

厚生労働科学研究費補助金「創薬基盤推進」
分担研究報告書

重症薬疹における病期ごとの Th2 サイトカインの検討

分担研究者 小豆澤 宏明

大阪大学大学院医学系研究科内科系臨床医学専攻情報統合医学皮膚学 助教

研究要旨 重症薬疹である Stevens-Johnson 症候群 (SJS)、中毒性表皮壊死症 (TEN) の患者では T ヘルパー 1 (Th1) 細胞が産生するサイトカインの関与が知られている。一方で薬剤性過敏症症候群 (DIHS) 患者ではヒトヘルペスウイルス 6 型 (HHV-6) をはじめとして、ヘルペスウイルスの再活性化がおこることが知られ、Th2 細胞が産生するサイトカインが関連している。我々は重症薬疹患者の血清を用いて、SJS/TEN 3 例、DIHS 3 例のそれぞれ急性期、回復期について血清中の IL-5、MDC (CCL22) の濃度を比較した。IL-5、MDC はともに重症薬疹の DIHS の急性期で高い傾向であった。MDC は TARC とならんでケモカイン受容体である CCR4 のリガンドでありバイオマーカーとして有用と考えられた。

A. 研究目的

Stevens-Johnson 症候群 (SJS)/中毒性表皮壊死症 (TEN) と薬剤性過敏症症候群 (DIHS) はともに重症薬疹である。DIHS は、通常薬疹とことなり、遷延することから、そのバイオマーカーは重要であると考えられる。

Ogawa らは thymus and activation-regulated chemokine

(TARC/CCL17) が DIHS の急性期に上昇することを報告し、バイオマーカーとしての有用性を報告している (引用文献 1)。TARC は、T 細胞が発現するケモカイン受容体である CCR4 のリガンドであることが知られ、アトピー性皮膚炎の病勢とも相関することから、アトピー性皮膚炎のバイオマーカーとして有用であることが知られ、保険適応の検査となっている。

IL-5 はヒトでは好酸球や好塩基球に作用し、好酸球の分化・増殖因子としてしられる。薬疹では、好酸球増多が見られることが多いことから、その病態には重要である。

Macrophage-derived chemokine (MDC, CCL22) は、マクロファージや樹状細胞により産生される CC ケモカインであり、Th2 サイトカインにより産生が増強されるアトピー性皮膚炎などのアレルギー性疾患でも上昇することが知られている。

今回我々は、Th2 サイトカインとしてしられる IL-5 と MDC について検討することで新たな重症薬疹のバイオマーカーの検討をお

こなった。

B. 研究方法

TEN3 例、DIHS3 例それぞれの急性期、回復期と健常人 3 名の血清を用いて IL-5、MDC (CCL22) の濃度を V-PLEX Kit (Meso Scale Discovery) により計測した。

(倫理面への配慮)

中毒疹が疑われた患者を対象にこの臨床研究への参加について説明と同意を得た上で採血を行った。「中毒疹における原因疾患の早期鑑別診断法の開発」という研究課題名で大阪大学医学部附属病院 臨床研究倫理審査委員会より平成 20 年 8 月 25 日より平成 28 年 7 月 31 日までの許可 (承認番号 08088-4) を受けた所定の説明書と同意書を用いた。

C. 研究結果

IL-5 は重症薬疹の急性期に高い傾向があり、SJS/TEN の急性期では平均 65pg/ml であったのに対して、DIHS の急性期で平均 318pg/ml であり高い傾向であった。(図 1) MDC については健常人で平均 3677pg/ml であったのに対して、DIHS の急性期では平均 21986pg/ml であった。(図 2)。

D. 考察

重症薬疹の中でもDIHSは薬剤によりT細胞が活性化することで、Th2サイトカインが強く誘導される。IL-5は、SJS/TENとDIHSの両方の急性期で上昇がみられたが、MDCはDIHSの急性期でのみ上昇がみられた。

E. 結論

今回の検討では症例数がすくないこともあり、TENやDIHSといった薬疹の臨床病型ごとの特徴を検討するには症例数を増やす必要があるが、MDCはDIHSの病勢におけるバイオマーカーとしての有用性が示唆された。

F. 健康危険情報

該当なし。

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H. 知的財産権の出願・登録状況(予定を含む)
該当ございません。

