皮，真皮への炎症細胞浸潤は乏しかった。

治療および経過：クロベタゾン酪酸エステル，タクロリムスの外用を開始したが，症状が続くため，トラスツズマブは継続したまま，パクリタキセルのみ中止したところ，紅斑は消退した。1ヵ月半後パクリタキセルを再開したが，現在まで，症状の再燃を認めていない。

考　察

パクリタキセルは，微小管蛋白重合を促進し，細胞分裂を阻害することで抗腫瘍効果をもつ化学療法薬で，乳癌，卵巣癌，非小細胞肺癌などの治療に用いられる。従来のパクリタキセルは，水に溶けないため，エタノールとポリオキシエチレンヒマシ油が添加されているが，アルブミン懸濁型パクリタキセル（アブラキサン®）は，アルコールを含まず，ヒト血清アルブミンに結合することで生理食塩水に懸濁することができる。パクリタキセルによる顔面紅斑の報告はStevens-Johnson症候群[1]，光線過敏症[2]，SLE[3]などの報告がある。またAdachiらはSS-A抗体陽性のシェーグレン症候群の患者においてパクリタキセル投与後に皮膚エリテマトーデスの紅斑が誘発された2症例を報告している[4]。しかし，われわれの症例は病理組織でinterface dermatitisの所見を欠き，臨床症状も含め，これらのいずれにも合致しない。Chuらは，タキサン系抗癌剤であるドセタキセルにより誘発されるFixed erythrodysaesthesia（あるいはerythrodysesthesia）plaqueの4症例を報告している[5]。薬剤の血管外や外傷と関係なく手背，前腕に出現し，紅斑はDusky，あるいはBizarre-shapedと表現される。ひりひりとした感覚を伴うことがあり，病理組織では表皮基底層の個細胞角化と核分裂像を伴い，これらは手足症候群の病理組織と類似する。一方で，Fixed erythrodysaesthesia plaqueは，手足症候群を合併せず，また，再投与で，紅斑が再現しないと報告されている[5]。もともと手足症候群はAcral erythemaのほかにPalmo-planter erythrodysaesthesiaとも呼ばれ，その手足以外の孤立性の皮疹をFixed erythrodysaesthesia plaqueと表現している。自験例2例でも，手足症候群の合併はみられなかった。われわれの調べえた限りではパクリタキセルによりFixed erythrodysaesthesia plaqueを生じたとの報告はなく，また，ドセタキセルにおいても，Fixed erythrodysaesthesia plaqueを顔面に生じたとの報告はなかった。2症例ともに紫外線量の多い時季に，パクリタキセルの投与が開始されていたが，紅斑を生じたのは秋から冬であり，本症が顔面といった露光部に生じた理由は紫外線といった光線が原因とは考えにくいものの，自験例2例は，臨床症状，経過，病理組織の特徴からFixed erythrodysaesthesia plaqueが，顔面に生じたものと考えられた。

自験例2例では，デキサメタゾンの全身投与を併用している間に紅斑が出現しており，症例1はステロイド外用にて改善がみられたが，症例2ではステロイド外用，タクロリムス外用でも改善がみられなかった。パクリタキセル開始から，症例1は1ヵ月半，症例2では7ヵ月してから発症したことは，通常のアレルギー機序の薬疹とは病態が異なることを示唆する。2症例ともに病理組織所見では炎症所見が乏しく，ステロイド外用の効果は表皮障害により二次的に起きた炎症を抑える可能性はあるものの，部分的と考えられる。Chuらの報告でもFixed erythrodysaesthesia plaqueの4症例において，1例のみベタメタゾンの外用を行っているが，本症に対するステロイド外用剤の有効性は不明である[5]。特徴的なのは，2症例ともにパクリタキセルの投与を，一時的に1ヵ月から1ヵ月半の間，中断したことにより，再投与しても紅斑の再燃はみられず，その後通常の投与間隔での継続投与が可能であった。このことからFixed erythrodysaesthesia plaqueの病態と考えられる細胞毒性あるいは表皮細胞の分化・成熟の異常は，必ずしも用量依存性ではなく，一時的な休薬によりリセットされるのではないかと推察される。

パクリタキセル投与中に生じる顔面の紅斑は，光線過敏症，エリテマトーデスによる場合は再投与が困難であるが，それ以外にもFixed erythrodysaesthesia plaqueのようにパクリタキセルの一時的な中断により，再投与しても再燃しない皮膚症状もあることから，患者の治療上の利益を損なわないためにも，再投与継続の可否について，慎重に見極めることが重要と考えられる。

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Two Cases of Facial Fixed Erythrodysaesthesia Plaque Induced by Paclitaxel

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Case 1 : A 67-year-old woman with metastatic breast cancer presented with bizarre-shaped erythema on the face and cubital fossa 6 weeks after biweekly albumin-bound paclitaxel for injectable suspension was started. Abraxane was discontinued for 4 weeks, and she was treated with topical steroid ointment. The erythema disappeared and did not recur after repeated readministration of paclitaxel for more than 6 months.

Case 2 : A 71-year-old woman with lymph node metastasis of breast cancer presented with erythema on the face with a tingling sensation 7 months after initiation of combination therapy with paclitaxel and trastuzumab. Although she was treated with topical steroids and tacrolimus, the symptoms did not improve. Only paclitaxel, but not trastuzumab, was discontinued for 6 weeks, and the erythema disappeared. Similar to case 1, facial erythema did not recur after the readministration of paclitaxel.

Skin biopsies of both cases showed epidermal dysmaturation with dyskeratotic cells, vacuolation degeneration, and inflammatory cell infiltration. Serum anti-nuclear antibody and anti-SSA/Ro antibodies were negative, indicating that lupus erythematosus induced by paclitaxel was unlikely. Therefore, we diagnosed these two cases as facial fixed erythrodysaesthesia plaque, which usually appears on the forearm by a similar pathological mechanism to the hand-foot skin reaction.

