

図 1.
DLST の原理
原因薬に反応するリンパ球が存在する場合は、細胞分裂を経てアイソトープを取り込み CPM (counts per minute) が高値となる。

まかなプロトコールを記す。

ヘパリン採血された末梢血を比重遠心にかけて、単核球分画を回収する。洗浄された細胞は、自己血漿を 20% 含む液体培地に、リンパ球数にして $1 \times 10^6/\text{ml}$ の濃度で調整する。このうち $100 \mu\text{l}$ (すなわち 1×10^5 個の単核球) を細胞培養用のウェルに分注する。被検査薬剤は 6 濃度で 2 重測定 (投与量の $1/190 \sim 1/46170$ の 3 倍希釈系列)、陰性コントロール (薬剤非添加) は 6 重測定、陽性コントロール (PHA $20 \mu\text{g}/\text{ml}$) は 2 重測定で行われている。

これを 4 日間培養し、培養終了 1 日前に ³H-thymidine を加え、次の日に細胞を回収し、液体シンチレーションカウンターで細胞に取り込まれた ³H-thymidine を定量化する。被検査薬剤を添加したウェルと陰性コントロールの CPM (counts per minute) の比で stimulation index を算出する。原因薬刺激によって細胞が分裂した際に ³H-thymidine が細胞に取り込まれるため (図 1)、陰性コントロールと比して CPM が高くなることが期待される。陰性コントロールの CPM 値を分母とし、薬剤を添加した検体の CPM 値との比が stimulation index (SI) 値である。SI 値 1.8 以上 (100 の係数を掛けている場合は 180) が陽性である。

DLST の読み方

表 1 に DLST 報告書の一例を示す。まず見るべきは陽性および陰性コントロールの CPM 値である。検査会社によっては陽性コントロールの値をルーチンには提供していないので、注意されたい。陽性コントロールである PHA 刺激の値は、BML 社では通常 5~6 桁のオーダーである。これに満たない場合は、リンパ球の viability が低下しており (いわゆる「生きが悪い」状態)、原因薬剤を添加しても有意な上昇が期待できないことが多い。このようなときの SI 値は不正確であり、もう一度行うことを検討されたい。化学療法を行った患者や、ステロイドパルス療法を行った患者でしばしば見られる所見である。

陰性コントロールである薬剤無添加検体においては、CPM 値が異常高値でないことを確認する。通常陰性コントロールは 100~800 程度であるが、ときおり陰性コントロールの CPM がこの値を超えるのを見受ける。なんらかの理由で末梢血リンパ球が既に活性化状態であることが疑われるが、このような活性化状態では原因薬剤が添加されても適切に刺激されないことが容易に予想される。

表 1.

DLST 結果報告書の見方

陰性コントロールが高いとき、陽性コントロールが低いときは解釈不能のことがあります。要注意。陽性コントロールは 50000 CPM 以上になり、6 桁に達することが多い。これらの値は検査会社もしくは測定機によって異なるため、絶対的な数値ではない。

希望系列：いずれかで SI 値 1.8 を超えたものが陽性と判定される

↓

【検査結果】									
依頼薬剤名	初濃度	薬剤希釈倍率	3 ⁰	3 ⁻¹	3 ⁻²	3 ⁻³	3 ⁻⁴	3 ⁻⁵	
注射用エフオーワイ	1/190 100 mg	CPM	63	779	769	309	291	229	
		SI	0.2	2.9	2.9	1.2	1.1	0.9	
依頼薬剤名	初濃度	薬剤希釈倍率	3 ⁰	3 ⁻¹	3 ⁻²	3 ⁻³	3 ⁻⁴	3 ⁻⁵	
フオイバン	1/190 100 mg	CPM	2057	2828	682	605	413	284	
		SI	7.7	10.6	2.5	2.3	1.5	1.1	

