖尿に加え，抗凝固剤などの多種類を複数服用されている．言い換えれば，被疑薬が1剤のみの症例は極めて稀である．薬疹では，被疑薬の精査，決定が有効な治療法の1つであることは言うまでもない．多種多剤を服用中の薬疹疑いの症例では，多種の薬剤の関与を1つずつ除外していく作業が重要であると考えられる．

薬疹が疑われる症例を初診した際に，複数の薬剤が同時に，あるいは連続性に服用され，被疑薬を絞り込む作業が困難なことは多い．例えば，感冒で発熱，関節痛，咳嗽などの各種症状の出現時，NSAIDsや去痰剤，抗生剤などが種類を変えて処方されていたり，降圧剤・抗高脂血症薬・糖尿病薬や抗凝固薬などの常用薬を複数服用されている高齢者は多い．日本総人口における65歳以上の人口の割合は年々増加し，27%を超えたとされる．また2015年の厚生労働省の統計では，院外処方1件あたりの薬剤数は2.9と，2010年から横ばいではあるが，高齢になるほど薬剤数は増加し，75歳以上では平均4種類以上の薬剤が処方されている(出典：社会保険医療診療行為別調査(平成26年6月審査分)第50表)．このような社会的背景もあり，多剤服用中の薬疹が疑われる高齢者の患者は今後も増加することが予測される．

このように服用薬剤数が多い症例では，薬剤の関与を完全に否定することは難しい．また，薬疹を強く疑う症例にかなりの時間をかけて検査しても，必ずしも原因薬を決定できるとは限らない．殊に最近では，DPP4阻害薬による水疱性類天疱瘡に代表されるような，従来の薬疹の概念に収まりきらない薬剤性の皮疹(薬剤性皮膚障害)も多く経験されるようになり，日常診療における薬疹の扱いを一層難しくしている．多種多様な薬剤を服用している薬疹疑いの患者への診療の極意はなく，近道もないように思われる．しかし，このような症例において被疑薬の精査，決定が有効な治療法の1つであり，必要であることは言うまでもない．最も簡単な方法は，疑わしい薬剤をすべて中止することであるが，実際には薬剤の中止は難しいことが多い．一つ一つ検査し除外を重ねていくことが重要であり，検査結果をどのように判断していくのか，検査法を中心に可能な範囲で記載してみたい．

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初診で多剤を服用されている薬疹疑いの症例をみた場合どうすればよいのか

多剤服用症例の対応が難しい一因として，被疑薬の絞り込みが難しいことが挙げられる．また，本当に多剤を服用しているから薬疹なのか？　という問題もある．殊に高齢者の場合には，元々の基礎疾患によるdermadromeとしての皮膚症状，加齢による湿疹続発性の紅皮症や中毒疹様症状な

表 1. 多剤の服用歴のある薬疹疑いの患者をみたときのチェック項目

1）内服薬剤の種類と期間の確認をする.
2）被疑薬による薬疹の報告の有無を確認する.
3）内科疾患による dermadrome を否定するために全身精査を行う(採血，CT など).
4）リンパ腫などの悪性を否定する，および診断確定のために生検を考慮する.
5）薬剤性の可能性が高ければ原因薬剤を精査する.
6）疑わしい薬剤から中止し，経過をみる.

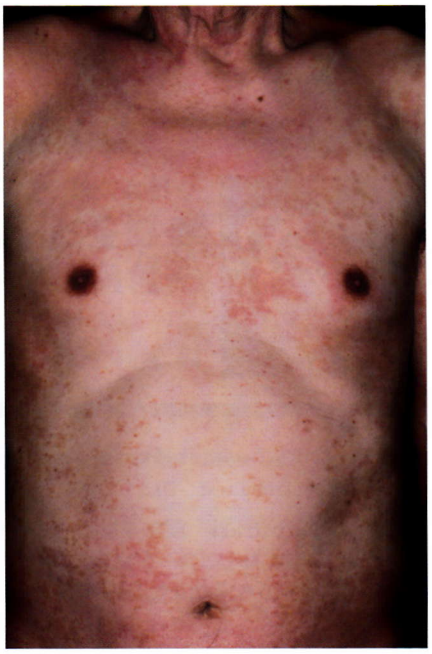

図 1. ▶
症例 1：70 代，男性
初診約 1 年前より躯幹を中心に瘙痒性の皮疹を認め，消長を繰り返す．高血圧，糖尿病，高脂血症に対して各種薬剤服用歴があり，薬疹を疑われて初診された．各種 DLST はすべて陰性のため内服は継続．痒疹(蕁麻疹様皮膚炎)の診断で，保湿剤とステロイド外用を併用し略治した．

ど，薬剤服用歴はあるが，薬剤とは無関係に皮疹を認めることも多い．しかし，近年明らかにされている DPP4 阻害薬による水疱性類天疱瘡に代表されるように，新規薬剤による新しい薬剤誘発性の皮膚症状もあり，最初から薬剤の関与を完全に否定してしまうことはできない．多剤服用症例では，第一に薬剤の関与を疑い，後述する薬剤アレルギーの精査を順次行う．その際に表 1 に記載した各項目をチェックしておくことが，診断の一助になると考えている(表 1)．

中毒性表皮壊死症(TEN)，Stevens-Johnson 症候群(SJS)，薬剤性過敏症症候群(DiHS)などの重症薬疹が疑われる場合には，被疑薬の中止を速やかに行う．高齢者では薬剤中止が生命予後に関わる場合も想定され，内科主治医との連携を密にとる必要性のあることは言うまでもない．発熱などの全身症状のない播種状紅斑丘疹型や扁平苔癬，乾癬型などの慢性の経過をとる症例では，原因薬が決定されるまでは服用を続けることも可能と考えている．

中村らは，高齢者の薬疹は紅斑丘疹型が多く，多形紅斑型，固定薬疹，光線過敏型薬疹に注意すること，原因薬として抗腫瘍薬，抗菌薬などを挙げている[1]．薬疹を疑われる高齢者の症例で鑑別すべき疾患として，慢性多形痒疹や蕁麻疹様皮膚炎が挙げられる．痒疹の一型に含まれる蕁麻疹様皮膚炎は，やや浮腫性の蕁麻疹様紅斑と丘疹，小結節などが混在する．難治性で全身に拡大しうるため，薬剤性を疑われやすい．しかし，各種薬剤の精査はすべて陰性で，内服薬中止や変更も奏効しない．このような症例の存在が，多剤服用者への対応を難しくしている可能性もある(図 1)．

被疑薬を絞り込む
—外来で行う検査法の実際と問題点—

原因薬精査の方法としては，薬剤添加リンパ球刺激試験(DLST)，パッチテスト，内服誘発テストの 3 つが挙げられる．各々一長一短があり，各々の特性を考えながら安全性を優先させて検査する．1 回の検査で必ずしも結論が出るとは限らないことが多く，偽陰性，偽陽性に注意しながら一つ一つ潰していく作業になる．

1．薬剤添加リンパ球刺激試験(drug-induced lymphocyte stimulation test；DLST)

外来診療で最も行いやすいのは，DLST である．欧米では LTT(lymphocyte transformation test)と呼ばれている．パッチテスト，内服テストが通院日数や患者本人に対する薬剤の直接的な反応を確認するのに対し，DLST は採血検体で行うことができるという安全面でのメリットからも，最初

表 2. DLST の結果をみる際の注意事項

1）コントロール値は問題ないか
2）ウイルス感染を起こしていないか
3）検査のタイミングは適切か
4）薬剤代謝産物が原因である可能性はないか

に勧められるべき検査方法である．その一方で，偽陰性や偽陽性をしばしば認めるため，信憑性に疑問を呈する意見も聞かれる．DLST の陽性率は40～15%と諸説がある[2]．大原らは，薬剤系統別の DLST 陽性率を検討し，マクロライド系，セフェム系の抗菌薬，消炎鎮痛剤・総合感冒薬などの NSAIDs の陽性率が高いことを報告している[2]．薬剤代謝産物が反応を起こしている場合では，現物を用いて DLST を施行しても陰性の場合があり，注意が必要である．DLST の問題点として，偽陰性や偽陽性の問題，検査のタイミングや結果の解釈の難しさが挙げられる．播種状紅斑丘疹型薬疹と鑑別が難しいウイルス性発疹症(伝染性紅斑，麻疹)では，さまざまな薬剤に対する反応が陽性になりやすいことが知られている[3]．伝染性単核球症発症時に認められるアンピシリン疹はその代表で，ウイルスによる反応が沈静化すると DLST も陰転化する[4]．また，検査のタイミングにも注意が必要で，播種状紅斑丘疹型，TEN，SJS では発症後 1 週間以内の早期に陽性率が高く，DiHS では 1～2 か月後に陽性になりやすいことが報告されている[5]．DLST は，SI(stimulation index)値としてコントロール値と比較した値での評価であるため(本邦では 180%以上が陽性とされている)，その結果はコントロール値の影響を受けることになる[3]．コントロール値が 100 cpm 以下の場合には，細胞がダメージを受けたために正確な反応がみられていない可能性が考えられる．一方，コントロール値が 1,000 cpm 以上の場合には，SI 値が 180 以下でも陽性の可能性があるとされている[3]．いずれの場合も，結果の解釈には熟慮が必要であり，結果が疑わしい場合には再検査や下記に示す別の方法を考慮する(表 2)．

2．パッチテスト

DLST がすべて陰性の場合，パッチテストを行うことになる．ほぼすべての薬剤が適応になりうるという利点の一方で，内服薬の標準試薬がないために規定の濃度がないことが問題点として挙げられている．また，薬剤の経皮感作の可能性があることも大事なポイントである[6]．播種状紅斑丘疹型，急性汎発性発疹性膿疱症(AGEP)，DiHS などの病型ではパッチテストが陽性になりやすいとされている(表 3)[7)~9)]．固定薬疹は薬剤反応性の T 細胞が病変部に常在するため，病変部でのパッチテストが陽性になりやすいことがよく知られている．中村らは，1980～2004 年の本邦薬疹患者のパッチテストの結果を詳細に検討し，薬剤系統別では抗痙攣剤(76.5%)，消炎鎮痛剤(59.5%)，抗菌薬(59.1%)，循環器治療薬(50.8%)が高く，薬剤別ではメキシレチン(100%)，カルバマゼピン(85.9%)，塩酸ジルチアゼム(78.6%)が多いことを報告している[7]．カルバマゼピンは 1～10%が至適濃度とされるが，薬剤により至適濃度は異なる可能性がある．一般的に 10%以上の高濃度は，内服試験と同様の反応を認めることがあり，注意が必要である．また，基剤の違いによる陽性率の検討において，エタノール基剤での陽性率がワセリン基剤での陽性率よりも高い薬剤があることが示されている．エタノール基剤はワセリン基剤に比較して皮膚透過性が高まるためと考えられているが[10]，近年注目されているアトピー性皮膚炎での経皮感作による食物アレルギー成立の報告をみても，皮膚バリアを壊しやすい基剤を用いたパッチテストには注意が必要である可能性が示唆される．

表 3. 薬疹の病型とパッチテスト陽性率

病　型	陽性率(%)[7)8)]
固定薬疹	100～80
多形紅斑	94～68
TEN	90～56
播種状紅斑丘疹型	85～65
DiHS	88～61
SJS	63～41

3．内服誘発テスト

1.，2. で被疑薬が絞れなかった症例を対象に行うことを考慮する．しかし，1.，2. とは異なり全身に皮疹を誘発することになる可能性もあるた

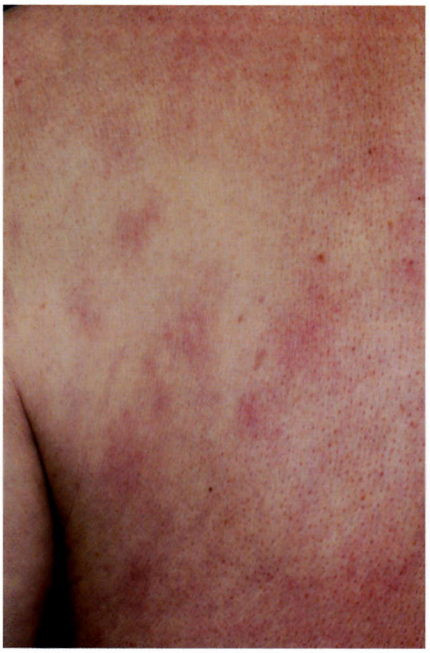

表 4. 症例 2：薬剤精査一覧

薬　剤	DLST SI(%)(CR)	パッチテスト(10%)	内服テスト
抗菌薬 A	119(536)，141(436)	—	陰性
抗菌薬 B	70(388)，76(78)	—	未施行
抗菌薬 C	72(1,040)，412(130)	—	陽性
抗生剤 D	60(1,040)	—	陰性
抗生剤 E	74(78)	—	未施行
抗生剤 F	213(130)	—	陰性

CR：コントロール，SI：stimulation index

◀図 2.
症例 2：80 代，男性
初診の約 2 年半前より A～F の薬剤服用後に，ときどき体のところどころに皮疹が出ることに気がついていた．3 日前から下腿の蜂窩織炎に対し抗菌薬 A を服用し，翌日より顔面躯幹に皮疹，さらに口腔粘膜にも拡大した．躯幹に類円形から長円形の淡紅色斑を示す．