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図 1

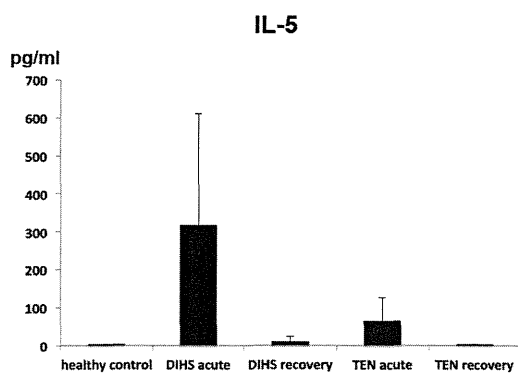
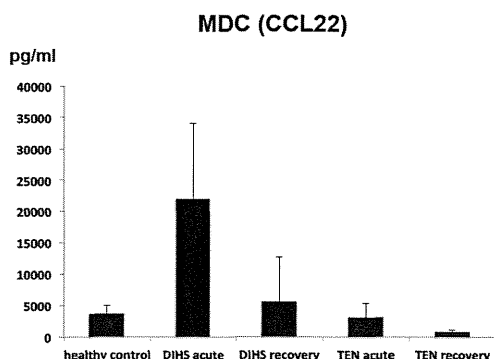


図 2



Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

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<u>小豆澤 宏明</u>	【薬物アレルギー-疑うべきポイントと対処法】 薬物アレルギーの発症機序	薬事	56	2135-2140	2014
<u>小豆澤 宏明</u>	重症薬疹の診断と治療 アップデート】 重症薬疹の検査	アレルギー・免疫	1	1240-1246	2014
<u>小豆澤 宏明</u> 、 横見 明典、 谷 守、 室田 浩之、 中山 貴寛、 玉木 康博、 野口 眞三郎、 片山 一朗	バクリタキセル投与中にみられた顔面紅斑の2例	Journal of Environmental Dermatology and Cutaneous Allergology	8	109-113	2014

IV. 研究成果の刊行物・別刷

CUTANEOUS DRUG REACTIONS

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

Nao Saito,¹ Hongjiang Qiao,¹ Teruki Yanagi,¹ Satoru Shinkuma,¹ Keiko Nishimura,¹ Asuka Suto,¹ Yasuyuki Fujita,¹ Shotaro Suzuki,¹ Toshifumi Nomura,¹ Hideki Nakamura,¹ Koji Nagao,² Chikashi Obuse,² Hiroshi Shimizu,^{1*} Riichiro Abe^{1*}

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 $16.1 \pm 2.1\%$ and $10.5 \pm 0.7\%$, 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

¹Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan. ²Graduate School of Life Science, Hokkaido University, Sapporo 060-0810, Japan. *Corresponding author. E-mail: aberi@med.hokudai.ac.jp (R.A.); shimizu@med.hokudai.ac.jp (H.S.)