【Control】		
PHA 無添加 (CPM)	PHA 刺激 (CPM)	SI (PHA)
268	60402	225.4

↑
陰性コントロール
(通常 100~800 程度)
↑
陽性コントロール
(通常 50000 以上)
↑
陽性/陰性の比

さらに、分母となる CPM 値が高いと、SI 値は見かけ上 1.8 未満となり、陰性という結果になる。DLST の結果が陰性であっても、陰性コントロールの CPM 値が高い場合、偽陰性の可能性を考える必要がある。陰性コントロールの CPM 値が高い原因として、感染症や炎症性疾患で活性化した T 細胞が末梢を循環している状態が考えられる。我々は DIHS の既往歴がある患者で DLST を施行した際に、陰性コントロールが常に 6000 CPM 程度を呈していた症例を経験しており、この患者では DLST による薬剤アレルギーの評価が不可能であった。また、上記の DLST プロトコールでは患者の自己血漿を 20% 含んでいる。血中濃度が安定している薬剤が原因だった場合、薬疹の急性期に採取した血漿にリンパ球を活性化させる程度の薬剤濃度が保たれていることも否定できない。

しばしば基準を少し超えた程度の検査結果を得て解釈に苦しむことがあるが、上記のごとく、SI 値 1.8 以上を本邦では陽性と解釈する(海外では 2.0 を基準としている)。

薬疹の病型と検査施行時期

DLST は薬疹の急性期に行うべきとの認識が従来からあった。Kano ら⁴⁾は薬疹を紅斑丘疹型 (MP)、スティーブンス・ジョンソン症候群/中毒性表皮壊死融解症 (SJS/TEN)、薬剤性過敏症症

候群 (DIHS) に分類して DLST の陽性率を検討した。すると、MP と SJS/TEN は急性期に陽性となる傾向を示し、従来の考えと合致する結果であった。しかし、DIHS においては急性期には陰性で、回復期に陽性転化する傾向を示し、MP および SJS/TEN とは明確な違いを認めた。この所見は DIHS の病態を表していることが推察され、臨床の現場では診断を補助する所見として DLST の陽性転化を積極的に確認すべきである。DIHS は今まで考えられていたよりも長い経過をとっていることが考えられるため、「回復期」を定義するのが難しいが、急性期を脱した後、すなわち 1 か月以降と考えればよいだろう。我々の経験では陽性転化するまで発症から 6 か月以上経過する症例もあり、DIHS においては長期の経過観察と検査のフォローアップが必要であると考えている。

薬剤の特性は DLST 結果に影響する

DLST の結果は被疑薬の特性により大きく影響を受け、偽陰性や偽陽性の結果を得る場合がある。偽陰性の代表的な例はアロプリノールである。アロプリノールは生体内でオキシプリノールに分解され、この分解産物に免疫源性があると報告されている。よって、*in vitro* 検査である DLST ではアロプリノールの分解産物が生じることは考えにくく、本薬と並列してオキシプリノールで施行す

べきである。他の薬剤においても分解産物が薬疹の発症に寄与している可能性は十分あるが、その情報は極めて限られているのが現状である。

DLSTは細胞増殖の度合いを定量化する検査であるため、細胞毒性の強い抗癌剤は*in vitro*でも毒性を発揮してしまい、解釈のできる結果が得られない可能性が高い。DLSTが依頼された際には、細胞毒性が最小限になるように希釈されて検査が行われるが、その分感度も低くなるようだ。事実、BML社の集計(表2)では多くの抗癌剤の陽性率は極めて低い。このような薬剤が被疑薬として疑われる場合は、パッチテストなどの検査を併用すべきであろう。一方、DNA合成を阻害する5-FUなどの代謝拮抗薬は、DNAの再利用機構であるサルベージ経路を活性化する⁵⁾。このためこれらの薬剤に曝露された細胞ではチミジンの取り込みがむしろ増加し、実際に細胞増殖をしていないのにCPMが高値となってしまう。その結果、メソトレキセートやTS-1などでDLSTを行うと高率に陽性反応が得られ、偽陽性が多く含まれている可能性が高い(表2)。5-FU系薬剤やメソトレキセートなどの代謝拮抗剤による薬疹を疑った際には、パッチテストや内服チャレンジテストなどを併用し、慎重に解釈すべきだろう。