め，TEN，SJS，DiHS などの重症薬疹では行うべきではない．通常，1/20～1/10，皮疹の程度などによっては 1/100 の少量から開始し，入院での検査が望ましい場合もある．朝 1 回服用から開始し，皮疹や瘙痒などの症状の発現がなければ徐々に服用量を増加させる．白血球数や肝機能などが動くこともあるため，可能であれば内服前後での検査データの確認が望ましい．扁平苔癬などの苔癬型では，皮疹が誘発されるまでに数日を要することも多く，常用量から開始することも可能である．

被疑薬がどうしても決まらない場合に

精査をしても原因薬を確定できない場合はどうすればよいのだろうか．もう一度，本当に薬剤性かを疑ってみることも必要であろう．コントロール値の影響で DLST が偽陰性や偽陽性になる症例などでは，精査にも限界がある．患者の立場とすれば，被疑薬を決定することと同じくらいに，服用可能な薬剤をみつけることや，原因と疑われていても決定できないのであれば服用を続けたいという希望をもっていることもある．医師は，原因を突き止めることに執心しがちであるが，被疑薬の決定が困難な場合には服用できる薬剤をみつけることを提案することもある．

被疑薬決定までには長い時間を要することもある

原因薬決定までには，患者が想像する以上に時間がかかることを最初に説明しておくのはとても重要なことである．DLST を一度に数種類行うことは実臨床ではさまざまな制約から難しいことや，パッチテストの施行には数回の通院が必要なことを患者本人に理解してもらう必要がある．

症例 2 は生来健康な 80 歳代の男性で，多発性の非色素沈着性の固定薬疹と診断した(図 2)．薬歴を確認したところ抗菌薬 A～F が被疑薬としてピックアップされた．薬剤精査の結果一覧を示す(表 4)．DLST 検査結果から抗菌薬 C，F の 2 剤が最終的には陽性を示した．パッチテストは全例陰性であり，DLST で陽性を示した抗菌薬 C，F を含め 4 剤の内服テストを 1/100 から開始したところ，抗菌薬 C 常用量服用 7 時間後に皮疹が確認された．時期をあけて行った DLST 陽性であった抗生剤 F の内服テストは陰性であり，抗菌薬 C が原因であったと確定した．最終結果が得られるまでに要した時間は 1 年ほどに及び，多剤を服用している症例での原因薬確定の難しさが痛感される．なお，CR 値が 1,000 以上では偽陰性になりやすい可能性が抗菌薬 C の 1 回目の DLST の結

果から伺える．

おわりに

多種多様な薬剤を服用し薬疹を疑われている症例の対応は難しい．多剤服用症例においては，一つ一つを丁寧に検査し精査していくことが結局は原因への近道ではないだろうか．その際，検査の特性を理解し慎重に判断していくことが求められている．

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CORRESPONDENCE

Local desensitization and progression of multiple fixed drug eruption

Fixed drug eruption (FDE) usually relapses at the same sites with each administration of the causative drug [1, 2]. However, there is a deviation from this characteristic sequence; certain sites are not involved with subsequent flare, while other sites are flared, creating a "wandering" appearance [3, 4]. Accumulating evidence indicates that FDE occurs following activation of skin-resident memory T cells (T_{RM}) [1] and its amelioration is associated with the influx of regulatory T cells (Tregs) [5]. Herein, we report a case of FDE due to acetaminophen (paracetamol), resembling ashy dermatosis (AD), with local desensitization for both patch test and an oral challenge test (OCT).

A 21-year-old woman presented with asymptomatic pigmented macules on her trunk and legs. The lesions initially appeared on the trunk one year previously and new lesions began to appear on the legs nine months later. Clinical presentation demonstrated numerous, ill-defined, ashy grey macules on her trunk and legs (*figure 1A*). Histopathological examination of the abdominal lesion showed pigment

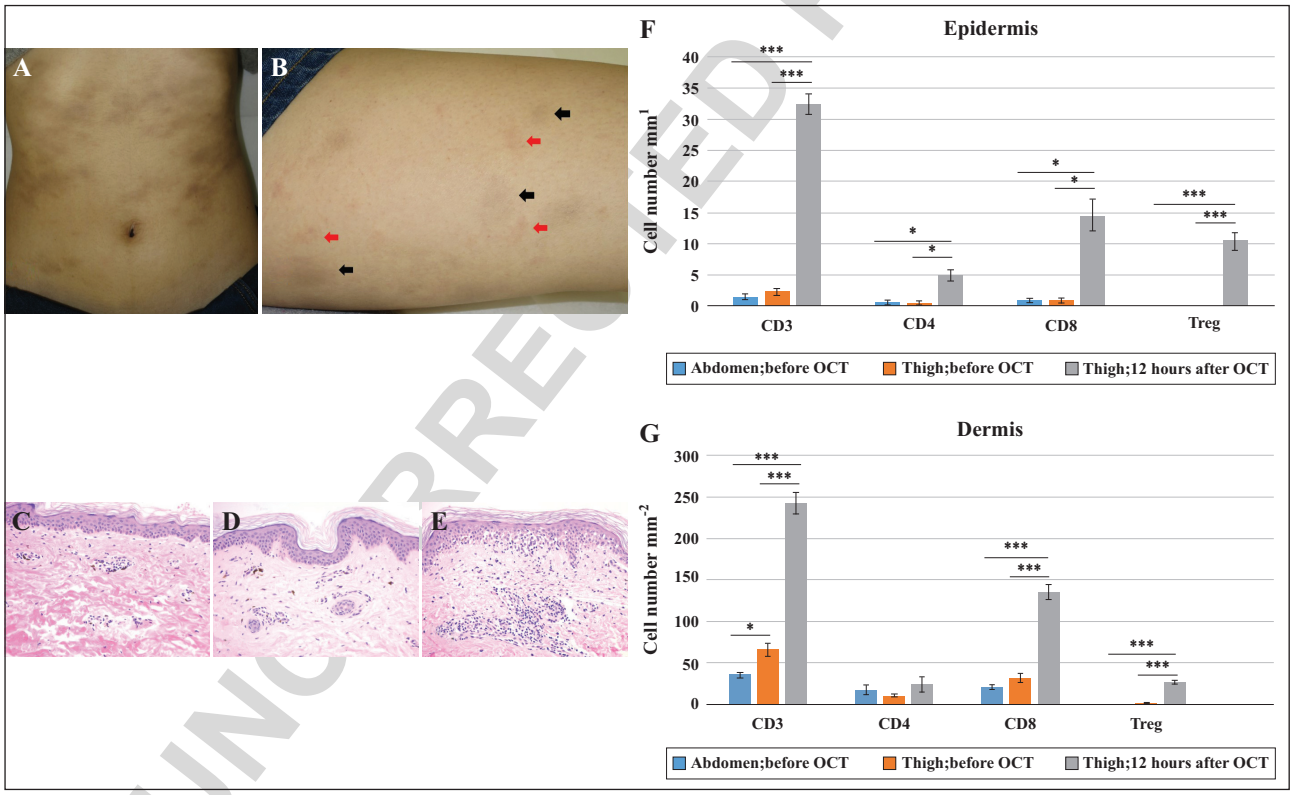

Figure 1. **A**) Multiple, ill-defined, confluent, brown macules with varying size on the abdomen during the resting phase. These lesions were refractory to both a patch test and an oral challenge test (OCT) with acetaminophen. **B**) Development of erythematous macules (red arrows) around the brown macules (black arrows) on the left thigh, two hours after OCT with acetaminophen. **C**) Histopathology of a brown macule on the abdomen in the resting stage showing increased pigmentation of the basal layer and pigment incontinence. (**D**) A brown macule on the thigh in the resting stage displaying increased pigmentation of the basal layer and pigmentary incontinence. (**E**) An erythematous macule on the thigh, 12 hours after OCT, displaying hydropic degeneration of the basal layer, dyskeratotic keratinocytes, and perivascular infiltration of lymphocytes in the dermis (hematoxylin-eosin; original magnification: ×200). **F**, **G**) Change in the distribution of CD3+, CD4+, CD8+, and Foxp3+ cells, which correspond to regulatory T cells (Tregs), in the lesional epidermis (**F**) and dermis (**G**) on the abdomen or thigh in the resting stage, and 12 hours after OCT on the thigh. Numbers of CD3+, CD4+, CD8+ and Foxp3+ cells/mm epidermal length and mm^2 dermis were counted. The data represent mean ± SEM of measurements obtained from four sections from a single sample. Statistical comparison between groups was performed using the Student's t-test. Statistical significance was set at $*p<0.05$ and $***p<0.01$.

doi:10.1684/ejd.2018.3297

To cite this article: Mitsui S, Katayama C, Aoyama Y, Mizukawa Y, Kuyama M, Kawakami Y. Local desensitization and progression of multiple fixed drug eruption. *Eur J Dermatol* 2018 (epub ahead of print) doi:10.1684/ejd.2018.3297

incontinence (*figure 1C*). She was taking acetaminophen twice a month for the treatment of migraine over a five-year period. Lymphocyte stimulation tests were negative with acetaminophen (stimulation index [SI]: 1.17; positive SI >1.8). Patch testing on the lesional pigmented macules of the abdomen in response to acetaminophen (1, 10, and 20% in petrolatum) was negative. OCT with acetaminophen (200 mg; half her single dose) was locally positive, with the patient developing erythematous macules around the pigmented macules on her thighs, two hours later (*figure 1B*), whereas the pigmented lesions on the abdomen remained unchanged. Histopathological evaluation of the erythematous lesion revealed hydropic degeneration of the basal layer and dyskeratotic keratinocytes (*figure 1E*). After a year of follow-up, following avoidance of acetaminophen, the pigmentation gradually faded.

Why did the erythematous macules appear only on the thighs after OCT, whereas abdominal lesions showed local desensitization for both OCT and the patch test? To date, one case of FDE due to acetaminophen resembling AD has been reported [6]. It is interesting that both this case and our case were negative for patch tests on the pigmented macules. We subsequently performed immunohistochemistry for CD3, CD4, CD8 and Foxp3 with specimens of the abdomen and the thigh in the resting stage before or long after OCT. For each sample, the mean number of intraepidermal immunoreactive cells was quantified per millimetre of epidermis and the number of dermal immunoreactive cells was quantified per mm^2 of dermis in four sections from a single sample.

No fundamental differences were found based on hematoxylin and eosin histological staining between those in the abdomen and the thigh in the resting stage (*figure 1D*). There was a significant decrease in the dermal infiltration of CD3+ cells at the abdominal site in the resting stage ($36.7 \pm 3.8/mm^2$), compared with those in the lesion on the thigh in the corresponding stage ($67.4 \pm 13.3/mm^2$) ($p<0.05$). However, there was no significant difference in the number of dermal CD4+ or CD8+ cells between the two sites. Dermal and epidermal infiltration of $Foxp3^+$ cells, which correspond to Tregs, was absent at the two sites (*figure 1F, G*). These results suggest an unknown pathogenesis, other than recruitment of Tregs, responsible for the local desensitization. Refractoriness of the FDE lesions to OCT could also be explained by a long refractory period of TRM, during which time TRM cannot respond to the antigen in a transient manner [7]. The duration of this refractory period may vary for each skin lesion. It is also interesting to note that there was a certain amount of CD8+ cells in the dermis of the thigh in the resting stage ($32.2 \pm 17.6/mm^2$), in comparison to the small number of epidermal CD8+ cells in the same sample ($0.93 \pm 0.7/mm$) (*figure 1F, G*). Although FDE is considered to be caused by activation of CD8+ T_{RM} in the epidermis [1], our results suggest that a dermal type of inflammation might play a part in the pathogenesis of FDE with an unusual clinical presentation.

Since this study was based on a sample from a single patient, our conclusions are clearly limited. Nevertheless, it is important to note that not all the sites of FDE are reactive. ■

Disclosure. *Acknowledgements: We would like to sincerely thank Wakako Oda, Satoko Maeda, Hiromi Watanabe, and Miho Mushiake for their assistance. Financial support: none. Conflict of interest: none.*

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doi:10.1684/ejd.2018.3297

Received: 7 January 2018 | Revised: 6 August 2018 | Accepted: 10 August 2018

DOI: 10.1111/cea.13252

WILEY

ORIGINAL ARTICLE

Basic Mechanisms in Allergic Disease

Monocytes are involved in the balance between regulatory T cells and Th17 cells in severe drug eruptions

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Funding information
Ministry of Education, Culture, Sports, Science and Technology (MEXT) (R.T., T.S.,); Ministry of Health, Labor and Welfare of Japan (T.S.); Japanese Research Committee on Severe Cutaneous Adverse Reaction (J-SCAR)

Abstract

Background: Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DiHS/DRESS) is a distinct phenotype of severe drug eruptions characterized by sequential reactivations of herpesviruses. Although a progressive loss of suppressive function in regulatory T cells (Tregs) occurred during the course of DiHS/DRESS, but not in Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), no previous studies investigated the mechanism. Given the recent finding that Treg development could be differentially regulated by $CD16^+$ patrolling monocytes (pMOs) and $CD14^+$ classical monocytes (cMOs), we can hypothesize that a differential fine-tuned interaction between Tregs and monocytes is the driving force behind the possible shift from Tregs to Th17 cells over a prolonged period of time in DiHS/DRESS.

Objective: To investigate whether the shift from Treg to Th17 could specifically occur during the course of DiHS/DRESS and to elucidate which subsets of monocytes could be involved in the shift.

Methods: We performed a prospective longitudinal study on the frequencies of Tregs, Th17 cells and monocyte subsets after onset of DiHS/DRESS and SJS/TEN, and long after their clinical resolutions. We next examined whether pMOs and cMOs could have a strong impact on the Th17/Treg differentiation and which cytokines could be crucial for the interaction between Th17/Tregs and MO subsets, by in vitro cocultures.