(J Environ Dermatol Cutan Allergol, 8 (2) : 109-113, 2014)

Key words : paclitaxel, facial erythema, fixed erythrodysaesthesia plaque

アレルギー 63(8),
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アレルギー用語解説シリーズ

p-i concept

Key words: hapten —— MHC —— p-i concept —— T cell receptor

概念と発見の経緯

薬剤の殆どは低分子（1KD以下）であり，それ自身は免疫原性をもたない．薬剤アレルギーを起こす条件として，これがハプテンとなって高分子の自己蛋白などと共有結合し，ハプテン抗原として樹状細胞によって処理（プロセシング）後，提示されて，反応する受容体をもった特異的T細胞に認識されることが必要とされていた（ハプテン説）．しかし，Pichilerらの樹立した薬剤反応性T細胞クローンの反応性は，ハプテン説では説明しえない奇異なものであった．この現象を説明するためには，薬剤がT細胞受容体，主要組織適合抗原（MHC）に緩く結合するだけでT細胞活性化を起こすことが必要であり，彼はこれをp-i (pharmacological interaction) conceptと名付けた[1]．

解　説

T細胞を介した薬剤アレルギーにおける抗原提示細胞（APC）の薬剤抗原認識様式として，1）ハプテン説（**図1A**）[2][3]，2）p-i concept（**図1BC**）[1][4]，および最近新たに提唱された3）薬剤結合MHCによるペプチドレパートリー変化によるもの[5][6]の3つがある．ハプテン抗原としては，ペニシリン，βラクタム系抗生剤，パラフェニレンジアミンなどが知られる．通常の蛋白抗原認識と同様に，ペニシロール基がMHCや自己蛋白のリジンに共有結合し，抗原として樹状細胞に取り込まれ，プロセッシングを経て感作T細胞に提示される．抗原に対する抗体も産生される．一方，p-i conceptでは，薬剤は緩くT細胞受容体（**図1B**）やMHC（**図1C**）に結合しただけで，速やかにT細胞を刺激する．この際，予めT細胞は薬剤感作を要さず，他の抗原によって感作された記憶T細胞であってもよい．薬剤抗原に対応する抗体は産生されない．緩い結合は共有結合と異なり，薬剤が受容体に結合するときに生じる結合と乖離を繰り返す特有のもので，イオン結合，水素結合，電気陰性度によるもの，ファンデルワールス力など種々総じてもたらされる親和性であることから，pharmacological interaction (p-i) とPichlerは呼んだ．臨床的には，初めての投与による薬剤アレルギー，代謝産物が反応性化合物でない薬剤による薬剤アレルギー，ハプテン抗原となりえない薬剤による皮膚貼付試験陽性は，p-i conceptによる反応を支持する所見である．in vitroでは，プロセッシングを必要としないAPCの抗原提示，薬剤添加後にAPC洗浄をおこなうとT細胞は無反応となること，薬剤添加後短時間にT細胞においてCaの流入やT細胞受容体の発現減少がおこることなどがみられる．現時点では，アバカビル，フルクロキサシン，カルバマゼピン，アロプリノール（オキシプリノール）において，p-i conceptによる反応の存在が証明されているが[7]，同じ薬剤のアレルギーに様々な薬剤認識様式が存在する可能性があり，これが臨床所見に多様性を与えていると考えられる[3]．

臨床的課題

p-i conceptの存在は容認されたが，検証された薬剤は少数である．薬剤アレルギーの頻度の高いものにおいて，本機構の存在の検索を広げる必要がある．本機構においては，その個体の感作T細胞，すなわち個体の経験が活性化するT細胞の数や質を決定付ける可能性がある．この機構によって生じる反応の詳細が明らかになれば，薬剤アレルギー治療や創薬の点からも新しい展望が開ける可能性がある．

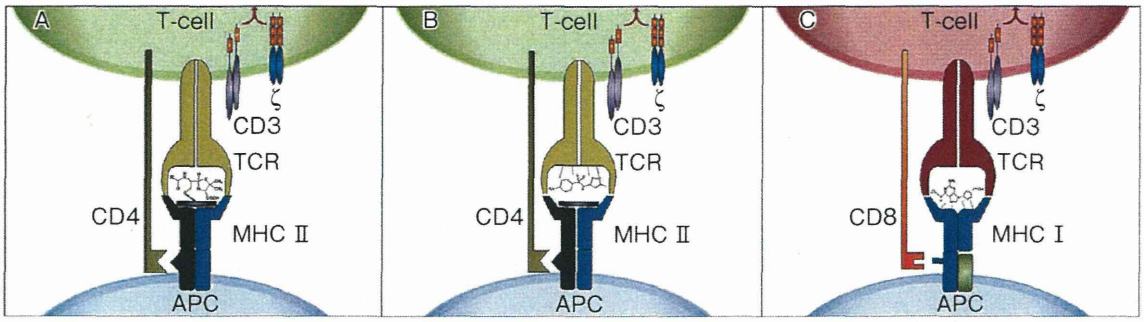

図1. ハプテン抗原認識（A）とp-i concept（B, C）. A. ハプテン抗原認識では，小分子薬剤は自己蛋白と共有結合することで新規抗原として認識される. B. サルファメキサゾールはT細胞受容体と緩く結合し，HLAとの相互作用でCD4陽性細胞を速やかに刺激する．厳格なHLA拘束は存在しない. C. アバカビルはHLA-Bに緩く結合し，T細胞受容体との接触によりT細胞を速やかに刺激する．（Adam J et al. Brit J Clin Pharm 2011より転載）

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★セルフチェック

1) p-i conceptを示唆する薬剤アレルギーの特徴はどれか？
   a) 抗原提示細胞による抗原プロセッシングを要する.
   b) MHC拘束性のT細胞反応である.
   c) 薬剤は自己蛋白と共有結合する.
   d) 薬剤添加によって速やかにT細胞受容体の発現減弱がみられる.
   e) 薬剤がMHCに結合し，T細胞は自己ペプチドを新抗原エピトープとして認識する.