解熱鎮痛剤(NSAIDs)は薬疹の原因として高頻度に報告されている。しかし、NSAIDは免疫系に作用する薬剤であることより、DLSTに影響を及ぼす可能性がある。例えば、ロキソニンは重症薬疹を起こす薬剤としてよく知られている。しかし、BML社のデータを参照すると、ロキソニンのDLST陽性率は52.4%であり、不自然に高い印象を覚える(表2)。アセトアミノフェンに関しても61.2%である。無論この2薬剤は高頻度に利用されており、多くの薬疹の原因となっていることが考えられるが、DLSTの結果に関しては、一部に偽陽性を含んでいる可能性も念頭に置き、慎重に解釈する必要がある。原因薬として決定する際には、パッチテストなどを併用すべきかもしれない。

漢方薬のDLSTも慎重に解釈する必要がある。表2に示すごとく、漢方薬は高頻度に陽性となり、小柴胡湯90.3%、防風通聖散74.1%、抑肝散69.6%、柴苓湯88.2%の陽性率を示す。これらの薬剤が一部の薬剤アレルギーで真の原因である可能性は否定できないが、DLSTに関しては漢方薬の免疫賦活作用により偽陽性を呈している可能性が高い。同様に、ワクチンや乳酸菌製剤などはアレルギーがなくてもDLST陽性となるのは当然の結果と言えよう。

DLST陰性率と存在しうる偽陰性を論じるのは難しいが、局所麻酔剤や造影剤などでは陽性率は極めて低い(表2)。アナフィラキシーが主たる病態であるためかもしれない。臨床の現場からすると、DLSTが唯一の検査法であるため提出せざるをえないのもうなずけるが、クリアな結果が出る望みは薄い。このような薬剤・病態に対しては後述の好塩基球刺激試験を試みる価値があるだろう。

このように、偽陽性の可能性を含む薬剤を認識するのは正しい診断を下すうえで極めて重要である。DLSTの陽性率とパッチテストの相関関係も検証する必要があるだろう。主要薬剤を対象に、正常人コントロールでのDLST陽性率を確認すると有益であるように思われる。

原疾患治療とステロイドの影響

原疾患とその治療はDLSTの結果に影響を及ぼすことがある。

高用量のステロイドに曝露されたリンパ球が原因薬剤に対して十分に活性化・増殖しないことは容易に予想される。その効果はステロイドパルスで顕著である印象をもつ。化学療法施行中もしくは施行後もDLSTの感度は落ちることが予想される。抗癌剤の直接作用も考えられるが、リンパ球の絶対数が低いことに起因するケースが多いだろう。これらの状態にある患者の薬疹を評価する際には、上述のPMAによる刺激に対してリンパ球が十分活性化していることを確認する必要がある。末梢血のリンパ球数にも留意されたい。

表 2. BML 社に 2005～2008 年の間に提出された各薬剤の DLST の陽性率を示す。陽性率が 50%以上のものを赤で示した。これらがすべて真の原因薬なのか、偽陽性を含むのかを今後検証する必要がある。