Results: Selective depletion of pMOs occurring at the acute stage of DiHS/DRESS was associated with the relative increase in the frequencies of cMOs producing IL-10 and it did drive Treg expansions. After clinical resolution, pMOs producing IL-6 were alternatively recruited and contributed to the eventual shift from a Treg to Th17 responses.

Conclusions and Clinical Relevance: The gradual shift from Treg to Th17 cell development observed during the clinical course of DiHS/DRESS is mediated by the predominance of cMOs at the acute stage and alternatively recruited pMOs at the resolution stage, respectively.

KEYWORDS
dermatology, drug allergy, lymphocytes, T cells, virus

1 | INTRODUCTION

Foxp3$^+$CD4$^+$ regulatory T cells (Tregs) can inhibit the function of T effector cells (Teff) at the site of microbial infections and allergic inflammation, thereby limiting severe immunopathology.[1-4] Numbers and functions of Tregs, therefore, should be under control depending on the phase of infections and inflammation, and the cytokine environment. In addition, impaired function of Tregs has been shown to contribute to the development of autoimmune diseases, such as type 1 diabetes mellitus,[5] multiple sclerosis,[6] systemic lupus erythematous[7] and acquired aplastic anaemia.[8] It remains unknown, however, when and how Tregs become impaired before or during very early disease development.

In this regard, drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS), a distinct phenotype of severe drug eruptions, offers a unique opportunity to link impaired Treg function to subsequent development of autoimmune disease: this is because DiHS/DRESS is characterized by expansions of fully functional Tregs associated with sequential reactivations of latent herpesviruses at the acute stage, followed by subsequent development of autoimmune manifestations occurring long after clinical resolution, possibly reflecting a progressive loss of Treg function.[4,9-12] Such a long time interval, however, makes it difficult to link a loss of Treg function and autoimmune manifestations.[13] Indeed, we are, at present, unable to determine which immunological alterations are prerequisite for a progressive loss of Treg function after resolution of DiHS/DRESS. Th17 and Treg cell differentiation is plastic,[14] and Tregs have the propensity to differentiate into Th17 cells in the absence of TGF-β_1,[14,15] after exposure to IL-6.[16] In addition, recent studies have also indicated that the expansion of Th17 cells is favoured by the progressive loss of Tregs leading to chronic graft-vs-host disease (cGVHD),[17] in which reactivations of herpesviruses can be typically observed in the same sequential order as observed in DiHS/DRESS.[18] Thus, we can postulate that resolution of DiHS/DRESS may be accompanied by a shift away from Treg differentiation towards Th17 cell differentiation.

We therefore performed a prospective longitudinal study on the frequencies of Tregs and Th17 cells after onset of the disease and long after clinical resolution. Prompted by recent reports on a close interaction between Tregs and monocyte subsets,[19] we also sought to characterize monocyte populations during the course of DiHS/DRESS, and determine whether monocyte subsets could have strong impact on the Th17 and Treg cell differentiation. To determine whether chronological changes in the balance of Tregs/Th17 cells and monocyte subsets are closely related to each other in patients with DiHS/DRESS but not in those with other severe drug eruptions, patients with Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) were also examined as controls. We found that predominance of classical monocytes (cMOs) associated with depletion of patrolling or proinflammatory monocytes (pMOs) observed in the acute stage of DiHS/DRESS can serve to expand Tregs and, upon resolution, pMOs alternatively recruited can drive a resultant shift away from a Treg to a Th17 response that is observed long after clinical resolution of DiHS/DRESS. Our findings provide evidence that the gradual shift from Treg to Th17 cell development is mediated by the predominance of different subsets of monocytes occurring during the prolonged latent period after clinical resolution.

2 | MATERIALS AND METHODS

2.1 | Subjects

Patients with DiHS/DRESS or SJS/TEN who visited our university hospital between 2008 and 2016 were enrolled: they included the acute stage and resolution stage of DiHS/DRESS and the acute stage and resolution stage of SJS/TEN, respectively. Seventeen healthy individuals were also included in our analyses as controls. The diagnosis of DiHS/DRESS and SJS/TEN was made based on their criteria, respectively.[10,20] Participant information is given in Table 1. This study was followed the guidelines for the ethical conduct of human research. This study was approved by the Ethical Committee of Kyorin University (ref. No.H22-077-07: Analysis of pathogenesis and risk factors for allergic inflammatory diseases/viral eruptions) and written informed consent was obtained from all of the participants prior to enrolment. This study was carried out in accordance with Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Ministry of Education, Culture, Sports, Science and Technology. The causative drugs, most of which were anticonvulsant drugs (eg carbamazepine, lamotrigine) and non-steroidal anti-inflammatory drugs, were withdrawn when the diagnosis of drug eruptions was made.

Blood and serum samples were obtained from these patients on or near the day of the initial presentation before starting treatment, and additional samples were subsequently obtained from these patients on a biweekly (before resolution) or several monthly basis (after resolution). Half of patients with DiHS/DRESS were treated with systemic corticosteroids while the vast majority of patients with SJS/TEN were treated with systemic corticosteroids. Samples obtained within 10 days after onset were defined as the acute stage samples while those obtained >37 days after onset were defined as the resolution stage samples; those obtained 11-36 days after onset were defined as the subacute stage samples: the resolution stage samples were obtained from patients who had no longer received oral prednisolone.

2.2 | Antibodies and reagents

Analysis of each lymphocyte and monocyte fraction of PBMCs was performed using antibodies against human CD4 (SK3, BD Biosciences, San Jose, CA), CD8 (Leu-2a, BD, New Jersey), CD14 (61D3, eBioscience/IM2580, Beckman Coulter, Pasadena, CA), CD16 (3G8, Biolegend, San Diego, CA, USA), CD19 (Leu-12, Beckman Coulter), CD25 (2A3, BD), CD45RA (HI100, Biolegend), CD56 (Leu-19, HCD56, Biolegend), Foxp3 (PCH101, eBioscience, San

TABLE 1 Clinical characteristics of DiHS/DRESS, SJS/TEN patients and healthy controls

	No. of cases	Age (y) mean ± SEM	*P* value*	Sex (male/female)	Causative drug	Treatment
DiHS/DRESS acute	31	53.8 ± 9.3	0.042	13/18	Anticonvulsant	N.A.
DiHS/DRESS reso	21	51.7 ± 11.6	0.051	9/12	Anticonvulsant	Corticosteroid (11) intravenous fluids (10)
SJS/TEN acute	18 SJS(12)TEN(6)	47.4 ± 18.5	0.078	9/9	Anticonvulsant, NSAIDs	N.A.
SJS/TEN reso	15 SJS(10)TEN(5)	52.8 ± 12.3	0.042	7/8	Anticonvulsant, NSAIDs	Corticosteroid (15)
Healthy controls	17	47.0 ± 10.1	N.A.	5/12		

DiHS, Drug-induced hypersensitivity syndrome; DRESS, drug reaction with eosinophilia and systemic symptoms; N.A., not applicable; NSAIDs, Non-Steroidal Anti-Inflammatory Drugs; reso, resolution; SEM, standard error of the mean; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.
Acute stage is <10 days from onset (before starting therapy).
Resolution stage is >37 days from onset.
*All *P* values are obtained by comparing with the age of healthy control. *P* values for categorical value were calculated by chi-square or Fisher's exact test.

Diego), IL-17A (eBio64DEC17, eBioscience), HVEM/CD270 (eBioHVEM-122, eBioscience) and 7-amino-actinomycin D (BD Bioscience). $CD3^+$ T cells and $CD4^+$ T cells were isolated using magnetic beads (CD3 microbeads isolation kit; and CD4 T cell isolation kit, Miltenyi Biotec, Bergisch Gladbach, Germany), respectively.

Antibodies used for the coculture of $CD3^+$ T cells and monocytes included antibodies against human CD3 (HIT3a, BD Bioscience), CD28 (37407, R&D, Minneapolis), IL-6 (MQ-213A5, Biolegend), IL-10 (JES3-19F1, Biolegend) and IL-17A.

These cells were cultured in triplicate and in RPMI 1640 medium (Sigma-Aldrich, St Louis) supplemented with 5% human AB serum (Sigma-Aldrich), or with foetal calf serum.

2.3 | Flow cytometric assay

All samples were analysed using a FACS Calibur or FACS Canto II flow cytometer (BD Biosiences). To detect intracellular Foxp3 expression, anti-human Foxp3 staining kit (PCH101, eBioscience) was used according to the manufacture's instructions. For intracellular IL-17A staining, cells harvested from stimulation cultures with PMA and ionomycin for 4 h were incubated in lysing solution and permeabilizing solution (BD Bioscience) and then incubated for 30 min with anti-IL-17A Ab.

2.4 | Identification of monocyte subpopulations

PBMCs were gated on the putative monocyte fraction, including a portion of the adjacent lymphocytes, as demonstrated previously.[4] Monocyte populations can be divided phenotypically, based on the surface expression of CD14 and CD16, into $CD14^+CD16^-$ cMOs and $CD14^{dim}CD16^+$ pMOs.[19,21-23]

2.5 | Cocultures of $CD3^+$ T cells and monocytes

For coculture studies, $CD14^{dim}CD16^+$ pMOs and $CD14^+$ cMOs were purified by sorting out the $CD14^{dim}CD16^+$ (purity > 93 ± 1.5%) and the $CD14^{++}$ (purity > 92 ± 2.1%), respectively. The FACS-sorted pMOs or cMOs (5×10^3 cells/well) were cocultured with allogeneic purified $CD3^+$ T cells (1.25×10^5 cells/well) derived from either DiHS/DRESS patients or healthy individuals; in some experiments these T cells were labelled with CFSE, according to manufacturer's protocol (Biolegend), before coculture and the cocultures were stimulated with CD3 and CD28 for 7 days in a 96-well plate and in RPMI 1640 medium supplemented with 5% human AB serum (both from Sigma -Aldrich). After harvesting, induction of IL-17A production in $CD4^+$ T cells was performed by stimulating with PMA and ionomycin for 4 hours at 37°C in a CO_2 incubator. The number or frequency of $CFSE^{low}$ cells in the IL-$17A^+$ cell fraction was measured using flow cytometry.

2.6 | Immunohistochemical detection of pMOs in skin lesions

Immunohistochemical detection of CD16 and paired immunoglobulin-like type 2 receptor α (PILR-α) on pMOs in skin lesions was performed using skin biopsy specimens from DiHS/DRESS and SJS/TEN skin lesions, as previously described.[4] Immunoreactivity was detected using AEC Liquid Substrate Chromogen (K3463, Dako). For detection of CD16 and PILR-α, mAb to CD16 purchased from Novocastra (2H7, Wetzlar, Germany) and mAb to PILR-α purchased from DENDRITICS (3642, Dardilly, France) were used, respectively. Stained sections were assessed in a blind fashion by two observers (Y.U. and Y.M.) independently; there was no significant differences assessment by the two. The numbers of infiltrated cells were quantified per mm^2 of epidermis/dermis. For each specimen, at least three randomly selected fields were assessed under ×40 magnifications.

2.7 | Statistical analysis

Data are expressed as mean ± SEM. Significance of differences between the groups was determined using Student's *t* test, Welch's *t* test and Fisher's exact probability test. To assess correlations,

Spearman's correlation coefficient was used. Significance was defined as *P* value of 0.05 or less for all tests.

3 | RESULTS

3.1 | Alterations in lymphocyte subsets during the acute stage of severe drug eruptions and after the resolution

As we previously demonstrated,[4] the significant decrease in the frequencies of $CD56^+$ NK cells and $CD19^+$ B cells (Figure S1) and the marked increase in the frequencies of Tregs were specifically found at the acute stage of DiHS/DRESS and returned to levels similar to those in healthy controls upon clinical resolution (Figure 1). Absolute numbers of each leucocyte fraction at the acute and resolution stage of DiHS/DRESS and SJS/TEN are also shown in Table S1.

A recent study[24] has demonstrated that $Foxp3^+$ Tregs could be classified into functionally distinct subpopulations, $CD45RA^+Foxp3^+$ resting/natural occurring Tregs (rTregs), $CD45RA^-Foxp3^{++}$ activated/induced Tregs (iTregs), both of which have potent suppressive function, and $CD45RA^-Foxp3^+$ non-suppressive T cells (non-Tregs). We therefore investigated which subpopulations could be expanded during the acute stage of DiHS/DRESS. As shown in Figure 2, the most remarkable increase was found in the iTreg fraction at the same stage. Upon clinical resolution, their frequency and number returned to values similar to those in healthy controls. Such dramatic alterations were not observed in patients with SJS/TEN throughout the observation period.

Because our recent studies[4,12,25] have suggested that a gradual loss of Treg function occurring upon clinical resolution of DiHS/DRESS would be a driving force in the subsequent development of autoimmune disease, we hypothesized that resolution of DiHS/DRESS could be associated with a shift away from Tregs to Th17 cell differentiation. Analysis of intracellular cytokine production by various lymphocyte subsets showed that the frequencies of Th17 cells were significantly increased after resolution of DiHS/DRESS as compared to those at the acute stage and healthy controls (Figure 3). In contrast, the frequencies of Th17 cells in patients with SJS/TEN, at either the acute or resolution stage, were not significantly different from those in healthy controls.