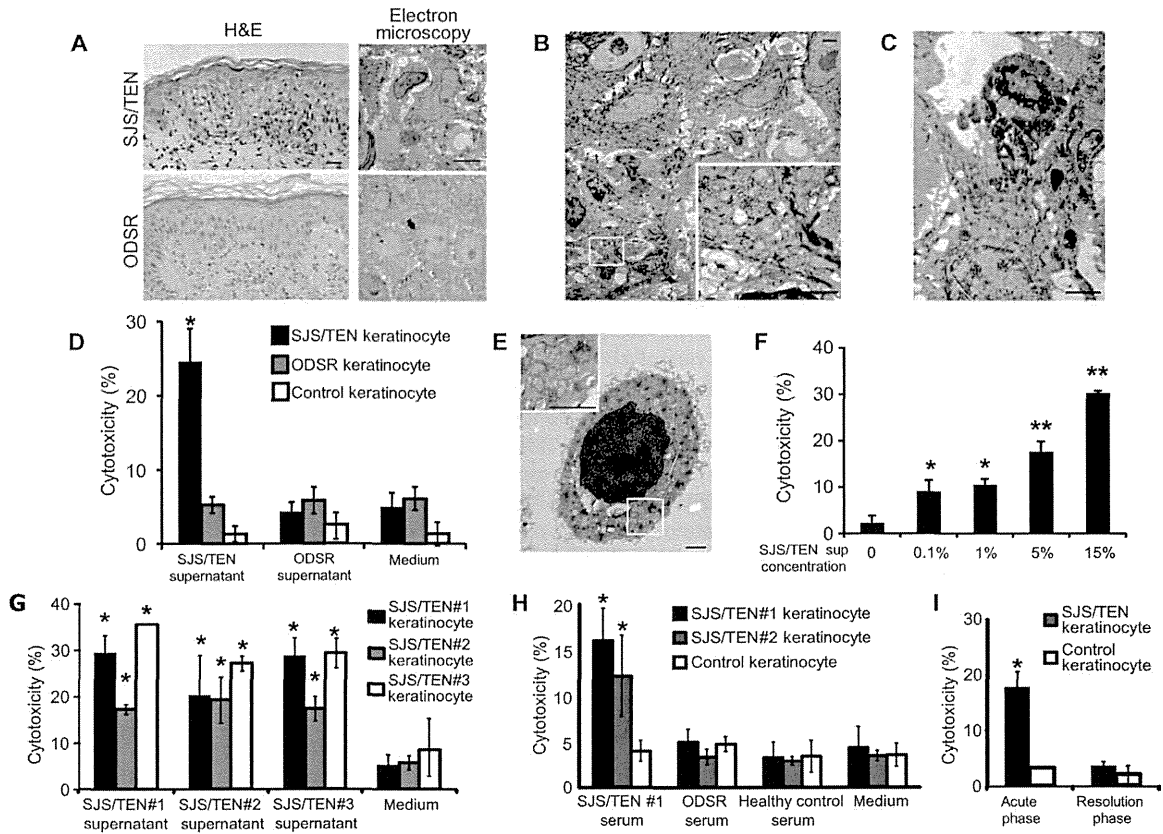


Fig. 1. Keratinocyte death in SJS/TEN shows necrotic and apoptotic morphology. (A) Morphological keratinocyte changes in SJS/TEN lesions and ODSRs were observed by hematoxylin and eosin (H&E) staining and electron microscopy, representative image. Scale bars, 10 μ m (H&E) and 3 μ m (electron microscopy). 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 μ m. (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 μ m. 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 con-

firmed 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 $76.7 \pm 5.8\%$ and $23.3 \pm 5.8\%$ 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

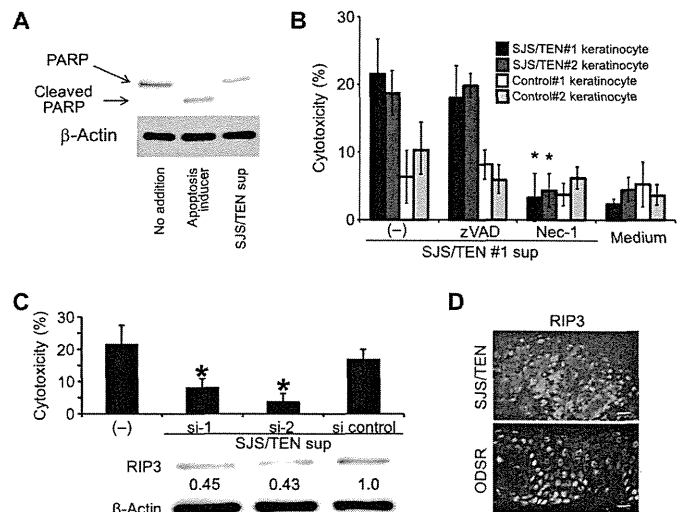


Fig. 2. Keratinocyte death by PBMC supernatant from SJS/TEN patients is mediated by necroptosis.

(A) PARP cleavage assay was performed with apoptosis inducer ($2 \mu\text{M}$ 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 ($50 \mu\text{M}$) or Nec-1 ($50 \mu\text{M}$) 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, $10 \mu\text{m}$. 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