DLST主要薬剤陽性率一覧(2008年度) 2005.1.1～2008.12.31

種類別	薬剤名称	一般名	陽性数	陽性率	疑陽性数	疑陽性率	
抗てんかん剤	テグレトール	カルバマゼピン	260	37.2%	55	7.9%	
	デパケン	バルプロ酸ナトリウム	38	14.4%	27	10.2%	
解熱鎮痛消炎剤	カロナール	アセトアミノフェン	476	61.2%	63	8.1%	
	ロキソニン	ロキソプロフェンナトリウム	734	52.4%	133	9.5%	
	セレコックス	セレコキシブ	293	47.3%	58	9.4%	
	ブルフェン	イブプロフェン	13	8.8%	9	6.1%	
	ボルタレン	ジクロフェナクナトリウム	80	17.0%	55	11.7%	
	バイアスピリン	アスピリン	110	16.0%	63	9.2%	
	バファリン	アスピリン・ダイアルミネート	48	17.5%	24	8.8%	
抗血小板剤	パナルジン	塩酸チクロピジン	25	4.2%	32	5.4%	
	ワーファリン	ワルファリンカリウム	147	46.2%	28	8.8%	
総合感冒剤	PL顆粒	合剤	182	45.4%	29	7.2%	
	バブロン	OTC製剤	130	61.3%	27	12.7%	
血圧降下剤	ディオバン	バルサルタン	136	39.2%	28	8.1%	
	アーチスト	カルベジロール	34	16.3%	12	5.8%	
	レニベース	マレイン酸エナラプリル	18	16.4%	10	9.1%	
	ニューロタン	ロサンタンカリウム	31	22.3%	12	8.6%	
	プロプレス	カンデサルタンシレキセチル	100	27.3%	44	12.0%	
	血管拡張剤	ヘルベッサ	塩酸ジルチアゼム	14	10.4%	7	5.2%
シグマート		ニコランジル	17	15.3%	9	8.1%	
ノルバスク		ベシル酸アムロジピン	37	9.6%	30	7.8%	
アムロジン		ベシル酸アムロジピン	31	9.0%	17	5.0%	
アダラート		ニフェジピン	47	22.0%	30	14.0%	
気管支拡張剤		テオドール	テオフィリン	7	10.6%	4	6.1%
	メブチン	塩酸プロテカロール	1	4.8%	2	9.5%	
ステロイド	セlestamin	ベタメタゾン・d-κ-ホルフェニラミンマレイン酸塩	2	5.4%	1	2.7%	
	プレドニン	プレドニゾロン	1	1.0%	5	4.8%	
消化器用薬	ナウゼリン	ドンペリドン	5	8.1%	6	9.7%	
	ビオフェルミン	乳酸菌製剤	40	54.1%	6	8.1%	
	ガスター	ファモチジン	42	10.7%	38	9.7%	
	ムコスタ	レバミピド	252	56.5%	44	9.9%	
	タケブロン	ランソプラゾール	169	21.7%	67	8.6%	
	バリエット	ラベプラゾールナトリウム	82	20.3%	41	10.1%	
	セルベックス	テブレノン	73	45.6%	15	9.4%	
抗癌剤	タキソール	バクリタキセル	3	7.5%	2	5.0%	
	イレッサ	ゲフィチニブ	3	7.1%	1	2.4%	
	パラプラチン	カルボプラチン	0	0.0%	0	0.0%	
	ランダ	シスプラチン	0	0.0%	2	18.2%	
	5-FU	フルオロウラシル	46	63.9%	2	2.8%	
	ティー・エスワン	デガフル・ギメラシル・オテラシル	175	72.9%	4	1.7%	
	抗菌剤	クラビット	レボフロキサシン	309	32.9%	123	13.1%
ジェニナック		メシル酸ガレノキサシン水和物	46	11.0%	34	8.2%	
ペントシリン		ピペラシリンナトリウム	13	22.8%	7	12.3%	
バクタ		スルファメトキサゾール・トリメトプリム	212	53.9%	25	6.4%	
メリアクトMS		メイアクト	38	10.3%	33	9.0%	
フロモックス		塩酸セフカペンピホキシル	88	12.8%	80	11.7%	
クラリス		クラリスロマイシン	117	12.7%	71	7.7%	
クラリシッド		クラリスロマイシン	28	8.4%	26	7.8%	
セフゾン		セフジニル	102	40.5%	26	10.3%	
ミノマイシン		塩酸ミノサイクリン	17	6.8%	18	7.2%	
ジスロマック		アジスロマイシン水和物	26	8.6%	24	8.0%	
サウシリン		アモキシシリン	294	47.0%	69	11.0%	
局所麻酔剤		キシロカイン	塩酸リドカイン	7	3.6%	10	5.2%
		シタネスト	塩酸プロピトカイン・フェリプレシン	8	7.1%	12	10.7%
	オーラ	塩酸リドカイン・酒石酸水素エピネフリン	4	3.8%	5	4.7%	
漢方薬	小柴胡湯		28	90.3%	0	0.0%	
	防風通聖散		63	74.1%	3	3.5%	
	抑肝散		32	69.6%	3	6.5%	
	葛根湯		54	54.0%	12	12.0%	
	柴苓湯		45	88.2%	2	3.9%	
	芍薬甘草湯		50	60.2%	6	7.2%	
	抗結核抗生物質	リファンピシン	リファンピシン	32	12.6%	24	9.5%
リファジン		リファンピシン	62	27.9%	23	10.4%	
リマクタン		リファンピジン	6	11.8%	4	7.8%	
結核化学療法剤	イスコチン	イソニアジド	141	29.4%	66	13.8%	
	エプトール	塩酸エタンブトール	44	18.6%	29	12.2%	
ワクチン	インフルエンザ		38	88.4%	4	9.3%	
	HBV		6	50.0%	6	50.0%	
高コレステロール血症	クレストール	ロスバスタチンカルシウム	54	15.9%	25	7.4%	
	リビトール	アトルバスタチンカルシウム水和物	33	8.6%	29	7.5%	
造影剤	イオパミロン	イオパミドール	1	4.0%	2	8.0%	
痛風治療剤	ザイロリック	アロプリノール	70	17.2%	36	8.8%	
抗リウマチ剤	リウマトレックス	メトトレキサート	165	77.1%	14	6.5%	
利尿剤	ラシックス	フロセミド	51	13.9%	25	6.8%	
消炎酵素剤	ダーゼン	セラペプターゼ	56	49.6%	6	5.3%	
全 平 均				28.8%		9.0%	