3.2 | Preferential depletion of pMOs at the acute stage of DiHS/DRESS

On the basis of available evidence to suggest a close interaction between Tregs and monocytes,[19, 22] we sought to characterize monocyte populations during the course of DiHS/DRESS. Human blood monocytes are heterogeneous populations and can be separated into distinct subsets: $CD14^+CD16^-$cMOs, and $CD14^{dim}CD16^+$ non-classical pMO (Figure S2).[19-23] Among them, pMOs have been reported to patrol the whole body for signs of infection[26,27] and control peripheral Treg development in immune thrombocytopenia.[19] We therefore investigated whether MOs, particularly pMOs, would be numerically or functionally impaired at the acute stage of DiHS/DRESS characterized by sequential reactivations of latent herpesviruses.[4,9,12,18] Surprisingly, pMOs have been specifically depleted from the circulation at the acute stage of DiHS/DRESS (Figure 4). pMOs were not significantly altered at either the acute or resolution stage of SJS/TEN as compared to those in healthy controls.

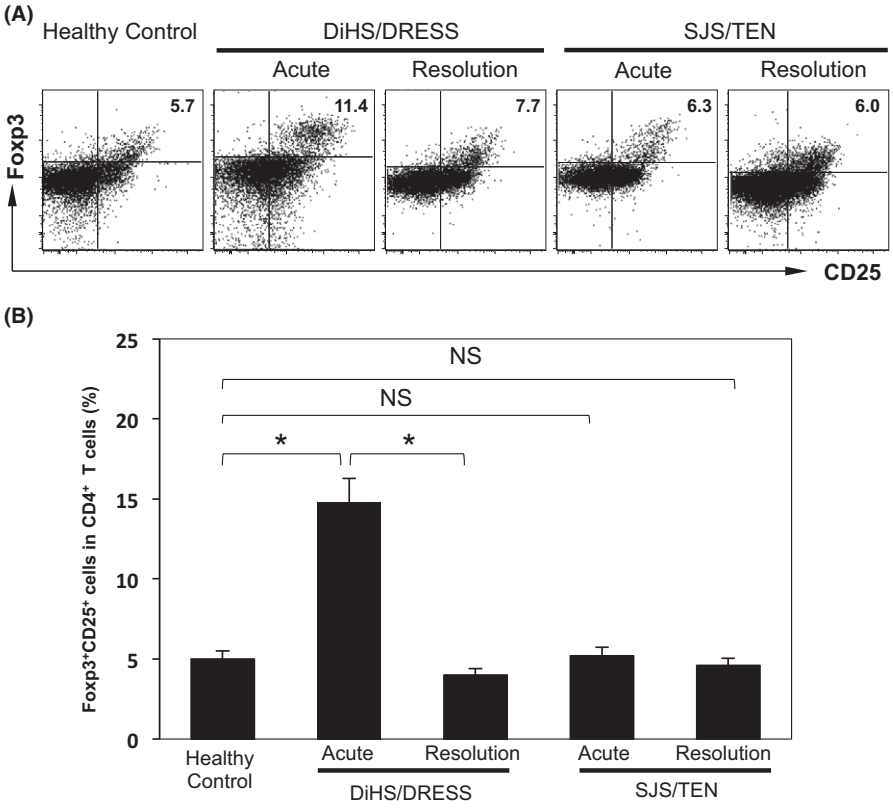

FIGURE 1 Expression of CD25 and Foxp3 in $CD4^+$ cells from DiHS/DRESS, SJS/TEN patients and healthy controls. A, Representative flow cytometry dot plots showing $Foxp3^+CD25^+$ regulatory T cells (Tregs) in $CD4^+$ T cells. B, The mean frequencies of Tregs in $CD4^+$ T cells in DiHS/DRESS at the acute stage (n = 31) and the resolution stage (n = 21) and SJS/TEN patients at the acute stage (n = 18) and the resolution stage (n = 15) and healthy controls (n = 17). Data are mean ± SEM. *, $P < 0.05$; NS, not significant

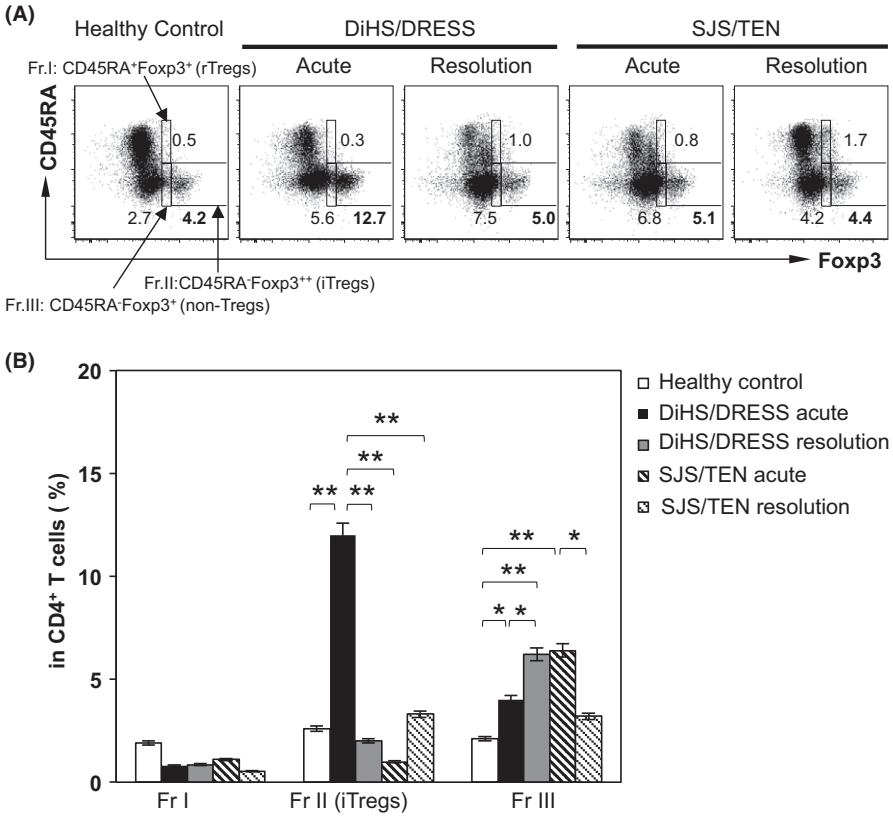

FIGURE 2 $CD4^+Foxp3^+$ cells can be divided into three functionally distinct subpopulations defined by Foxp3 and CD45RA expression: $CD45RA^+Foxp3^+$ rTregs (Fr.I), $CD45RA^-Foxp3^{++}$ iTregs (Fr.II) and $CD45RA^-Foxp3^+$ non-Tregs (Fr.III). A, Representative flow cytometry dot plots showing each subpopulation of Tregs in DiHS/DRESS and SJS/TEN patients at different stages and healthy controls. B, The mean frequencies of subpopulations of $Foxp3^+CD25^+$Tregs in patients with DiHS/DRESS at the acute stage (n = 31) and at the resolution stage (n = 21) and SJS/TEN at the acute stage (n = 18) and the resolution stage (n = 15) and healthy controls (n = 17). Results are expressed as the mean ± SEM. Fr., Fraction *,$P < 0.05$, **,$P < 0.01$

This selective depletion of pMOs with potent antiviral activity may explain herpes simplex virus (HSV) reactivation observed at the acute stage of DiHS/DRESS.[28,29] Consistent with this view, pMOs were found to preferentially express herpes virus entry mediator (HVEM) and PILR-α, which specifically bind to glycoprotein B (gB) and glycoprotein D (gD) of HSV, respectively[30] (Figure S3).

Recent studies have demonstrated that pMOs are involved in the epidermal damage in SJS/TEN,[31] while their role in the pathogenesis of DiHS/DRESS is not known. We therefore investigated whether preferential depletion of pMOs could be also observed in the skin lesions of DiHS/DRESS. As shown in Figure S4, the frequencies of pMOs in the skin lesions of DiHS/DRESS were generally eightfold lower as compared to those in the SJS/TEN lesions: the number of pMOs as evidenced by PILR-α expression was profoundly reduced in the DiHS/DRESS lesions (9.0 ± 1.4/mm^2; $P < 0.01$), while pMOs were abundantly infiltrated into the epidermal tissue of SJS,/TEN lesions (71.0 ± 7.2/mm^2), suggesting that no epidermal damage in DiHS/DRESS lesions is largely attributable to depletion of pMOs. These results indicate that pMOs capable of binding to HSV were selectively depleted from skin tissues as well as the circulation at the acute stage of DiHS/DRESS.

3.3 | Sequential analysis of Tregs and pMOs in DiHS/DRESS

To demonstrate the relationship between Tregs and pMOs, we undertook a sequential analysis of Treg and pMO frequencies on different occasions during the course of DiHS/DRESS. As shown in Figure 5A-E, the mean frequencies of pMOs were at the nadir during the first 1-2 week after onset (acute stage) and then remained fairly constant during the subacute stage, day 11-36. The frequencies of pMOs were gradually approaching to baseline levels observed in healthy controls and achieved it on day 37 onward, during the resolution stage. These dynamic changes in the frequencies of pMOs were never observed in patients with SJS/TEN (data not shown). The increase in pMOs was positively correlated with Th17 cells (r^2=0.93; P = 0.0001) and negatively correlated with Tregs (r^2=–0.81; P = 0.0007) (Figure 5D,E).

3.4 | Potent ability of cMOs to expand iTreg in vitro

To examine whether the dynamics of pMOs could have an impact on Treg expansions, we initially defined the most efficient conditions for expansion of Tregs stimulated with CD3 plus CD28 in the presence of MOs. Freshly purified T cells from healthy controls were cocultured with purified allogeneic MO subpopulations obtained from either healthy controls or DiHS/DRESS patients at the acute stage, and the cocultures were stimulated with CD3 plus CD28 for 7 days. As shown in Figure 6A, CD3 plus CD28 most efficiently stimulated iTreg proliferation as well as Teff proliferation, when purified T cells from healthy controls and those from DiHS/DRESS patients at the acute stage were used as responder cells: proliferation of iTregs was detected by an increase in the frequencies of $Foxp3^{++}CD45RA^-$ iTregs (Figure 6 and Figure S5).

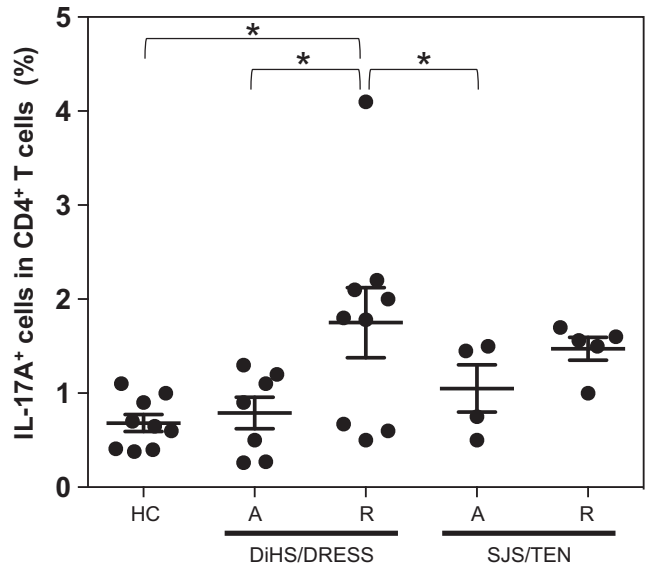

FIGURE 3 Intracellular expression of IL-17A in $CD4^+$ T cells in those patients and healthy controls. DiHS/DRESS patients (n = 12), SJS/TEN patients (n = 9) and healthy controls (n = 9). HC, healthy controls; A, acute stage; R, resolution stage. *, $P < 0.05$

The ability of cMOs to expand iTregs was significantly higher than that of pMOs when cMOs from healthy controls were used (Figure 6A,C). As shown in Figure 6B,D, cMOs obtained from DiHS/DRESS patients at the acute stage had more potent ability to expand iTregs than those from healthy controls and those from the resolution stage of DiHS/DRESS, and their potent ability was inhibited by the addition of anti-IL-10 Ab (Figure S5). These results suggest that cMOs obtained at the acute stage of DiHS/DRESS could serve to expand iTregs via the release of IL-10.

We next examined whether pMOs recruited upon clinical resolution of DiHS/DRESS or their products could inhibit expansions of iTregs obtained from DiHS/DRESS patients at the resolution stage. Anti-IL-6 mAb significantly reversed the inhibitory effect of pMOs on iTregs (Figure 6E). These results indicate that IL-6 derived from pMOs recruited upon clinical resolution of DiHS/DRESS could serve to inhibit expansions of iTregs.

3.5 | Potent ability of pMOs to increase the frequency of Th17 cells

To assess whether the increase in the frequencies of Th17 cells observed at the resolution stage of DiHS/DRESS was also mediated by pMOs alternatively recruited upon clinical resolution, the CD3-stimulated cocultures were further stimulated with PMA and ionomycin for 4 h to detect intracellular expression of IL-17A. For analysis of Th17 responses, we compared the potency of pMOs derived from healthy controls to expand Th17 cells with that of pMOs recruited upon clinical resolution of DiHS/DRESS, because our kinetic analysis of Th17 cells demonstrated that the increase in the frequencies of Th17 cells appeared to occur coincident with restoration of pMOs.

As shown in Figure 7, pMOs recruited upon clinical resolution of DiHS/DRESS had much more potent ability to expand Th17 cells than that of pMOs obtained from healthy controls. The frequencies of $CFSE^{low}$Th17 cells were significantly decreased by the addition of anti-IL-6 mAb at the start of the cocultures: the decrease by anti-IL-6 mAb was more remarkable, when pMOs recruited upon clinical resolution of DiHS/DRESS were used, than those from healthy controls. These results indicate that pMOs alternatively recruited upon resolution of DiHS/DRESS can drive Th cells to differentiate further to a Th17 phenotype through the release of IL-6.