正解

d)

Science Translational Medicine
AAAS

An annexin A1–FPR1 interaction contributes to necroptosis of keratinocytes in severe cutaneous adverse drug reactions

Nao Saito *et al.*

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Editor's Summary

Subduing a Severe Skin Side Effect

Certain pain relievers and antiepileptic drugs can cause a very rare, but sometimes fatal, side effect in which skin painfully blisters and peels, caused by the patients' immune response to the drug. Saito *et al.* now find that, in susceptible patients, the drug causes secretion of the protein annexin A1 from immune cells, with deadly effect on skin cells. Annexin acts on these cells to cause necroptosis, a programmed form of cell death. The authors confirmed their results in mice, showing that an inhibitor of necroptosis blocked skin blistering. With these findings, Saito *et al.* lay the groundwork for a countermeasure to this dangerous side effect of otherwise extremely beneficial drugs.

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CUTANEOUS DRUG REACTIONS

An annexin A1–FPR1 interaction contributes to necroptosis of keratinocytes in severe cutaneous adverse drug reactions

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Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening, cutaneous adverse drug reactions that are accompanied by keratinocyte cell death. Dead keratinocytes from SJS/TEN lesions exhibited necrosis, by morphological criteria. Supernatant from peripheral blood mononuclear cells (PBMCs) that had been exposed to the causative drug from patients with SJS/TEN induced the death of SJS/TEN keratinocytes, whereas supernatant from PBMCs of patients with ordinary drug skin reactions (ODSRs) exposed to the same drug did not. Keratinocytes from ODSR patients or from healthy controls were unaffected by supernatant from SJS/TEN or ODSR PBMCs. Mass spectrometric analysis identified annexin A1 as a key mediator of keratinocyte death; depletion of annexin A1 by a specific antibody diminished supernatant cytotoxicity. The necroptosis-mediating complex of RIP1 and RIP3 was indispensable for SJS/TEN supernatant–induced keratinocyte death, and SJS/TEN keratinocytes expressed abundant formyl peptide receptor 1 (FPR1), the receptor for annexin A1, whereas control keratinocytes did not. Inhibition of necroptosis completely prevented SJS/TEN-like responses in a mouse model of SJS/TEN. Our results demonstrate that a necroptosis pathway, likely mediated by annexin 1 acting through the FPR1 receptor, contributes to SJS/TEN.

INTRODUCTION

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare, life-threatening adverse drug reactions characterized by extensive detachment of the epidermis. They are considered as part of the same spectrum of diseases, but SJS patients have skin detachment on less than 10% of the body surface area, whereas TEN patients have more extensive lesions (*1*). After causative drug intake, the eruptions show erythema, and then the skin lesions spread to the whole body and become erosions. Mucous membranes are involved in about 90% of patients. Although SJS and TEN are rare (seven cases and two cases per million population for SJS and TEN, respectively), the mortality rates are high: up to 5 and 30% for SJS and TEN (*1*). The cause is thought to be the induction of an immunological reaction by the causative drugs. In patients with SJS/TEN, $CD8^+$ T cells are the predominant cell population that infiltrates the epidermis of the lesions (*2*), and drug-specific $CD8^+$ T cells proliferate predominantly in peripheral blood (*3*). Keratinocyte death in SJS/TEN has been thought to result from the action of cytotoxic cells or soluble factors such as soluble FasL or granulysin (*2*, *4*, *5*). Keratinocytes have been suggested to die by apoptosis (*4*), although the precise mechanism of keratinocyte death in SJS/TEN remains unclear.

Cell death generally has been thought to be initiated by a regulated signaling pathway, known as apoptosis, or by an unregulated process resulting from cellular damage, known as necrosis. This paradigm has been challenged by findings that necrosis can also result from programmed signaling (*6*). Under some conditions, stimulation with Fas ligand or tumor necrosis factor–α (TNF-α) can induce cell death that has the morphological features of necrosis (*7*, *8*). Recently, the RIP1/RIP3 complex was found to play a major role in necroptosis, and multiple small-molecule inhibitors of necroptosis, or "necrostatins," were discovered (*9*, *10*). Necroptosis is now recognized as a cellular defense mechanism against viral infections and as being critically involved in ischemia-reperfusion damage (*9*). Paneth cells in Crohn's disease have been reported to show programmed necrosis (*11*, *12*).

Here, we investigate how keratinocytes die in SJS/TEN. Drug-specific lymphocytes exist in patients who have recovered from drug allergies, including SJS/TEN (*13*–*15*). We searched for cytotoxic agents that might be secreted from these drug-specific lymphocytes by examining the supernatant of causative drug–exposed peripheral blood mononuclear cells (PBMCs) from recovered SJS/TEN patients.

RESULTS

SJS/TEN keratinocyte death by necroptosis

To investigate the nature of keratinocyte death in SJS/TEN, we examined the morphological changes in active lesions of SJS/TEN that showed marked epidermal cell death by electron microscopy (Fig. 1, A to C). To ensure that keratinocyte death was not necrosis resulting from ischemic or mechanical stress, we obtained all samples from erythematous lesions of SJS/TEN that showed nonbullous skin eruptions clinically and no epidermis-dermis detachment histologically. We found that some keratinocytes showed necrotic morphology, including membrane breakdown and numerous swollen cellular organelles (Fig. 1B). Other keratinocytes showed a reduction of cellular volume and chromatin condensation, features compatible with apoptotic morphology (Fig. 1C). Necrotic and apoptotic cells represented 16.1 ± 2.1% and 10.5 ± 0.7%, respectively, of all keratinocytes ($N = 80$) in the SJS/TEN lesions. Therefore, morphological apoptosis and necrosis both occur in erythematous lesions of SJS/TEN.