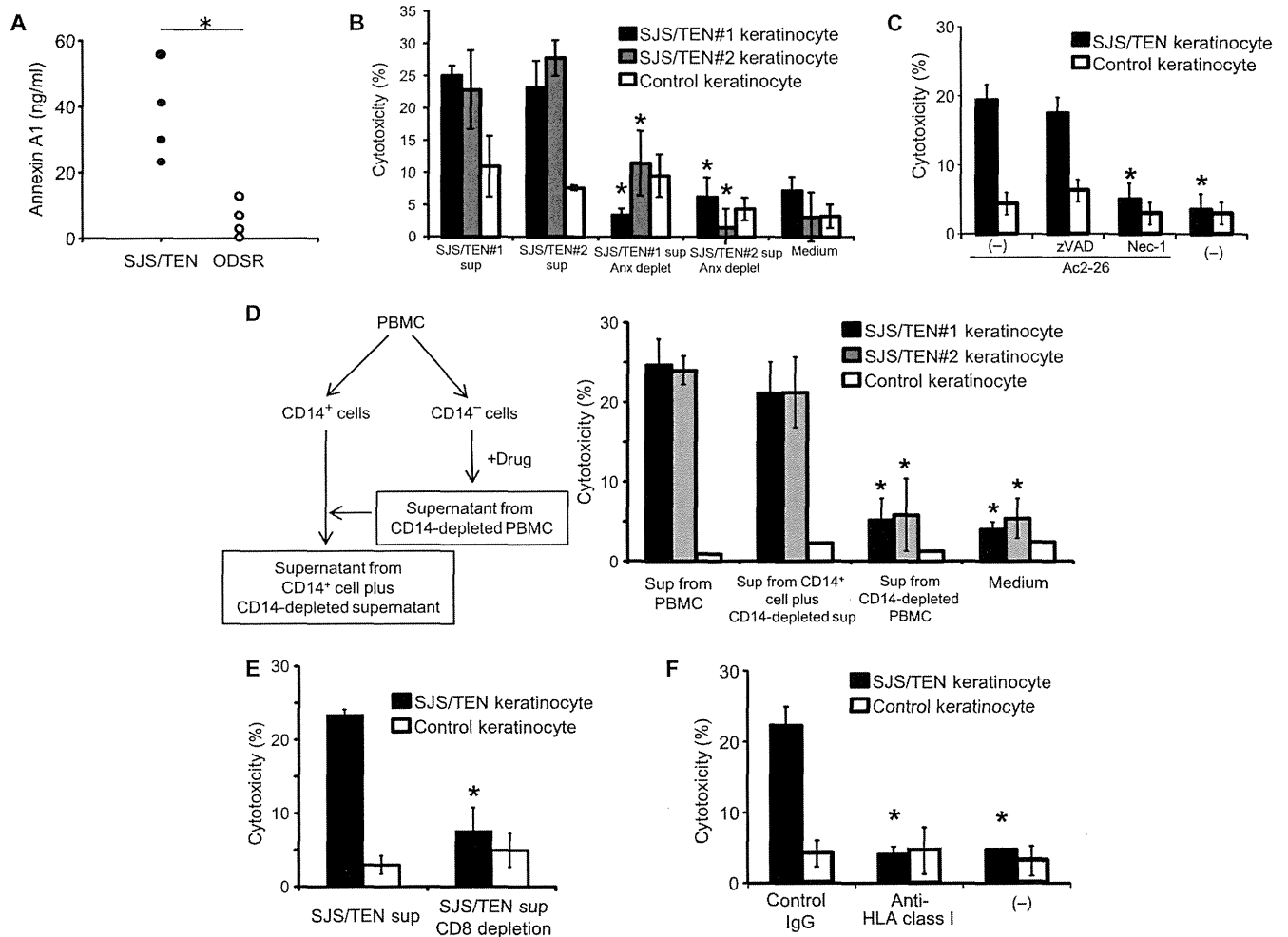


Fig. 3. Annexin A1 mediates necrosis caused by PBMC supernatant from SJS/TEN patients. (A) Annexin A1 concentrations were measured by annexin A1 peptide enzyme-linked immunosorbent assay (ELISA) in supernatants collected from causative drug-exposed PBMCs of SJS/TEN ($n = 4$) and ODSR ($n = 4$) patients. $*P < 0.05$. 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 super-