DLSTに必要な細胞数は1薬剤目200万個、2薬剤目以降、1薬剤増えるごとに120万個の加算を目安とする。よって、検査提出の直近に得られた末梢血のデータである程度の予測はできるので、可能なときには確認すべきである。ただし、実際の末梢血単核球の回収率は基礎疾患の有無、個体、年齢(小児>成人)によって大きく異なり、低めに見積もって25%程度と考えていたほうが安全のようだ。BML社では最終的な細胞数が少ない症例で、どうしても施行しなければならないものは、提出医の了承のもと測定ポイントを減らしたり(希釈系列を少なくする)、細胞数を2割減で行ったりする。この際には感度が低下することを認識しておかなければならない。

新しい検査法

さて、薬疹の診療においてDLSTは欠かせない存在であるが、比較的低感度なのが問題である。現在のところ代替検査法は確立されていないが、我々は好塩基球刺激試験(basophil activation test:BAT)に注目している。BATはDLST同様の*in vitro*検査であり、食物アレルギーなどの即時型反応では比較的確立されている⁶⁾。近年薬剤アレルギーにも応用される例が散見されているが⁷⁾、DLSTと比較した報告は存在しない。この試験では、DLST同様患者末梢血を1時間ないし24時間薬剤に曝露させ、好塩基球上に発現される活性化マーカーのCD203cをフローサイトメトリーにて定量する手法である。現在検討を重ねている段階であるが、BATはアナフィラキシーのみならず遅延型の薬疹にも応用できそうだ。また、BATとDLSTはそれぞれ単独陽性となる傾向を示し、両検査法を併用することで原因薬剤の同定率が上がることを期待している。

結語

本稿は主としてDLSTの原理、結果の読み方、およびいわゆるpitfallについて述べた。これらをよく理解して初めて薬疹の原因薬を正しく同定

できるのではないかと考えている。いくつかの薬剤が呈しうる偽陽性は重要な問題であり、今後正常人で検証するなど、システマティックな解析が望まれる。

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Stevens-Johnson syndrome/toxic epidermal necrolysis mouse model generated by using PBMCs and the skin of patients

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Background: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening cutaneous reactions caused by drugs or infections and exhibiting widespread epidermal necrosis. Currently, there is no animal model that reproduces SJS/TEN symptoms.

Objective: We sought to develop a novel mouse model of SJS/TEN by using PBMCs and skin from patients who had recovered from SJS/TEN.

Methods: For our mouse model, patients' PBMCs were injected intravenously into immunocompromised NOD/Shi-*scid*, IL-2R γ^{null} (NOG) mice, followed by oral administration of a causative drug. Subsequently, to replace human skin, unaffected skin specimens obtained from patients who had recovered from SJS/TEN were grafted onto NOG mice, after which patient-derived PBMCs and the causative drug were applied.

Results: Mice injected with PBMCs from patients with SJS/TEN and given the causative drug showed marked conjunctival congestion and numerous cell death of conjunctival epithelium, whereas there were no symptoms in mice injected with PBMCs from patients with ordinary drug skin reactions. CD8⁺ T lymphocyte-depleted PBMCs from patients with SJS/TEN did not elicit these symptoms. In addition, skin-grafted mice showed darkening of the skin-grafted areas. Cleaved caspase-3 staining showed that dead keratinocytes were more numerous in the skin-grafted mice than in the healthy control animals.