We next hypothesized that, upon initial drug stimulation, drug-specific lymphocytes secrete a soluble factor that induces widespread cutaneous

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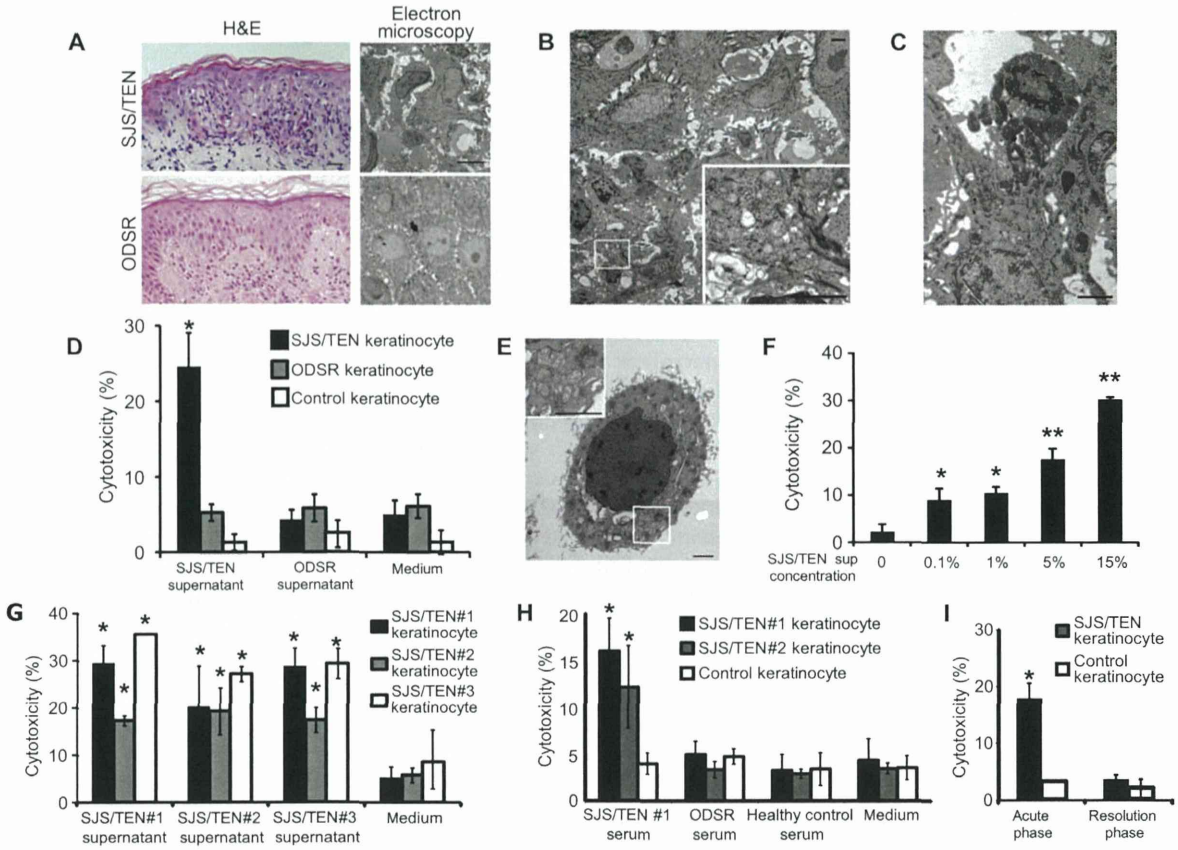

Fig. 1. Keratinocyte death in SJS/TEN shows necrotic and apoptotic morphology. (**A**) Morphological keratinocyte changes in SJS/TEN lesions and ODSRs were observed by hematoxylin and eosin (H&E) staining and electron microscopy, representative image. Scale bars, 10 μm (H&E) and 3 μm (electron microscopy). Skin samples were obtained from patient nos. 10 (early lesion) and 18 (early lesion). (**B** and **C**) Representative images of necrotic (B) and apoptotic (C) keratinocyte changes seen in SJS/TEN lesions by electron microscopy. (Inset) Swollen mitochondria. Scale bar, 5 μm. (**D**) Keratinocytes from SJS/TEN patients, ODSR patients, or healthy controls were exposed to supernatants from causative drug–exposed PBMCs for 8 hours. Cytotoxicity was measured by trypan blue staining ($n = 4$). *$P < 0.01$. Keratinocytes and PBMCs were obtained from patient no. 3 (postlesional skin), patient no. 14 (postlesional skin), and healthy control no. 6. (**E**) Representative image of SJS/TEN supernatant–exposed SJS/TEN keratinocytes showing necrotic morphology including swollen mitochondria (insert) by electron microscopy. Scale bar, 5 μm. Keratinocytes were obtained from patient no. 3 (postlesional skin). (**F**) Dose dependence of cytotoxicity by SJS/TEN supernatant (sup) was analyzed ($n = 4$). *$P < 0.05$; **$P < 0.01$. Keratinocytes and PBMCs were obtained from patient no. 5 (nonlesional skin). (**G**) Cytotoxicity of SJS/TEN supernatant (5%) on SJS/TEN keratinocytes from three patients. Each experiment was repeated five times. *$P < 0.01$ versus medium. Keratinocytes and PBMCs were obtained from patient nos. 3 (postlesional skin), 4 (nonlesional skin), and 8 (nonlesional skin). (**H**) Keratinocytes from SJS/TEN patients or healthy controls were exposed to sera of patients with SJS/TEN or ODSR, and healthy controls for 8 hours. Cytotoxicity was measured by trypan blue staining ($n = 4$). *$P < 0.01$. Keratinocytes were obtained from patient no. 4 (nonlesional skin), patient no. 10 (postlesional skin), and healthy control no. 9. Sera were obtained from patient no. 4, patient no. 18, and healthy control no. 2. (**I**) Cytotoxicity of SJS/TEN serum during disease onset (acute phase) and after recovery (resolution phase) ($n = 5$). *$P < 0.01$. Keratinocytes were obtained from patient no. 1 (postlesional skin) and healthy control no. 5. Sera were obtained from patient no. 1.

detachment through keratinocyte death. The lymphocytes that specifically reacted with the causative drug then may remain in peripheral blood of recovered SJS/TEN patients, and upon reexposure to the causative drug, these lymphocytes would again secrete the key soluble factor(s). To test for the presence of causative drug–specific lymphocytes in peripheral blood in recovered patients, we collected PBMCs from patients ($n = 6$) who had recovered from SJS/TEN 1 to 5 years before. Enzyme-linked immunospot (ELISPOT) analysis of human interferon-γ (IFN-γ) secretion was conducted to detect antigen-specific human cells; we detected causative drug–specific lymphocytes (fig. S1). After in vitro reexposure to the causative drug, the number of drug-specific lymphocytes increased markedly (fig. S1). These data confirmed that, even after the resolution of SJS/TEN, drug-specific lymphocytes still circulate, as reported (*13*, *14*).