natant 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

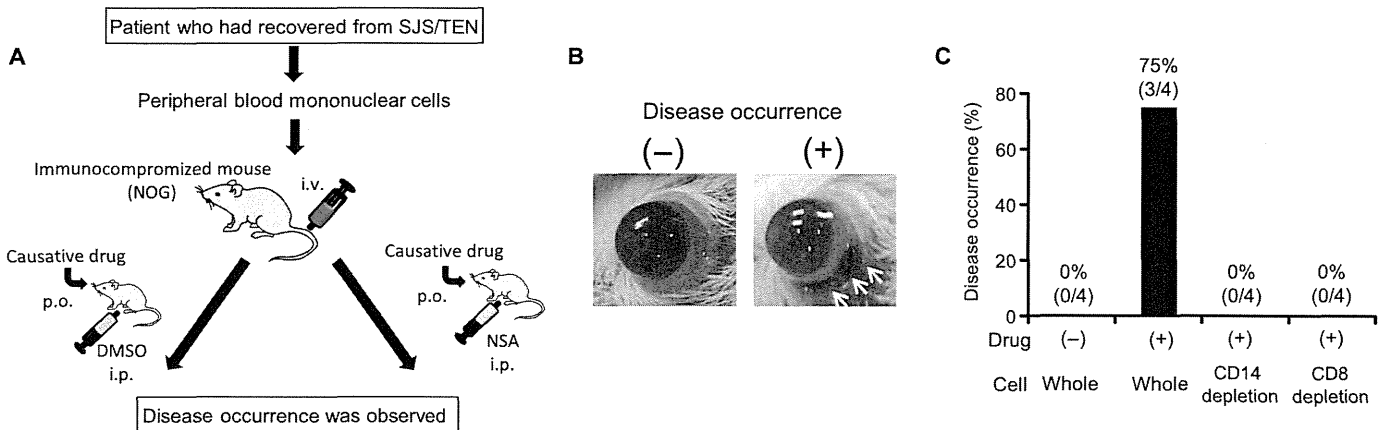


Fig. 4. CD14⁺ and CD8⁺ cells are required for pathogenesis in a mouse model of SJS/TEN model mice. (A) PBMCs were obtained from patients who had recovered from SJS/TEN. PBMCs, CD14⁺-depleted PBMCs, or CD8⁺-depleted PBMCs (2 × 10⁶) were injected intravenously into NOG [nonobese diabetic (NOD)/Shi-scid, 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).

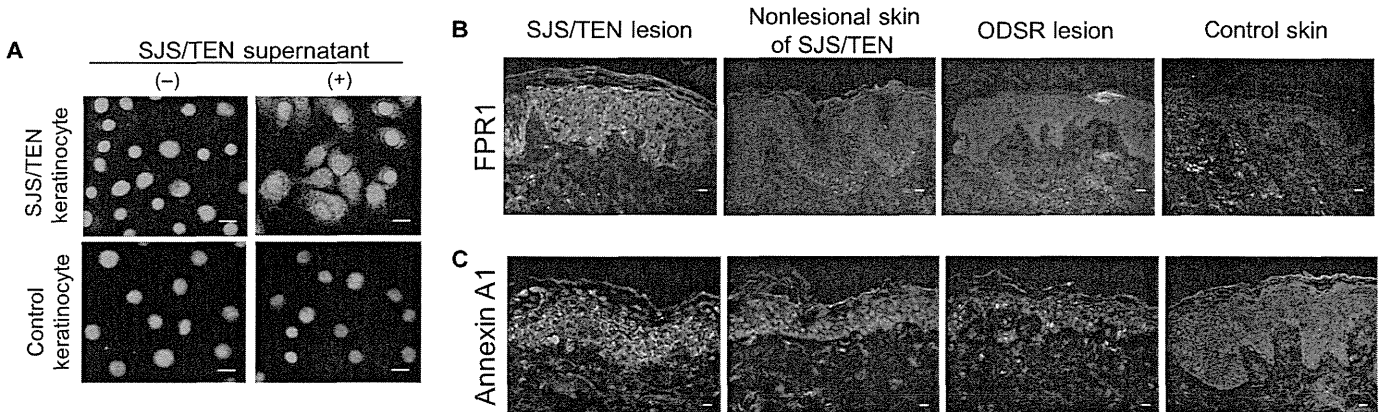


Fig. 5. SJS/TEN keratinocytes express FRP1. (A) Representative images of FRP1 in cultured keratinocytes from SJS/TEN patients or healthy controls. Cultured cells were treated with or without SJS/TEN supernatant (5%) (4 hours) and were stained for FRP1 with an antibody (n = 3). Representative data are 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.