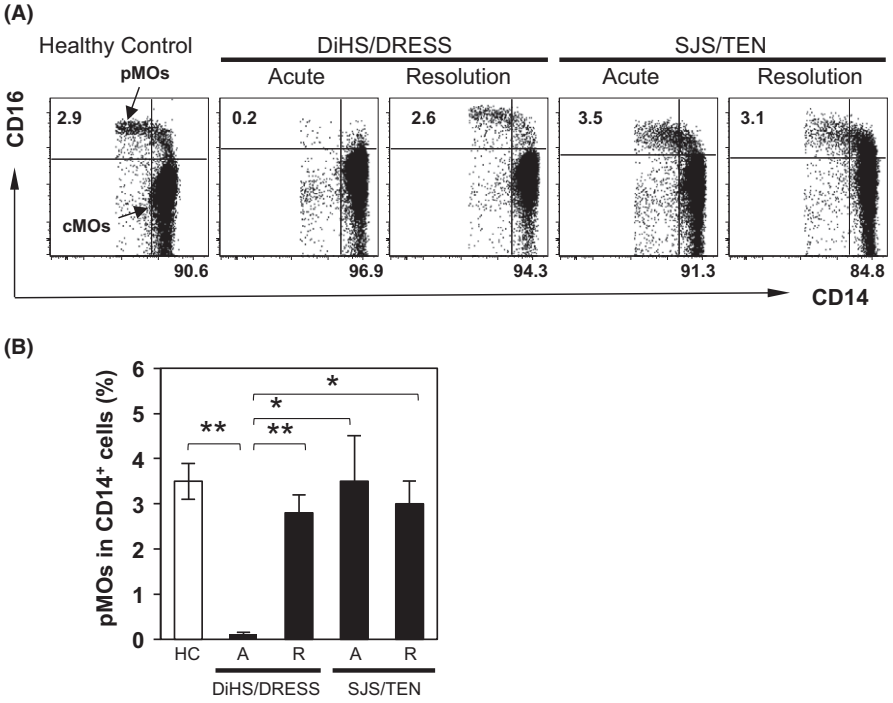

FIGURE 4 The frequencies of patrolling monocytes (pMOs) in DiHS/DRESS and SJS/TEN patients at different stages and healthy controls. A, Preferential depletion of $CD14^{dim}CD16^+$pMOs at the acute stage of DiHS/DRESS. Representative dot plots showing the expression of CD16 vs CD14 in DiHS/DRESS and SJS/TEN patients, and healthy controls. B, The mean frequency of pMOs and classical monocytes (cMOs) in $CD14^+$ cells from DiHS/DRESS patients at the acute stage (n = 31) and the resolution stage (n = 21), SJS/TEN patients at the acute stage (n = 18) and the resolution stage (n = 15) and healthy controls (n = 17). Data are mean ± SEM. HC, healthy controls; A, acute stage; R, resolution stage *, $P < 0.05$, **, $P < 0.01$

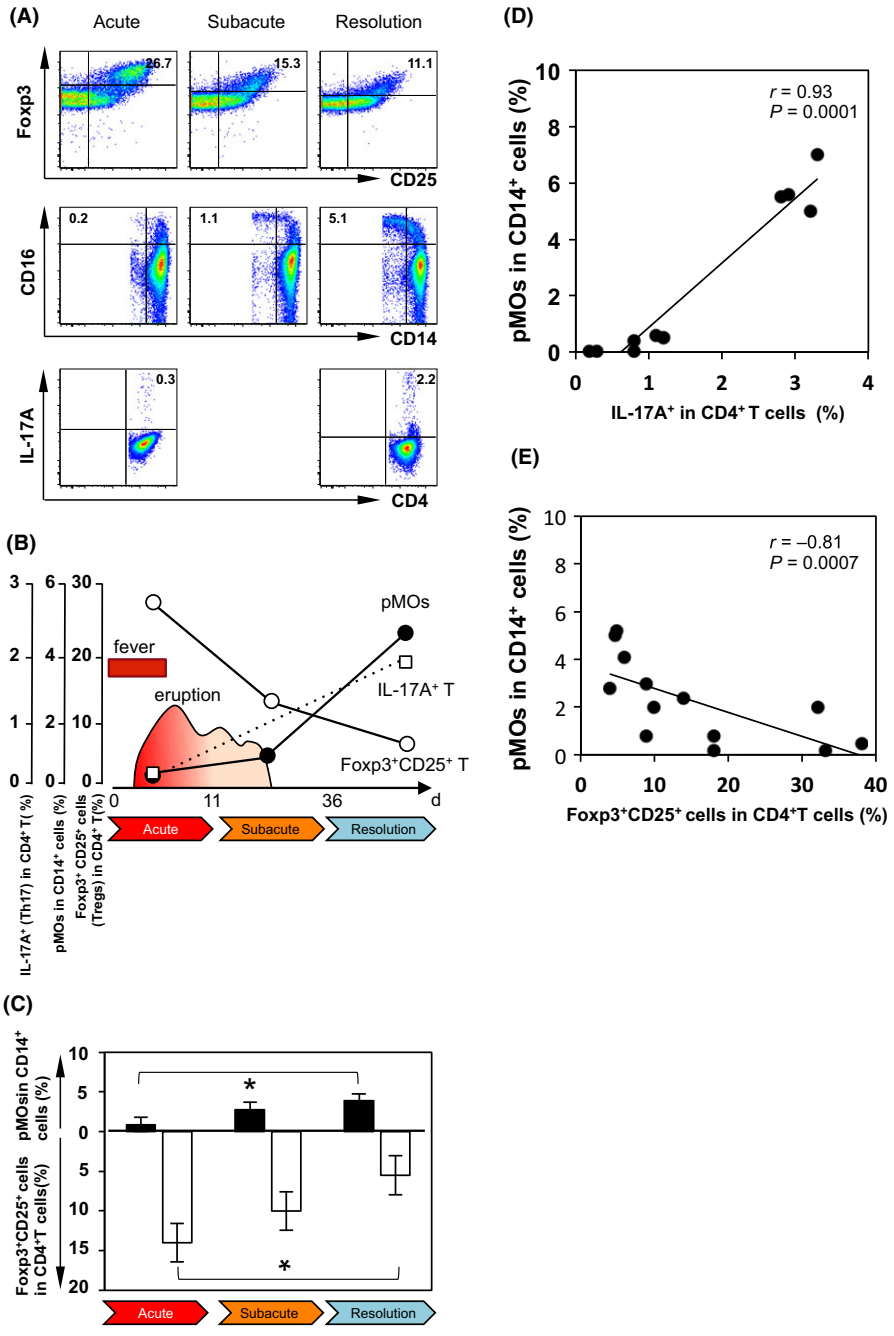

FIGURE 5 Longitudinal analysis of regulatory T cell (Treg), patrolling monocyte (pMO), and Th17 cell frequencies in DiHS/DRESS patients at the different stages. A, Representative flow cytometry dot plots showing the frequencies of regulatory T cells (Tregs), patrolling monocytes (pMOs) and Th17cells at the different stages. B, Representative clinical course of symptoms of a DiHS/DRESS patient in relation to their frequencies. C, The mean frequencies of pMOs and Tregs from DiHS/DRESS patients at the acute (n = 15), subacute (n = 6) resolution stage (n = 9). Data are mean ± SEM. *, $P < 0.05$ (D) A positive correlation between the frequency of pMOs and Th17 cells from DiHS/DRESS patients, as determined by Spearman's correlation test. (n = 10) (E) An inverse relationship between the frequencies of pMOs and Tregs from DiHS/DRESS patients, as determined by Spearman's correlation test. (n = 13)

4 | DISCUSSION

Clinical pictures and outcomes of DiHS/DRESS and SJS/TEN are generally thought to be fundamentally different despite the weight of evidence for drug Ag-specific Teff as a common principal pathogenic mediator in both diseases. DiHS/DRESS is characterized by activation of fully functional Tregs, whereas SJS/TEN is a pathogenic consequence of aberrant activation of Teff driven by impaired suppressive function of Tregs.[4] Thus, the difference between the two diseases appears to reside within the Tregs rather than the Teff; nevertheless, we could not exclude the possibility that some of Teffs could be also different between the two diseases. Interpreted within the framework of current knowledge on a possible interaction between Tregs and other immune cells, these findings suggest that impairment of Treg-mediated immunosuppression differentially observed in the two diseases could not be intrinsic to Tregs themselves but may be secondary to the paucity of signals necessary for Treg development. In view of recent studies indicating that Treg development can be differentially regulated by pMOs and cMOs,[19,22] it is logical to ask whether alterations in monocyte subsets could be responsible for the expansions and subsequent loss of Tregs depending on the stage of DiHS/DRESS.

In this study, we have demonstrated that selective depletion of pMOs at the acute stage of DiHS/DRESS causes the relative increase in the frequencies of cMOs with the potent ability to expand Tregs through the production of IL-10, which may in turn direct Treg

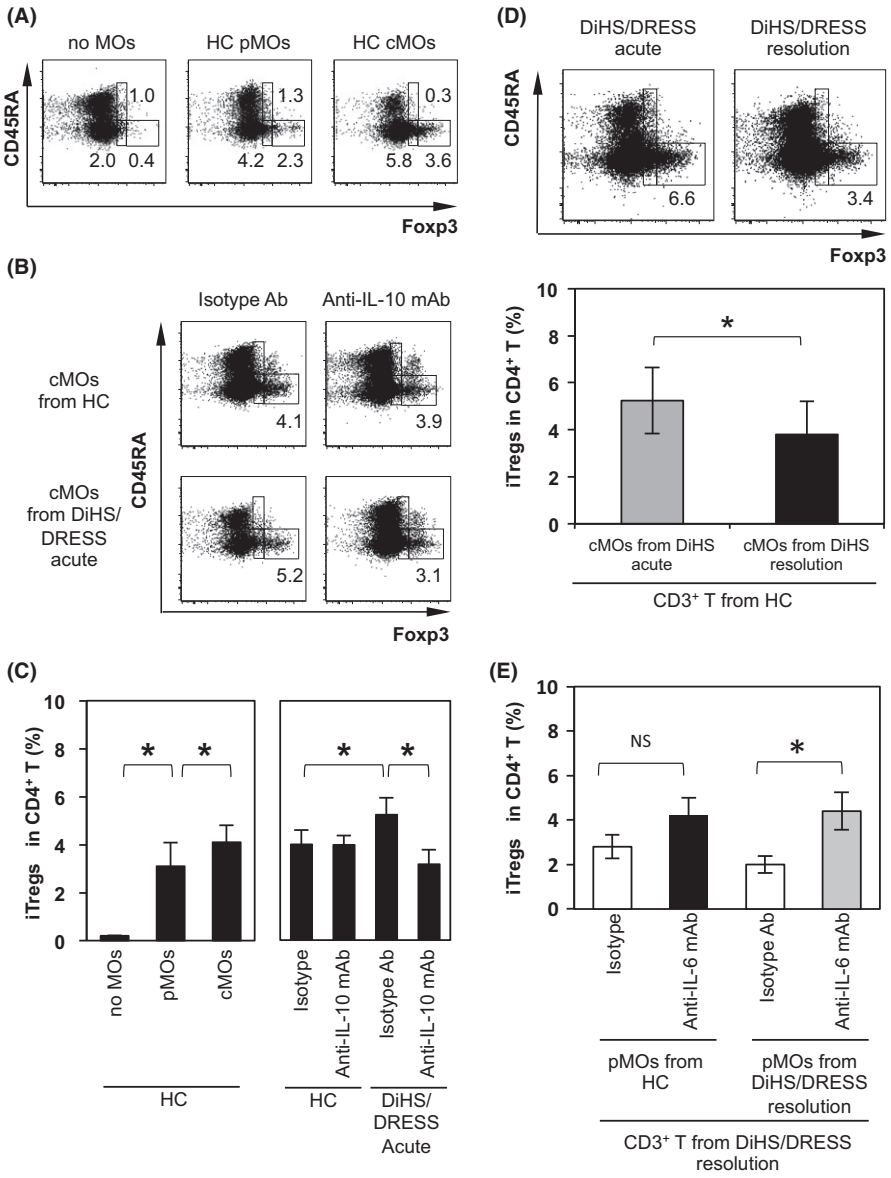

FIGURE 6 Potent ability of monocytes (MOs) to alter activated/induced regulatory T cells (iTregs) and the effect of anti-IL-10/IL-6 monoclonal antibodies (mAbs). A, Representative dot plots and frequencies of iTregs in cocultures of $CD3^+$T cells from healthy controls and no monocytes (no MOs) and pMOs from hea[l]thy controls and classical monocytes (cMOs) from healthy controls. B, Representative dot plots and frequencies of iTregs in cocultures of cMOs from acute stage of DiHS/DRESS and cMOs from healthy controls and the effect of anti-IL-10 mAb on iTregs. C, The mean frequencies of iTregs in $CD4^+$ T cells in cocultures of $CD3^+$T cells from healthy controls and no MOs (n = 10) and pMOs from healthy controls (n = 10) and cMOs from healthy controls (n = 10) (left panel) and in cocultures of cMOs from healthy controls (n = 10) or cMOs from DiHS/DRESS patients at the acute stage (n = 6) and the effect of anti-IL-10 mAb on iTregs (right panel). D, Representative dot plots and frequencies of iTregs in cocultures of $CD3^+$T cells from healthy control and cMOs from DiHS/DRESS patients at the acute stage and the resolution stage. The mean frequencies of iTregs in cocultures of $CD3^+$T cells from healthy control (n = 10) and cMOs from DiHS/DRESS patients at the acute stage (n = 6) and resolution stage (n = 6) (lower panel). E, The mean frequencies of iTregs in cocultures of $CD3^+$T cells from resolution stage of DiHS/DRESS and pMOs from healthy controls (n = 10) or pMOs from resolution stage of DiHS/DRESS (n = 6) and the effect of anti-IL-6 mAb on iTregs. Results are expressed as the mean ± SEM. *, $P < 0.05$; NS, not significant

expansions. However, pMOs alternatively recruited upon selective depletion of "original" pMOs could contribute to the shift away from a Treg to a Th17 responses that is observed during the resolution stage of DiHS/DRESS, probably through their production of IL-6. In view of the crucial role of pMOs for mediating innate host defence responses,[26,27] their delayed recruitment into the host response would provide a temporal window for the establishment and spread of invading pathogens. Nevertheless, alternatively recruited pMOs have been shown to have "pathogenic" features characterized by potent ability to produce IL-6: our ongoing studies on MOs obtained from patients with *Mycoplasma pneumoniae* (*MP*) infection show that *MP* MOs, either cMOs or pMOs, have the impaired ability to expand