To test for toxic agents that might be secreted by drug-specific lymphocytes, we exposed PBMCs from recovered SJS/TEN patients to the causative drugs and then collected the supernatants. Treatment of keratinocytes from SJS/TEN patients with SJS/TEN supernatant resulted in cell death, whereas treatment of SJS/TEN keratinocytes with supernatant from PBMCs from patients with ordinary drug skin reactions (ODSRs) and other types of severe adverse drug reactions [drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS)] had no effect on keratinocytes (Fig. 1D and fig. S2). Keratinocytes from

ODSR patients or healthy controls were unaffected by SJS/TEN and ODSR supernatant.

We examined the morphological changes in supernatant-treated keratinocytes by electron microscopy. The supernatant-exposed keratinocytes showed swollen mitochondria and blebbing of the cellular membrane, reactions compatible with necrotic morphology (Fig. 1E). Necrotic and apoptotic cells accounted for 76.7 ± 5.8% and 23.3 ± 5.8% of dead keratinocytes (n = 35), respectively.

SJS/TEN supernatant induced cytotoxicity against SJS/TEN keratinocytes in a dose-dependent manner (Fig. 1F). SJS/TEN supernatant from three recovered patients and SJS/TEN keratinocytes from the same patients showed significant cytotoxicity in each combination (Fig. 1G). Furthermore, we analyzed the cytotoxicity of supernatants from PBMCs of a SJS/TEN patient (case 5 in table S2) exposed to an irrelevant drug (amoxicillin). The supernatants from PBMCs exposed to the irrelevant drug did not induce cytotoxicity (fig. S3).

Because the previous experiments used samples from patients who had recovered from SJS/TEN, we tested whether a cytotoxic soluble factor was also present in peripheral blood during the active phase of SJS/TEN. Sera from patients with active SJS/TEN were incubated with SJS/TEN keratinocytes and found to cause cytotoxicity, whereas sera from patients with ODSR did not (Fig. 1H). The keratinocytes of ODSR patients or healthy controls were unaffected by SJS/TEN and ODSR sera. In addition, we investigated the direct cytotoxicity of sera from patients who were in the SJS/TEN recovery phase. These sera did not induce cytotoxicity (Fig. 1I).

To determine whether keratinocytes taken from healed postlesional skin and keratinocytes taken from nonlesional skin differed in sensitivity to the putative toxic agent, we compared SJS/TEN PBMC supernatant–induced cytotoxicity in keratinocytes from these two sites (fig. S4A). We obtained the cultured keratinocytes from normal-appearing skin that had been lesional during the acute phase but that had returned to normal (postlesional; n = 3) or from normal-appearing skin that was never lesional (nonlesional; n = 4). SJS/TEN PBMC supernatant induced comparable cytotoxicity in both cases (fig. S4B).

Apoptosis is dependent on the activation of caspases; necroptosis is not influenced by caspase inhibition but is blocked by necrostatin-1 (Nec-1), an inhibitor of the kinase activity of RIP1 and by RIP3 inhibition (*9*). To test whether SJS/TEN supernatant–induced cytotoxicity is apoptosis, we investigated the cleavage of poly(adenosine 5′-diphosphate–ribose) polymerase (PARP), a substrate of cleaved caspase-3. Cleaved PARP was not detected in keratinocytes treated with SJS/TEN supernatant (Fig. 2A).

To further investigate the mechanism of SJS/TEN supernatant–induced cytotoxicity, we examined the effect of the pan-caspase inhibitor zVAD and the necroptosis inhibitor Nec-1 on SJS/TEN supernatant–induced keratinocyte death. Although zVAD did not inhibit cytotoxicity, Nec-1 completely inhibited cytotoxicity (Fig. 2B). In addition, to clarify the role of RIP3 in our cytotoxic process, we knocked down RIP3 with small interfering RNA (siRNA), which significantly decreased the cytotoxicity of the SJS/TEN supernatant (Fig. 2C). In the SJS/TEN lesions, the keratinocytes showed abundant RIP3 expression, just as necroptotic Paneth cells do in Crohn's disease (*12*). In contrast, no cells showed RIP3 expression in ODSR lesions (Fig. 2D). These data suggest that necroptosis can contribute to keratinocyte death in SJS/TEN.

Some cell lines are capable of undergoing necroptosis in response to cytokines of the TNF family (*16*). In this pathway, TNF-α reacts with the TNF-α receptor, forming a complex with FADD (Fas-associated protein with death domain) and RIP1/RIP3, after MLKL phosphorylation (*17*, *18*). To investigate whether these molecules also control keratinocyte-programmed necrosis, we analyzed the expressions of RIP1, RIP3, FADD, and CYLD in keratinocytes. Expression levels of these molecules varied among keratinocytes from SJS/TEN patients, ODSR patients, or healthy controls (fig. S5), indicating that the levels of these molecules were not regulating the susceptibility to keratinocyte necroptosis.

Necroptosis by annexin A1–formyl peptide receptor 1 interaction

To try to identify the necroptosis mediators in the SJS/TEN supernatant, we tested apoptosis inducers such as granulysin and necroptosis agents such as TNF-α, poly(I:C) (polyinosinic-polycytidilic acid), or LPS (lipopolysaccharide), but found that they failed to induce SJS/TEN keratinocyte death (fig. S6). Therefore, we performed mass spectrometry [liquid chromatography–tandem mass spectrometry (LC-MS/MS)] of SJS/TEN and ODSR supernatants and identified the protein annexin A1 as significantly more abundant in SJS/TEN supernatant than in ODSR supernatant (table S1 and Fig. 3A). To test the importance of annexin A1, we depleted it from SJS/TEN supernatant using a specific annexin A1 antibody, which significantly blocked SJS/TEN supernatant–induced keratinocyte death (Fig. 3B). Moreover, the annexin A1–mimetic peptide Ac2-26 induced cytotoxicity in SJS/TEN keratinocytes, but not in healthy control keratinocytes