Conclusion: We have established a novel human-oriented SJS/TEN mouse model and proved the importance of CD8⁺ T lymphocytes in SJS/TEN pathogenesis. The mouse model promises to promote diagnostic and therapeutic approaches. (*J Allergy Clin Immunol* 2013;131:434-41.)

Key words: Stevens-Johnson syndrome, toxic epidermal necrolysis, animal models

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Abbreviations used

APC:	Antigen-presenting cell
CTL:	Cytolytic T lymphocyte
DC:	Dendritic cell
FITC:	Fluorescein isothiocyanate
GVHD:	Graft-versus-host disease
GVHR:	Graft-versus-host reaction
NK:	Natural killer
NOG:	NOD/Shi- <i>scid</i> , IL-2R γ^{null}
ODSR:	Ordinary drug skin reaction
PE:	Phycocerythrin
sFasL:	Soluble Fas ligand
SJS:	Stevens-Johnson syndrome
TEN:	Toxic epidermal necrolysis
TUNEL:	Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare, life-threatening mucocutaneous reactions characterized by extensive detachment of the epidermis.¹ They are considered part of the same spectrum of diseases but with different severities. Patients with SJS have skin detachment on less than 10% of the body surface area, whereas patients with TEN have more extensive lesions.² The overall incidences of SJS and TEN have been estimated at 1 to 6 cases per million person-years and 0.4 to 1.2 cases per million person-years, respectively. The mortality associated with TEN is 25%. The eruptions are initially distributed on the face, trunk, and extremities but can rapidly extend to the whole body within just a few hours. Mucous membrane involvement is observed in approximately 90% of cases. Approximately 85% of patients have conjunctival lesions. Ocular complications include chronic conjunctivitis, conjunctival scarring, corneal vascularization, and corneal damage, which can lead to blindness. Ocular morbidity and visual loss can be caused by acute corneal complications, and progressive conjunctival scarring is also significantly associated with subsequent loss of vision.³ Several treatments have been attempted, including high-dose corticosteroids, intravenous immunoglobulin, and plasmapheresis; however, some cases are resistant to these therapies.⁴ In some cases only supportive therapy is applied out of concern over the immunosuppressive effect of these treatments.⁵

The pathologic mechanisms of SJS/TEN are not fully known.⁶ Several mediators to induce SJS/TEN have been proposed, such as Fas ligand,⁷ soluble Fas ligand (sFasL),⁸ perforin, granzyme B,⁹ and granulysin.^{10,11} These mechanisms can induce massive epithelial cell death. Nevertheless, no one has been able to explain why these systemic "cell-death mediators" affect skin

exclusively and result in widespread mucocutaneous erosions without dysfunction of other organs. Indeed, serum sFasL levels are increased not only in patients with SJS/TEN but also in those with viral infections¹² and graft-versus-host disease (GVHD).¹³

Because there is only 1 available animal model, basic research on SJS/TEN is still quite preliminary. Azukizawa et al^{14,15} generated transgenic mice that express the foreign antigen ovalbumin only on keratinocytes. Injections of ovalbumin-specific cytotoxic T cells induced erosive skin manifestations and numerous apoptotic keratinocytes. Although this model showed widespread erosions and partially elucidated the pathomechanisms of skin lesions in patients with SJS/TEN, the model did not reproduce the drug-specific immune reactions that occur in the patients' blood cells and skin component cells. A more precise drug-triggered SJS/TEN mouse model is urgently required for a more complete understanding of SJS/TEN pathomechanisms and preclinical studies for novel therapeutic strategies.

In light of this, we aimed to develop a relevant animal model of SJS/TEN using patients' tissue samples to reproduce a reaction identical to that of SJS/TEN. Using immunocompromised mice, we successfully evoked the same reactions between the causative drug and human immune cells.