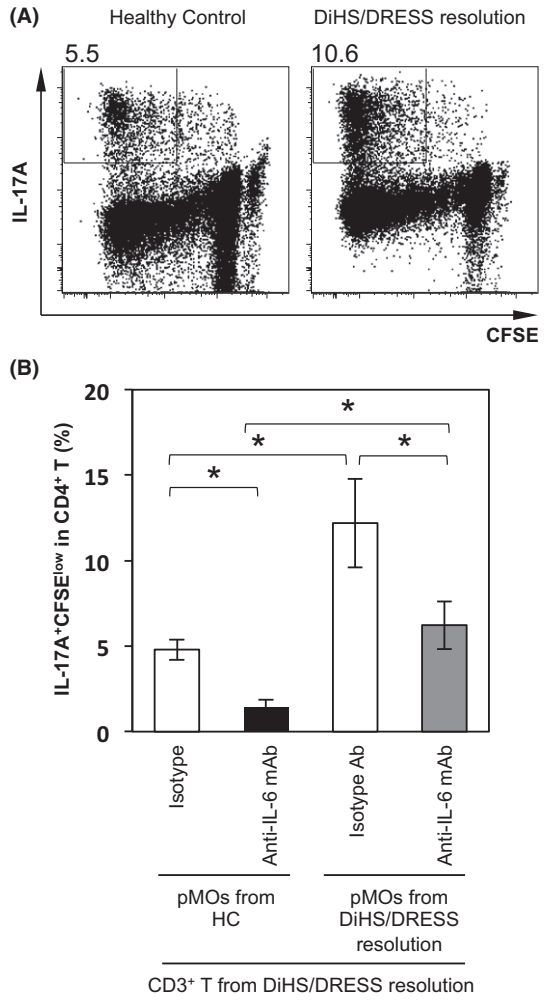

FIGURE 7 Potent ability of patrolling monocytes (pMOs) from healthy controls or pMOs from DiHS/DRESS at the resolution stage to expand $Th17^+$ cells and its inhibition by anti-IL-6 mAb. A, Representative dot plots showing frequencies of carboxyfluorescein diacetate succinimidyl ester (CFSE) ^{low} Th17 cells in cocultures of T cells from DiHS/DRESS patients at the resolution stage and pMOs from either healthy controls or DiHS/DRESS patients at the resolution stage. B, The mean frequencies of $CFSE^{low}$ Th17 cells in cocultures of T cells from DiHS/DRESS patients at the resolution stage (n = 6) and pMOs from either healthy controls (n = 10) or DiHS/DRESS patients at the resolution stage (n = 6) and its inhibition by anti-IL-6 mAb on iTregs. Results are expressed as the mean ± SEM.CFSE, *, $P < 0.05$

Tregs while enhancing Th17 development (unpublished data), thereby driving the shift from a Treg to a Th17 response, as demonstrated in pMOs recruited during the resolution stage of DiHS/DRESS. Consistent with this view, recent studies have shown that iTregs and Th17 cells are not at the final stage of their differentiation and have the potential to convert into Th17 and Tregs, respectively, depending on MO subsets.[32] Nevertheless, no previous studies have demonstrated the existence of such a close interaction between Tregs/Th17 cells and MOs in a given disease setting. Although a more complex scenario than that provided by our study could exist in a number of diverse physiological and pathological settings, this complex interaction should be a therapeutic target and the outcome of the disease may depend on how we can control the interaction.

A previous study proposed an inflammatory role for pMOs in the pathogenesis of SJS/TEN.[31] Although this study indicated that pMOs can directly contribute to the pathological process by promoting epidermal damage, pMOs may act, either singly or in concert with other cells, to precipitate loss of Treg functions even if pMOs themselves do not always cause sufficient tissue destruction. In contrast, because pMOs are major effectors involved in the innate immune response to viral infections,[26,27] their numerical and functional defects would leave the host vulnerable to viral infections. In support of this view, a profound loss of this population was specifically found in the acute stage of DiHS/DRESS and associated with sequential occurrence of several herpesvirus reactivations.[12,18] Indeed, we demonstrate that PILR-α and HVEM, which can specifically bind to HSV gB and gD, respectively, are preferentially expressed on pMOs, suggesting the importance of pMOs in mediating anti-viral role. Depletion of pMOs sensing HSV at the acute stage of DiHS/DRESS would provide an explanation for why sequential reactivations of herpesviruses are specifically observed in patients with DiHS/DRESS.

An intriguing question about this syndrome is why pMOs had been depleted from the circulation at onset. It remains unknown whether pMOs and cMOs represent two independent functional cell types or whether pMOs could derive from cMOs in the presence of macrophage colony-stimulating factor (M-CSF). If pMOs arise from cMOs, one possibility is that depletion of pMOs in the acute stage of DiHS/DRESS would represent the blockade of the differentiation of cMOs to pMOs due to the paucity of M-CSF. In support of this possibility, our sequential analyses of serum cytokine levels after onset of DiHS/DRESS demonstrated that serum levels of M-CSF were significantly lower in the acute stage of DiHS/DRESS than those in the acute stage of other severe drug eruptions (unpublished data). Alternative possibility is that pMOs, which are suggested to play an anti-viral role, could be exhausted by repeated reactivations of herpesviruses, which may occur in an unrecognized fashion far before onset of DiHS/DRESS. Another possibility is that pMOs would be removed from the circulation by their preferential infection with HSV because of their HVEM and PILR-α expression. Nevertheless, we could not totally exclude the possibility that part of pMOs may have been trapped in the tissue microvasculatures before onset.

Th17 cells can be induced by IL-6 and TGF-β and propagated by IL-23 and IL-21,[33] although Th17 cells and Tregs share a common requirement for TGF-β in their differentiation requirements. Thus, the relative amounts of IL-6 or TGF-β would determine whether Th17 cells or Tregs emerge as the dominant phenotype: high IL-6 levels in the presence of low TGF-β levels could shift the Treg/Th17 balance towards a Th17 response. Our findings help to clarify the role of monocyte subsets and their products, IL-6, on the Treg/Th17 balance (Figure S6). Alternatively, recruited pMO-mediated enhancement of Th17 cell development was inhibited by anti-IL-6 mAb and associated with the inhibition of Treg cell development. Our findings

indicate that anti-IL-6 mAb may have a beneficial effect in patients with DiHS/DRESS at the resolution stage, in which impaired Tregs and increased Th17 cells play a key role in sustaining the inflammatory responses, although we have to consider the potential side effects. Consistent with this view, our ongoing studies on serum cytokine levels at the initial presentation of severe drug eruption show that the IL-6 levels are reliable biomarkers predictive of the progression to SJS/TEN.[34]

Corticosteroids have been shown to reduce various proinflammatory cytokine levels and expand Tregs.[35,36] We therefore evaluated the effect of systemic corticosteroids on pMO and Treg frequencies during the course of DiHS/DRESS. The remarkable difference between corticosteroid-treated and non-corticosteroid-treated groups was found in pMO and Treg frequencies during the subacute stage, days 11-36. Although expanded Tregs were contracted and pMOs increased in frequency in both treatment groups, the decrease in Treg frequencies and the restoration of pMOs occurred much more rapidly in the non-corticosteroid-treatment group than those in the corticosteroid-treated group (unpublished data), suggesting that corticosteroids could serve to prevent a rapid recovery of pMOs and a rapid contraction of Tregs, both of which may result in the development of immune reconstitution inflammatory syndrome (IRIS). In this regard, corticosteroids would play an important role in potentially delaying a rapid recovery of immune cells and successful management of DiHS/DRESS might need a combination of biological agents targeting Tregs and pMOs sequentially and concomitantly.

In conclusion, we found that MOs from the acute stage of DiHS/DRESS are more prone to expand iTregs due to the relative increase in cMOs with the potent ability to expand iTregs to cMOs, whereas those at the resolution stage are likely to induce Th17 cell development due to reappearance of pMOs preferentially producing IL-6 (Figure S6) that may be different from the original population. Our study supports the concept that the major therapeutic target for severe drug eruptions is the MO subsets that can drive the shift from a Treg to a Th17 development.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) (R.T., T.S.,) and the Ministry of Health, Labor and Welfare of Japan (T.S.), and Japanese Research Committee on Severe Cutaneous Adverse Reaction (J-SCAR).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Ushigome Y, Mizukawa Y, Kimishima M, et al. Monocytes are involved in the balance between regulatory T cells and Th17 cells in severe drug eruptions. *Clin Exp Allergy*. 2018;48:1453–1463.
https://doi.org/10.1111/cea.13252

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms severity score: A useful tool for assessing disease severity and predicting fatal cytomegalovirus disease

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Tokyo and Saitama, Japan

Background: The prognosis of drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) is highly unpredictable. Severe complications, either related or unrelated to cytomegalovirus (CMV) reactivation, are a highly probable cause of death.

Objectives: The aim was to establish a scoring system for DiHS/DRESS that can be used to monitor severity, predict prognosis, and stratify the risk of developing CMV disease and complications.

Methods: A retrospective analysis of 55 patients with DiHS/DRESS was performed. A composite score was created using clinical data. DiHS/DRESS patients were also stratified into 3 groups based on the scores to predict the risk of CMV reactivation and complications.

Results: This scoring system made it possible to predict CMV disease and complications. Scores ≥4 were associated with the later development of CMV disease and complications, while no patients with scores <4 developed complications.

Limitations: This was a single-institution study with a relatively small patient cohort that lacked a validation cohort.

Conclusions: Our scoring system may be useful for predicting CMV-related complications, and early intervention with anti-CMV agents should be considered in patients with scores ≥4 or with evidence of CMV reactivation. (J Am Acad Dermatol 2019;80:670-8.)

Key words: CMV reactivation; disease severity; drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS); prognosis; scoring system.

Patients with drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) may experience repeated exacerbations beyond the point where the causative drug would be expected to be eliminated from the body,[1-4] reflecting sequential reactivations of herpesviruses. In particular, cytomegalovirus (CMV) reactivation occurring 3 to

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Supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology and the Health and Labour Sciences Research Grants (Research on Intractable Diseases) from the Ministry of Health, Labour and Welfare, Japan (Dr Shiohara).
Conflicts of interest: None disclosed.

Accepted for publication August 30, 2018.
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Published online September 18, 2018.
0190-9622/$36.00

https://doi.org/10.1016/j.jaad.2018.08.052

7 weeks after the onset of DiHS/DRESS may result in uncontrolled viral replication that can lead to fatal disease, including pneumonia, hepatitis, and gastroenteritis[5]; CMV disease could be regarded as the most important factor for the prognosis of these patients. In addition, complications such as myocarditis are more common than are generally realized.[6] Therefore, the severity of clinical symptoms at onset provides only a guide to prognosis. Various clinical symptoms and fatal complications that have traditionally been regarded as being either related or unrelated to severe drug eruptions or CMV reactivation may develop at various time points after onset, indicating difficulties to predict its prognosis.[1-3,6] Because these complications have been underrecognized and are often fatal if not promptly treated,[5-15] there is a clear need to identify effective parameters for assessing disease severity and predicting prognosis of the disease in the early stage.

CAPSULE SUMMARY

- The clinical course of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms is unpredictable.
- We developed a scoring system to predict development of cytomegalovirus disease and complications. Using this scoring system, cytomegalovirus disease and complications could be preventable by prompt treatment with anticytomegalovirus agents.

Unfortunately, no previous studies have established a scoring system by which severity and treatment efficacy can be assessed and complications can be predicted at any time. Our aim in this study was to establish a scoring system for DiHS/DRESS that would be useful for monitoring severity and predicting patient prognosis, especially fatal complications. We hypothesized that DiHS/DRESS patients who later go on to develop fatal complications differ from those who can overcome these complications.

METHODS

Patients

Of patients admitted to our hospital for DiHS/DRESS between 1998 and 2016, 55 patients (22 men, 33 women) were selected if information sufficient for retrospective analyses was available from the medical records (ethics approval number 125-01). The mean age was 54.5 ± 20.0 years (range 14-88 years). Clinical symptoms of DiHS/DRESS developed 44.0 ± 6.9 days (range 10 days to 1 year) after starting the culprit drugs, and these drugs were withdrawn 6.8 ± 1.4 days (range 1-60 days) after the onset of clinical symptoms. Most patients were followed longitudinally for ≥1 year after clinical resolution. The diagnosis of DiHS/DRESS was made according to the criteria for DiHS (including atypical DiHS[16]) and DRESS (including probable and definite DRESS[17,18]) on clinical grounds. Inclusion criteria were as follows: age >12 years and only patients with no symptoms suggestive of DiHS/DRESS before drug exposure in the medical record.