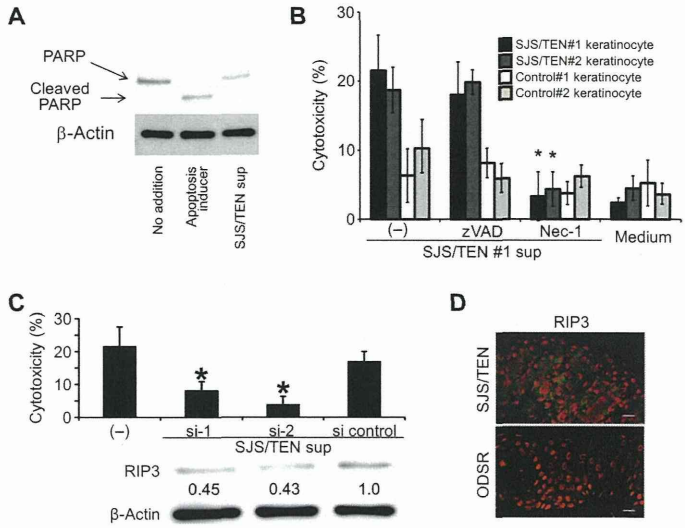

Fig. 2. Keratinocyte death by PBMC supernatant from SJS/TEN patients is mediated by necroptosis. (**A**) PARP cleavage assay was performed with apoptosis inducer (2 μM staurosporine) or SJS/TEN supernatant (5%). Keratinocytes and PBMCs were obtained from patient no. 3 (postlesional skin). The experiments were repeated three times, and representative data are shown. (**B**) Effects of the pan-caspase inhibitor zVAD (50 μM) or Nec-1 (50 μM) on cytotoxicity were analyzed (n = 4). *P < 0.01 versus SJS#1 sup alone. Keratinocytes were obtained from patient no. 3 (postlesional skin), patient no. 4 (nonlesional skin), healthy control no. 8, and healthy control no. 10, and PBMCs from patient no 3. (**C**) RIP3 was knocked down with siRNA (si-1 or si-2) in SJS/TEN keratinocytes, and SJS/TEN supernatant–induced cytotoxicity was analyzed (n = 4). Densitometric values are shown as percent optical density of RIP3 in siRNA-transfected cells after β-actin normalization. *P < 0.05 versus siRNA control. Keratinocytes and PBMCs were obtained from patient no. 3 (postlesional skin). (**D**) Representative image of RIP3 expression in SJS/TEN lesions and ODSRs. Nuclei were stained with propidium iodide (PI). Scale bars, 10 μm. Skin samples were obtained from patient nos. 10 (early lesion) and 18 (early lesion).

(Fig. 3C). Although annexin A1 is an intracellular molecule, it is also secreted from $CD14^+$ monocytes, where it acts as an immunosuppressant (*19*). To test for the source of annexin A1, we depleted $CD14^+$ monocytes from SJS/TEN PBMCs that were exposed to the causative drug, and these PBMCs failed to induce SJS/TEN keratinocyte death (Fig. 3D), confirming $CD14^+$ cells as the likely source of annexin A1. Indeed, $CD14^+$ cells are present in SJS/TEN skin lesions (*20*).

$CD14^+$ monocytes can be divided into at least two populations: $CD14^{bright}$ classical monocytes and $CD14^{dim}$ proinflammatory monocytes. Both cell types induced cytotoxicity (fig. S7). Monocyte activation is not mediated by a specific antigen. However, we and other groups have reported that $CD8^+$ cells and major histocompatibility complex (MHC) class I are indispensable in SJS/TEN pathogenesis (*4*, *15*). Therefore, we suggest that $CD8^+$ cell activation by a specific antigen (the causative drug) or by MHC class I is critical for the secretion of annexin A1 from monocytes. First, supernatant from $CD14^+$-depleted PBMCs failed to induce cytotoxicity (Fig. 3D). Furthermore, supernatant from $CD14^+$ plus CD14-depleted supernatant succeeded in killing keratinocytes (Fig. 3E). Supernatant