METHODS

Patients' samples

A total of 6 patients with SJS/TEN participated in this study (the patient information is detailed in the Table E1 in this article's Online Repository at www.jacionline.org). The causative drugs were acetaminophen in 4 patients, amoxicillin in 1 patient, and phenytoin in 1 patient. Blood samples were taken from patients with SJS/TEN at least 6 months to 3 years after complete remission of symptoms. Skin biopsy specimens were taken at least 1 year after complete remission of symptoms. The patients had received no systemic glucocorticoids before the study. Ordinary drug skin reactions (ODSRs) in our experiments included the maculopapular type and excluded other adverse drug reactions, such as drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms and acute generalized exanthematous pustulosis. Samples were obtained from Hokkaido University Hospital. The collection of samples was approved by the local ethics committee and the institutional review board of Hokkaido University, and each patient provided written informed consent.

Mice

Immunocompromised NOD/Shi-*scid*, IL-2R γ^{null} (NOG) mice at 6 to 7 weeks of age were purchased from the Central Institute for Experimental Animals (Tokyo, Japan). With human PBMCs, NOG mice have been used as models of human disease, such as HIV infection.¹⁶ All the animal experiments were performed under the approval of the ethics committee for animal studies of Hokkaido University.

Analysis of graft-versus-host reactions

A graft-versus-host reaction (GVHR) was induced by means of intravenous injection of human PBMCs. Whole PBMCs (1×10^7) were obtained from healthy control subjects, suspended in 0.1 mL of PBS, and then injected intravenously into NOG mice. Skin, ocular, and mucous manifestations were observed. Body weight was monitored. Peripheral blood and splenocytes were analyzed by using flow cytometry to detect human cells. Skin and ocular lesions were investigated histopathologically.

ELISpot IFN- γ assay

PBMCs were prepared from patients' blood and isolated by using Ficoll-Isopaque (Pharmacia Fine Chemicals, Piscataway, NJ) density

gradient centrifugation. Mouse peripheral cells and splenocytes were also isolated. The number of IFN- γ -producing cells was determined by using an ELISpot assay kit (Human IFN- γ ELISpot PVDF-Enzymatic; Diaclone, Besancon, France). Ninety-six-well nitrocellulose plates were washed 3 times with PBS before use, and PBMCs (2×10^5 in 100 μL) were incubated overnight with causative drugs in RPMI-1640 medium supplemented with 2 mmol/L L-glutamine, 25 mmol/L HEPES buffer, and 10% heat-inactivated autologous serum. Plates were washed 3 times with PBS, incubated for 2 hours with a biotinylated anti-IFN- γ antibody, and extensively washed. IFN- γ spot-forming cells were developed by using streptavidin-alkaline phosphatase, incubated for 2 hours, and washed before addition of the substrate (5-bromo-4-chloro-3-indolyl-phosphate). The number of spots was counted by using a dissecting microscope (SMZ1500; Nikon, Tokyo, Japan), and the frequency of IFN- γ lymphocytes was defined as the number of spots in 2×10^5 mononuclear cells. The drug-specific reactions between antigen-presenting cells (APCs) and antigen-specific T cells resulted in production of IFN- γ from drug-specific lymphocytes (ie, the IFN- γ -producing T cells are antigen-specific [causative drug-specific] T cells). Using the ELISpot assay, we detected causative drug-specific T cells.

SJS/TEN mouse model using patients' PBMCs

PBMCs were obtained from patients who had recovered from SJS/TEN. In some experiments isolated PBMCs were restimulated with causative drugs in completed RPMI media for 6 days. In other experiments CD4⁺ or CD8⁺ cells in PBMCs were depleted by using a magnet-activated cell sorter (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany). PBMCs (2×10^6) were injected intravenously into the NOG mice, followed by oral administration of the causative drugs (acetaminophen, amoxicillin, or phenytoin, 100 μL). The dosage used in the model mice was based on milligrams per kilogram of body weight converted from the adult human normal dose. We administered the drug to the mice once daily. In addition, we confirmed that the dosage was under the median lethal dose in mice. Drug dosage was estimated by dose conversion by body weight. We checked for any changes of the skin, eyes, and mucosa, such as skin color or mucous hemorrhage. Peripheral blood and splenocytes were analyzed by using flow cytometry to detect human cells. Skin, ocular, and liver lesions were investigated by means of histopathologic examination and immunohistochemical staining.