Composite score

A composite score was created using demographic data, medical history, and clinical variables (Table I); this scoring was constructed based on previously published and unpublished data, including our own,[5,6,18-23] particularly on the effect of age and clinical variables on the development of CMV disease and complications.[5,24-29] A score of 1 was given whenever allopurinol was given, because allopurinol has been regarded as one of the factors involved in disease severity and renal insufficiency.[19,23] The severity score was determined routinely on at least 2 occasions, at the early stage (days 0-3 after the initial presentation, early score) and at a later stage (2-4 weeks after the initial presentation, late score).

Various other parameters, as shown in Table I, were graded from −1 to 3 to measure disease severity. The scores obtained at any time were compared with the early score to assess whether patients had progressive disease or were resolving.

All patients analyzed were CMV immunoglobulin G−positive. CMV reactivation was defined as ≥20 genome copies in 10^6 peripheral leukocytes or the detection of CMV-C10/11 antigenemia.[30] CMV disease was defined as having symptoms consistent with an infection and an inflammatory condition, temporally related to CMV reactivation, as previously described.[27] In contrast, complications were defined as severe clinical symptoms, such as pneumonia, peritonitis, and intestinal bleeding, either temporally or clinically related or unrelated to CMV reactivation. Complications were divided into 2 types, as previously indicated: early-onset (≤2 weeks after the initial presentation) and late-onset (>2 weeks after the initial presentation).[6] Most early-onset complications were regarded as comorbidities; most late-onset complications included myocarditis, gastrointestinal bleeding, and pneumonia, which could have generally been related to treatment.

The composite scores obtained were compared between CMV^+ cases and CMV^- cases with the

Table I. Composite scores for evaluating the severity of drug-induced hypersensitivity syndrome and drug reaction with eosinophilia and systemic symptoms and predicting the disease outcomes

Parameters	Grade/extent	Score
Fixed		
Age, y	≤40/41-74/≥75	−1/0/2
Duration of drug exposure after onset, days	0-6/≥7	0/1
Allopurinol exposure	Yes	1
Variable		
Pulsed prednisone*	Yes	2
Skin involvement		
Erythema, % BSA	<70/≥70/erythroderma	0/1/2
Erosion, % BSA	<10/10-29/≥30	0/1/3
Fever ≥38.5°C, days duration	0 or 1/2-6/≥7	0/1/2
Appetite loss (≤70% of regular food intake), days	0-4/≥5	0/1
Renal dysfunction (creatinine), mg/dL	<1.0/1.0-2.0/≥2.1 or HD	0/1/3
Liver dysfunction (ALT), IU/L	<400/400-1000/>1000	0/1/2
C-reactive protein, mg/dL	≤2/<2-<10/≥10-<15/≥15	−1/0/1/2

Each variable parameter was determined at early (days 0-3 after the initial presentation) and later times (2-4 weeks after the initial presentation), and on an as-needed basis.
ALT, Alanine aminotransferase; *BSA*, body surface area; *HD*, hemodialysis.
*Intravenous methylprednisone use ≥500 mg/day for 3 days.

Mann-Whitney U test (Table II). Receiver operator characteristic curves were used to test the performance of the composite score to predict CMV reactivation in DiHS/DRESS cases.

RESULTS

Early and late scores

Early scores usually represented the severity of the disease before treatment. In contrast, because approximately half of the patients received systemic corticosteroids, late scores represented the severity of the disease after starting corticosteroid and non-corticosteroid treatment.

Of the 55 patients investigated for the presence of CMV in sequential blood samples by polymerase chain reaction (PCR) or antigenemia assay, 44 remained quantitatively CMV^- throughout the period of surveillance. In CMV^+ cases, CMV DNA or antigenemia was initially detected at 27.2 days (range 16-45 days) after the initial presentation. CMV^+ DiHS/DRESS cases were significantly older and had more complications associated with a fatal course (Table II). CMV^+ cases were found to run a more severe and protracted course, as evidenced by longer periods of hospitalization; CMV^+ cases had a longer average hospital length of stay (56.9 ± 6.1 vs 25.3 ± 1.9 days, $P < .01$). There was no significant difference in the DRESS score[18] between them (data not shown). Among variable laboratory parameters, C-reactive protein levels at the initial presentation were significantly linked to later development of CMV reactivation (Supplemental Table I; available at http://www.jaad.org). Total doses of corticosteroids used until 8 weeks after initial presentation were higher in CMV^+ than CMV^- cases (Table II), although there was no significant difference. Most importantly, either early or late scores were significantly higher in CMV^+ than CMV^- cases (Table II), even when an elderly population ≥60 years of age and corticosteroid-treated cases after initial presentation were analyzed (data not shown).

CMV-related and -unrelated complications

Five of 11 CMV^+ patients who were quantitatively CMV PCR- or antigenemia-positive developed CMV disease or complications, while the remaining 6 patients, who were quantitatively CMV PCR- or antigenemia-positive, had no evidence of CMV disease and complications at any time (Table III). None of the 44 CMV^- patients developed any late-onset complications. CMV disease such as enterocolitis or complications developed immediately after the detection of CMV reactivation (mean 33.2 ± 6.2 days after the initial presentation) in all cases, usually 2 to 28 days after the detection of CMV reactivation (mean 10.0 ± 4.8 days). CMV viral loads were usually determined once every 2 weeks before, during ganciclovir (GCV) or valganciclovir (VGCV) therapy, and after cessation of anti-CMV therapy. Anti-CMV therapy was initiated after positive PCR or antigenemia results were obtained and was continued until negative PCR or antigenemia results were obtained. Treatment delay defined as an interval of ≥3 days from the first positive PCR or

Table II. Demographic and clinical characteristics of patients with CMV^+ and CMV^- drug-induced hypersensitivity syndrome and drug reaction with eosinophilia and systemic symptoms

	CMV^+ (n = 11)	CMV^- (n = 44)
Age, y, mean ± SEM	73.3 ± 3.4*	49.8 ± 2.9
Gender, M:F	7:4	16:28
Underlying disease (n)	Arrhythmia (1), brain tumor (2), bullous disease (1), cerebrovascular disease (1), dementia (1), epilepsy (2), Guillain–Barre syndrome (1), and hyperuricemia (3)	Brain tumor (1), cerebrovascular disease (2), epilepsy (12), fibromyalgia (1), hyperuricemia (7), neuralgia (3), psychological illness (17), and restless legs syndrome (1)
Causative drug (n)	Allopurinol (2), carbamazepine (5), dapsone (1), mexiletine (1), phenytoin (1), and trimethoprim/sulfamethoxazole (1)	Allopurinol (5), carbamazepine (27), lamotrigine (9), and phenytoin (3)
Early score, median (IQR)	6 (4-7)*	2 (1-4)
Late score, median (IQR)	3 (1-5)*	−1 (−2 to 0)
Duration of causative drug exposure before onset, mean ± SEM	42.9 ± 8.1 days	44.3 ± 8.1 days
Hospitalization period, mean ± SEM (range)	56.9 ± 6.1 days* (28-81 days)	25.3 ± 1.9 days (6-54 days)
Total doses of systemic corticosteroids before initial presentation, mean ± SEM (range) [n]†‡	673.3 ± 600.8 mg (75-1875 mg) [3]	120.3 ± 64.5 mg (7.5-690 mg) [10]
Starting doses of systemic corticosteroids after initial presentation, mean ± SEM (range) [n]‡	54.3 ± 4.8 mg (40-80 mg) [9]*	45.5 ± 5.1 mg (10-70 mg) [11]
Total doses of systemic corticosteroids until 8 weeks after initial presentation, mean ± SEM (range) [n]‡§	1928.9 ± 127.0 mg (1290-2470 mg) [9]*	1729.1 ± 232.1 mg (260-3200 mg) [11]
No. of cases with CMV disease/complications‡	5*	0
Mortality rate (no. of deaths)‡	27.3% (3)*	0% (0)

CMV, Cytomegalovirus; *CRF*, chronic renal failure.
*$P < .01$.
†No. of cases treated with corticosteroid before presentation.
‡Fisher's exact test used.
§No. of cases undergoing corticosteroid therapy at 8 weeks.

antigenemia result until the initiation of antiviral therapy was associated with the development of CMV disease or complications. The interval was longer in patients with complications than in those without complications (10.3 ± 6.1 vs 2.2 ± −0.2 days, $P = .08$). In case 5, CMV disease or complications developed 2 days after cessation of anti-CMV therapy. In cases 2 and 4, VGCV or GCV was started after the development of complications. All but case 1 received GCV or VGCV 2 to 28 days after the detection of CMV reactivation. Fatal outcomes were found exclusively in CMV^+ cases, especially those in whom GCV was initiated ≥3 days after the detection of CMV reactivation, while most cases in whom GCV or VGCV was started within 2 days after detection recovered fully, indicating a great need for starting GCV or VGCV immediately. Importantly, all cases who later developed serious complications, such as pneumonia and intestinal bleeding, had CMV reactivation 10.0 ± 4.8 days before onset of the complications.

We next asked whether we could identify patients who were at a greater risk of developing CMV disease and complications by using the composite score. Early or late scores ≥4 were associated with the later development of CMV disease and complications (Table III). Receiver operator characteristic curves confirmed that this composite score had the

Table III. Demographic and clinical characteristics of cytomegalovirus DNA$^+$ cases with or without complications

Case no., age, y/sex	Causative drug	Underlying disease(s)	Score early	Score late	CMV, C10/11* or DNA†	Time to CMV reactivation (factors thought to be the trigger)	Complications (day of detection)/outcome	Time to initiation of anti-CMV therapy after detection of CMV	Duration of anti-CMV therapy (anti-CMV agents)
1, 88/F	Allopurinol	Hyperuricemia	4	3	ND, 4.4 × 10	28 days (unknown)	Pneumonia (day 32)/death	N/A	N/A
2, 79/M	Carbamazepine/ allopurinol	Guillain—Barre syndrome/ hyperuricemia	11	6	5/4, ND	29 days (2 days after tapering systemic corticosteroids)	Peritonitis (day 31)/death	3 days	8 days (VGCV)
3, 81/M	Allopurinol	Hyperuricemia	8	5	4/2, ND	24 days (1 day after discontinuation of IVIG)	Intestinal bleeding (day 28)/recovery	8 days	24 days (GCV)
4, 74/M	Mexiletine	Arrhythmia	4	3	149/112, 3.7 × 10	16 days (3 days after tapering systemic corticosteroids)	Intestinal bleeding (day 44)/death	28 days	22 days (GCV + IVIG)
5, 48/M	TMP/SMX	Brain tumor	6	1	17/18, ND	19 days/31 days (2 days after tapering systemic corticosteroids/2 days after discontinuation of VGCV)‡	Intestinal bleeding (day 31)/recovery	2 days/2 days	8 + 15 days‡ (VGCV)
6, 67/F	Carbamazepine	Epilepsy	1	6	7/3, ND	45 days (10 days after tapering systemic corticosteroids)	No/recovery	2 days	15 days (VGCV)
7, 83/F	Carbamazepine	Dementia	6	3	1/2, ND	26 days (10 days after tapering systemic corticosteroids)	No/recovery	2 days	8 days (VGCV)
8, 80/F	Phenytoin	Epilepsy	7	1	0/1, 2.0 × 10	25 days (1 day after tapering systemic corticosteroids)	No/recovery	2 days	21 days (VGCV)
9,§ 63/M	Carbamazepine	Brain tumor	4	5	ND, 9.0 × 10	25 days (7 days after tapering systemic corticosteroids)	No/recovery	N/A	N/A
10, 73/M	Carbamazepine	Cerebrovascular disease	7	0	2/1, ND	35 days (15 days after tapering systemic corticosteroids)	No/recovery	3 days	8 days (VGCV)
11, 70/M	Dapsone	Bullous disease	5	0	4/2, ND	27 days (6 days after tapering systemic corticosteroids)	No/recovery	2 days	18 days (VGCV)

Cases 1-5 indicate those with complications, either CMV-related or CMV-unrelated drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms.
CMV, Cytomegalovirus; *F*, female; *GCV*, ganciclovir; *IVIG*, intravenous immunoglobulin; *M*, male; *N/A*, not applicable; *ND*, not done; *TMP/SMX*, trimethoprim/sulfamethoxazole; *VGCV*, valganciclovir.
*Data on the first positive of antigenemia (no. of CMV$^+$ cells/slide).
†Data on the first positive polymerase chain reaction study (≥20 genome copies in 10^6 peripheral leukocytes).
‡VGCV was administered from day 21 to day 28 (for 8 days) 2 days after detection of CMV reactivation, and from day 33 to day 47 (for 15 days), 2 days after intestinal bleeding, respectively.
§Pulsed prednisone used before the initial presentation.