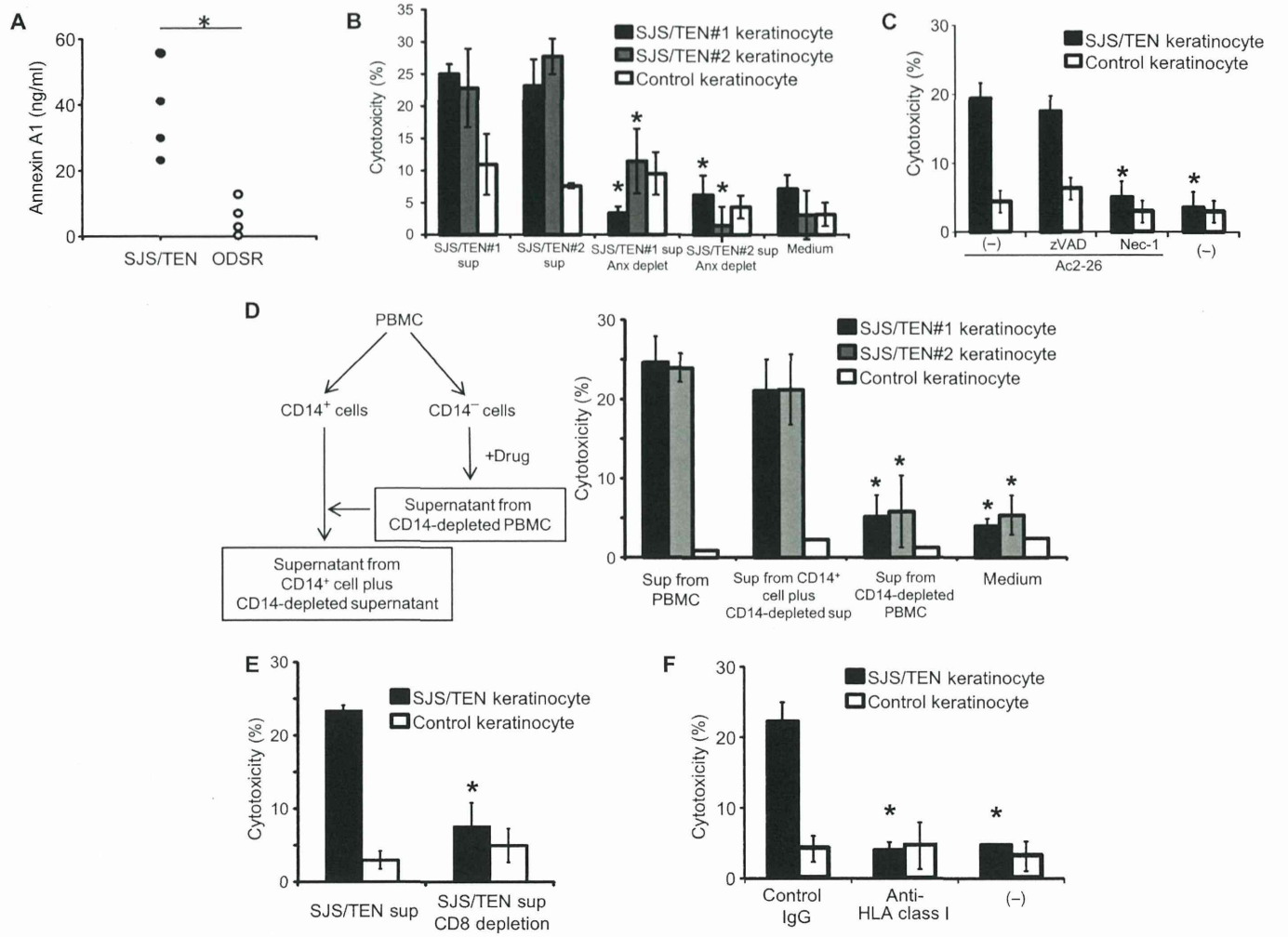

Fig. 3. Annexin A1 mediates necrosis caused by PBMC supernatant from SJS/TEN patients. (**A**) Annexin A1 concentrations were measured by annexin A1 peptide enzyme-linked immunosorbent assay (ELISA) in supernatants collected from causative drug–exposed PBMCs of SJS/TEN ($n = 4$) and ODSR ($n = 4$) patients. *$P < 0.05$. PBMCs were obtained from patient nos. 3, 4, 5, 6, 11, 12, 14, and 17. Each point was measured three times. (**B**) Cytotoxicity assay using annexin A1–depleted SJS/TEN supernatant ($n = 5$). *$P < 0.05$ versus SJS/TEN sup. Keratinocytes and PBMCs were obtained from patient no. 3 (postlesional skin) and no. 5 (nonlesional skin). Keratinocytes were obtained from healthy control no. 4. (**C**) Effect of annexin A1 peptide (Ac2-26) (50 ng/ml) on cytotoxicity in SJS/TEN keratinocytes ($n = 5$). *$P < 0.05$ versus Ac2-26. Keratinocytes were obtained from patient no. 5 (nonlesional skin) and healthy control no. 3. (**D**) Effect of CD14-depleted supernatant and supernatant from $CD14^+$ cells plus CD14-depleted supernatant on cytotoxicity of keratinocytes. ($n = 5$). *$P < 0.01$ versus supernatant from PBMC. Keratinocytes were obtained from patient no. 3 (postlesional skin), patient no. 5 (nonlesional skin), and healthy control no. 7. PBMCs were obtained from patient no. 3. (**E**) Effect of $CD8^+$ cells on SJS/TEN PBMC supernatant–induced cytotoxicity. Supernatant from $CD8^+$ cell–depleted SJS/TEN PBMCs with causative drug exposure was analyzed for cytotoxicity ($n = 5$). *$P < 0.05$ versus SJS/TEN supernatant. Keratinocytes were obtained from patient no. 3 (postlesional skin) and healthy control no. 8. PBMCs were obtained from patient no. 3. (**F**) Effect of anti–MHC class I antibody on SJS/TEN supernatant–induced cytotoxicity. SJS/TEN PBMCs were preincubated for 30 min at 37°C with anti–MHC I antibody (10 μg/ml) or control mouse IgG ($n = 5$). *$P < 0.05$ versus SJS/TEN supernatant. Keratinocytes were obtained from patient no. 10 (postlesional skin) and healthy control no. 5. PBMCs were obtained from patient no. 2.

from $CD8^+$-depleted PBMCs did not induce cytotoxicity (Fig. 3F). In addition, we collected supernatant from causative drug–exposed PBMCs that had been cultured with neutralizing MHC class I antibody (W6/32). The cytotoxicity of the supernatant was greatly decreased; in contrast, the supernatant from causative drug–exposed PBMCs that had been cultured with control mouse immunoglobulin G (IgG) did not show reduced cytotoxicity (Fig. 3G). Together, these data show that $CD8^+$ cell activation by a specific antigen (the causative drug) or by MHC class I is critical to cytotoxicity.

Finally, we investigated the roles of $CD14^+$ and $CD8^+$ cells in SJS/TEN model mice that we have recently developed (*15*) (Fig. 4A). These mice, generated by using SJS/TEN PBMCs and causative drugs (*15*), show eye manifestations of disease (marked conjunctival congestion) (Fig. 4B). If we used $CD14^+$-depleted PBMCs or $CD8^+$-depleted PBMCs during generation of these model mice, the development of the conjunctival congestion was prevented. $CD8^+$-depleted PBMCs also failed to induce SJS/TEN-like symptoms (conjunctival epithelial cell death) in the model mice (Fig. 4C).