Table IV. Risk stratification of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms cases based on the early score

	Mild (1>) (n = 5)	Moderate (1-3) (n = 23)	Severe (4≤) (n = 27)
No. of CMV^+ cases[§]	0	1*	10 *
Late score, median (IQR)	−2 (−2 to −1.5)	−1 (−1 to 0)	1 (0-5)[†]
No. of cases with CMV disease/complications[§]	0	0	5[†]
Mortality rate (no. of dead patients)[§]	0% (0)	0% (0)	11.5% (3)
Hospitalization period, days, mean ± SEM (range)	16.2 ± 3.7 (9-27)	23.2 ± 2.4 (6-65)	41.7 ± 3.8[†] (14-81)
Total doses of systemic corticosteroids until 8 weeks after initial presentation, mean ± SEM (range) [no. of cases][§‖]	0 mg (0 mg) [0]	1459.2 ± 247.8 mg* (260-2170 mg) [6]	1973.2 ± 154.6 mg* (620-3200 mg) [14]
WBC (cells/μL), mean ± SEM	5740.0 ± 1059.1	8708.7 ± 1521.8	10,388.9 ± 972.0
Plt (μL), mean ± SEM	21.8 ± 2.0	19.9 ± 1.6	29.8 ± 7.8
ALT (IU/L), mean ± SEM	115.0 ± 32.2	201.9 ± 48.9	80.4 ± 15.2
CRP (mg/dL), mean ± SEM	0.8 ± 0.2	2.9 ± 0.5	7.4 ± 1.1*
Early NLR, mean ± SEM	1.6 ± 0.6	4.3 ± 0.6	6.5 ± 1.3
Late NLR, mean ± SEM	1.4 ± 0.2	2.1 ± 0.3	3.2 ± 0.4

ALT, Alanine aminotransferase; *CMV*, cytomegalovirus; *CRP*, C-reactive protein; *DiHS/DRESS*, drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms; *IQR*, interquartile range; *NLR*, neutrophil/lymphocyte ratio; *WBC*, white blood cell count.
*$P < .05$ (mild vs moderate, severe).
†$P < .05$.
§Fisher's exact test.
‖No. of cases undergoing corticosteroid therapy at 8 weeks.

potential to identify CMV disease and complications in patients with DiHS/DRESS (Supplemental Fig 1).

Risk stratification of CMV reactivation

We next stratified disease severity into 3 groups based on early scores to predict the risk of CMV reactivation or CMV disease and complications (Table IV). Mild disease was defined as scores <1 (n = 5), moderate disease as scores 1 to 3 (n = 23), and severe disease as scores ≥4 (n = 27). CMV reactivation including CMV disease and complications occurred most frequently in the severe group: CMV disease and complications developed exclusively in the severe group. Median CRP levels were significantly different among the 3 categories, with the severe group having the highest. The average hospital length of stay was also the longest in the severe group. Mild and moderate disease groups had no evidence of progression to overt CMV disease or severe complications after 12 months of follow-up. Adjusted analyses of variance across the 3 disease severity groups showed that disease severity from mild to moderate to severe was associated with increasing periods of hospitalization and increasing white blood cell counts and CRP levels. A diagnostic and prognostic algorithm based on early and late scores is proposed in Fig 1.

DISCUSSION

Complications occurring during DiHS/DRESS have been extensively described in previous studies[5-14] and have received attention as the cause of mortality in DiHS/DRESS. These complications include myocarditis, *Pneumocystis jirovecii* pneumonia, sepsis, and gastrointestinal bleeding,[5-14] most of which lead to significant morbidity and mortality if unrecognized and untreated.[5-7,10,12,13] Therefore, the establishment of a scoring system using routinely obtained parameters by which disease severity and treatment efficacy can be assessed at any time and disease progression to more aggressive stage can be predicted is urgently needed for the successful management of DiHS/DRESS. By comparing the composite score in the early phase (days 0-3) with that at any time after starting therapy, the composite score can provide clinicians with clues for optimizing therapeutic efficacy and preventing treatment-related relapse. Not only worsening of clinical symptoms but also lack of improvement can be also evaluated by comparing these scores on different occasions. This scoring system might be useful in predicting beneficial treatment results in DiHS/DRESS (Table III). In addition to the utility of the scoring system for monitoring the extent of disease severity, this report supports the potential

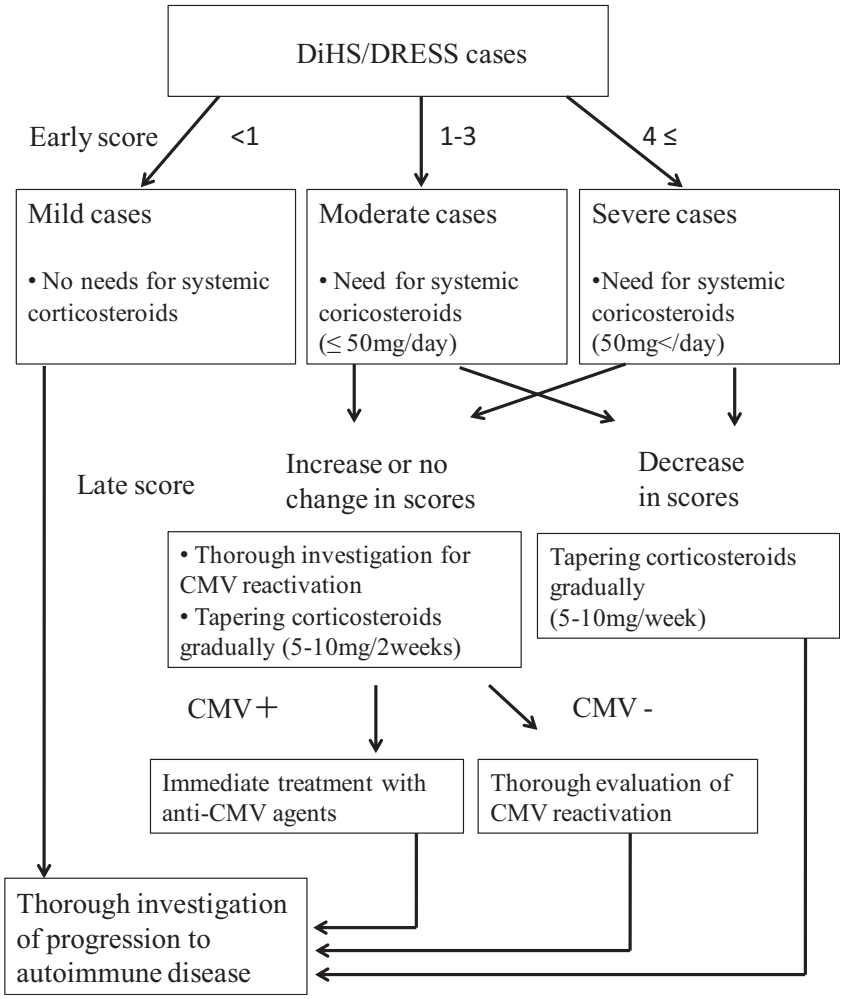

Fig 1. Proposed flow diagram for the diagnosis and outcome prediction of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms based on early and late scores. *CMV*, Cytomegalovirus; *DiHS/DRESS*, drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms.

use of this scoring system for identifying individuals with a likelihood of CMV reactivation and complications, either CMV-related or CMV-unrelated, with fatal outcomes. Indeed, the present data show that patients with an early or late score ≥4 were significantly more likely to later develop CMV disease and complications; 5 of 26 patients with scores ≥4 developed CMV disease and complications, while no patients with a score <4 developed severe complications.

The immunosuppressive effects of corticosteroids are more pronounced in elderly patients, resulting in an increased risk of CMV reactivation. Indeed, there was a positive relationship between age of DiHS/DRESS onset and the score ($P < .01$). Indeed, in these patients, especially those treated with systemic corticosteroids and elderly patients ≥60 years of age, frequent relapses and worsening of clinical symptoms were observed with reduction of the corticosteroid dose. In this regard, previously reported cases suggest that the use of pulsed prednisone may be related to later development of CMV reactivation.[24,31] It is probable that a large reduction of prednisone doses needed immediately after pulsed prednisone may paradoxically induce a rapid recovery of immune responses that could in turn contribute to the development of CMV reactivation as a manifestation of immune reconstitution inflammatory syndrome.[32] To prevent a rapid immune recovery, we recommend that systemic corticosteroids be initiated at a sufficient dose of 40 to 60 mg per day prednisone equivalent in DiHS/DRESS and be followed by a gradual dose reduction of prednisone at least over >8 weeks (Fig 1). Tapering more gradually

over a prolonged period is recommended to achieve the optimum therapeutic result in patients with DiHS/DRESS.[1,3]

As pointed out by Bourgeois et al,[6] the present data also show that late-onset complications, such as myocarditis, which occur months after the rash and laboratory abnormalities have resolved and long after withdrawal of the causative drug, are more likely associated with fatal outcomes than early-onset complications. In view of our finding that CMV reactivation occurred 27.2 ± 2.3 days after onset while complications developed 33.2 ± 6.2 days after onset in CMV^+ cases, most late-onset complications would be caused by CMV reactivation. Because previous studies indicated that CMV reactivation could be the cause of several complications, such as gastrointestinal bleeding,[5,14] renal dysfunction,[7] myocarditis,[10] and sepsis,[12,13] it is likely that fatal complications occurring in the late stage of DiHS/DRESS could be preventable with anti-CMV therapy. In support of this possibility, a delay in initiating anti-CMV therapy after the first detection of CMV reactivation was likely to reduce efficacy; cessation of anti-CMV therapy was temporarily associated with the development of CMV disease or complications, as shown in case 5. Therefore, no development of CMV disease and complications during anti-CMV therapy and its transient resurgence soon after cessation of anti-CMV therapy suggest that anti-CMV therapy is effective in preventing the development of not only overt CMV disease but also complications that are generally regarded as being CMV-unrelated. Because CMV is thought to affect the other herpesviruses, such as Epstein–Barr virus,[33,34] the present data concur with previous observations that anti-CMV therapy may have been also effective at curtailing the risk of other herpesvirus-related complications; anti-CMV therapy has been reported to exert beneficial anti-Epstein–Barr virus or human herpesvirus-6 effects,[34,35] although the efficacy of anti-CMV therapy against other members of the herpesvirus family, either directly or indirectly, has not been compared. Given that mortality in DiHS/DRESS is estimated to be 10% among patients in previous studies,[22] the mortality rate of 5% in the present cohort appeared to be the lowest, to the best of our knowledge, reflecting a clear benefit of the prompt recognition of CMV disease and appropriate management.

Our study is limited by its retrospective nature, single-institution assessment, the lack of a validation cohort, and the limited follow-up time, although it is the largest longitudinal retrospective cohort study to date in patients with DiHS/DRESS. Studies with large numbers of patients with DiHS/DRESS pooled from multiple centers and long-term follow-up would be of further significant value in assessing outcomes associated with CMV reactivation.

In conclusion, our scoring system offers the possibility of screening high-risk patients before the development of CMV reactivation followed by CMV-related or CMV-unrelated complications; this scoring system can guide treatment options and help predict outcomes. Patients with scores ≥4 at any time are candidates for a more thorough clinical evaluation of CMV reactivation.

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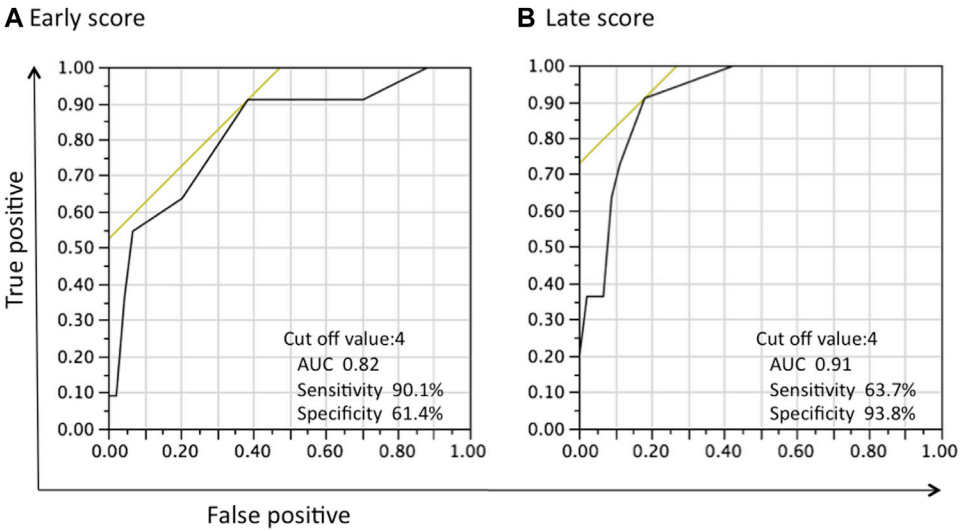

Supplemental Fig 1. Receiver operating characteristic curves and diagnostic performance of the predicted probability for detection of the later development of CMV disease and complications based on early and late scores.

Supplemental Table I. Laboratory findings of CMV^+ and CMV^- cases at the initial presentation

	CMV^+ (n = 11)	CMV^- (n = 44)
WBC (cells/μL), mean ± SEM	9418 ± 952	9225 ± 992
Lymphocytes (μL)	1170 ± 166	1482 ± 185
Eosinophils (μL)	1205 ± 503	1454 ± 359
Plt (μL), mean ± SEM	37.8 ± 18.5	21.4 ± 1.2
Cr (mg/dL), mean ± SEM	1.4 ± 0.5	1.0 ± 0.2
ALT (IU/L), mean ± SEM	80.5 ± 19.9	147.8 ± 40.1
CRP (mg/dL), mean ± SEM	8.5 ± 2.1*	3.9 ± 0.6
NLR, mean ± SEM	5.8 ± 0.9	4.9 ± 0.9

ALT, Alanine aminotransferase; *CMV*, cytomegalovirus; *Cr*, creatinine; *CRP*, C-reactive protein; *NLR*, neutrophil/lymphocyte ratio; *WBC*, white blood cell count.
*$P < .05$.