Annexin A1 binds to formyl peptide receptor 1 (FPR1) and acts via that receptor (*19*). FPR1 is in the family of G protein (heterotrimeric guanine nucleotide–binding protein)–coupled receptors and is associated with tissue damage (*21*). When treated with SJS/TEN supernatant, SJS/TEN keratinocytes expressed abundant FRP1 in vitro, whereas

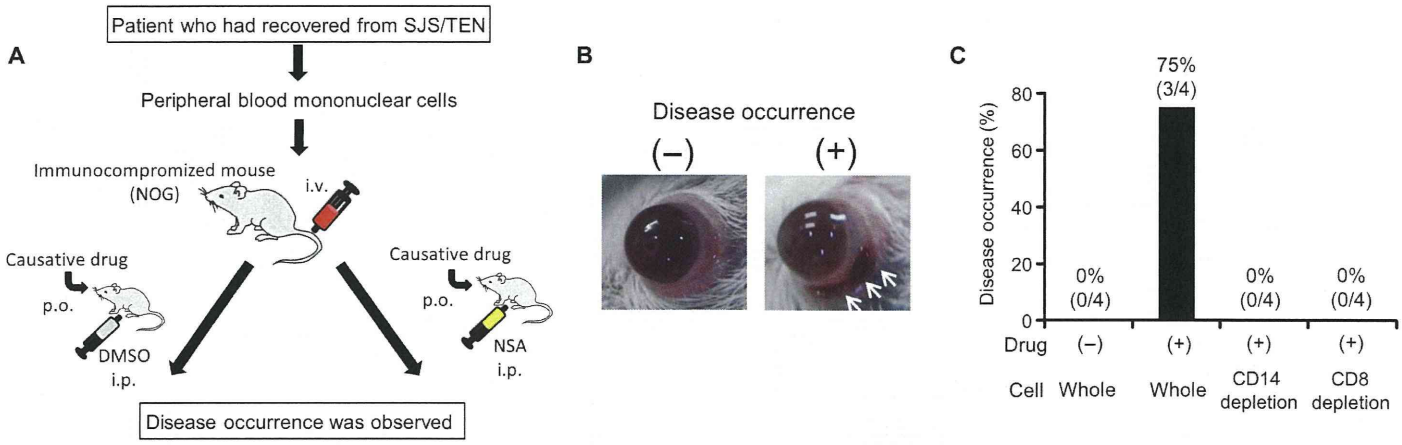

Fig. 4. $CD14^+$ and $CD8^+$ cells are required for pathogenesis in a mouse model of SJS/TEN model mice. (**A**) PBMCs were obtained from patients who had recovered from SJS/TEN. PBMCs, $CD14^+$-depleted PBMCs, or $CD8^+$-depleted PBMCs (2×10^6) were injected intravenously into NOG [nonobese diabetic (NOD)/Shi-scid, interleukin-1 receptor (IL-2R) null] mice, followed by oral administration of the causative drug. The dosage used in the model mice was based on mg/kg body weight converted from human adult normal dose. We administered the drug to the mice once daily. In addition, these mice received necrosulfonamide (NSA) or dimethyl sulfoxide (DMSO) intraperitoneally. The mice were observed for eye manifestations of disease. PBMCs were obtained from patient nos. 2 and 3. (**B**) SJS/TEN model mice were established by intravenous injection of PBMCs obtained from SJS/TEN patients and oral administration of the causative drugs. SJS/TEN model mice showed eye dysfunction (marked conjunctival congestion), as shown in the representative photos. PBMCs were obtained from patient no. 3. (**C**) Effect of CD14 and CD8 depletion on the ability of SJS/TEN PBMCs to cause SJS-like disease in model mice ($n = 4$).

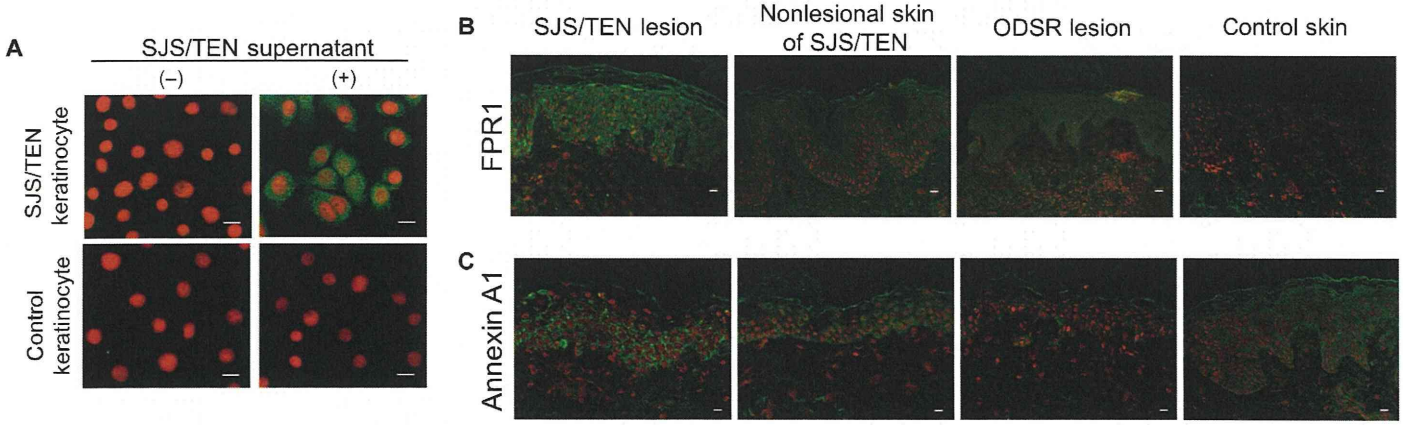

Fig. 5. SJS/TEN keratinocytes express FRP1. (**A**) Representative images of FRP1 in cultured keratinocytes from SJS/TEN patients or healthy controls. Cultured cells were treated with or without SJS/TEN supernatant (5%) (4 hours) and were stained for FRP1 with an antibody ($n = 3$). Representative data are shown. Nuclei were stained with PI. Scale bars, 5 μm. Keratinocytes were obtained from patient no. 3 (postlesional skin) and healthy control no. 4. (**B** and **C**) Representative images showing expression of FRP1 and annexin A1 in SJS/TEN lesions, nonlesional skin of SJS/TEN patients, ODSR lesions, and control skin. Nuclei were stained with PI. Scale bars, 10 μm. Skin samples were obtained from patient no. 10 (acute lesion and nonlesional skin), patient no. 18 (acute lesion), and healthy control no. 4.