Flow cytometric analysis

Cells were stained with the following antibodies: phycoerythrin (PE)-conjugated mouse CD45, fluorescein isothiocyanate (FITC)-conjugated human CD45, peridinin-chlorophyll-protein complex-conjugated human CD3, FITC-conjugated human CD4, PE-conjugated human CD8, or PE-Cy7-conjugated human CD56 (BD Biosciences, San Jose, Calif). Analysis was performed by using a FACSAria with BD FACSDiva software (BD Biosciences).

Immunohistochemistry

Immunostaining of ocular, skin, and liver tissues was performed with antibodies to cleaved caspase-3 (Cell Signaling Technology, Beverly, Mass) and human CD4 and CD8 (BD Biosciences). FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa) and TRITC-conjugated rabbit anti-rat IgG (Sigma-Aldrich, St Louis, Mo) were used as secondary antibodies. The nuclei were counterstained with propidium iodide. Fluorescence staining was detected with a confocal laser scanning fluorescence microscope (Fluoview FV1000; Olympus, Tokyo, Japan). We counted the number of stained cells from 5 separate fields, and the average was shown. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) is a method for detecting apoptotic cells with DNA fragmentation by labeling the terminal end of nucleic acids. The TUNEL assay was performed according to the manufacturer's protocol (Takara Bio, Shiga, Japan).

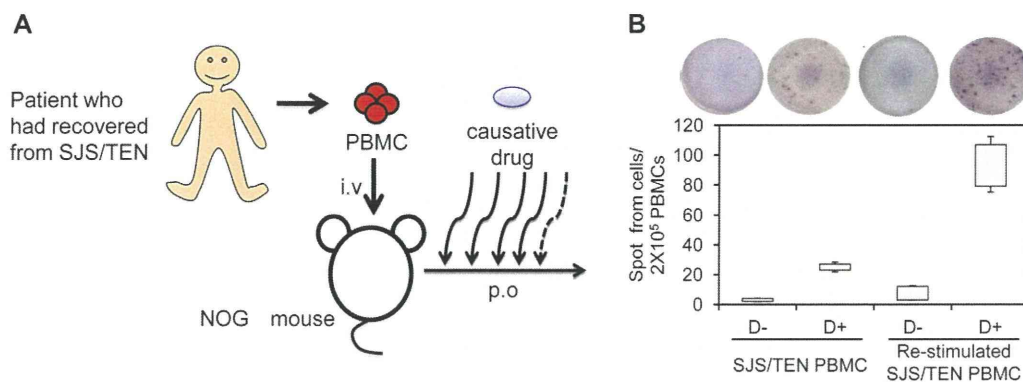


FIG 1. Development of SJS/TEN model mice by using PBMCs of patients who had recovered from SJS/TEN. **A**, Scheme of model mice development. PBMCs were obtained from patients who had recovered from SJS/TEN. These PBMCs (2×10^6) were injected intravenously into NOG mice, followed by oral administration of the causative drugs. **B**, Causative drug-specific cells were detected by using the human IFN- γ ELISpot. When the causative drug was added to cultured PBMCs of patients who had recovered from SJS/TEN (patient 2), causative drug-specific lymphocytes were detected (30 spots per 2×10^5 cells). After 2 stimulations of the causative drug (restimulation), the number of drug-specific lymphocytes increased (90 spots per 2×10^5 cells). Samples from patients 1 to 6 with SJS/TEN were analyzed, and representative data (patient 2) are shown. *i.v.*, Intravenous; *p.o.*, by mouth.

Measurement of human granulysin and human/mouse sFasL

Human granulysin and human/mouse sFasL in supernatants of PBMCs or sera from SJS/TEN model mice were measured by using ELISA. Human granulysin ELISA (BML, Tokyo, Japan) was performed as previously reported.¹¹ Human/mouse sFasL levels were measured by using an ELISA kit from R&D Systems (Minneapolis, Minn).

SJS/TEN mouse model using patients' PBMCs and skin

Full-thickness skin grafts from healthy control subjects or patients who had recovered from SJS/TEN or an ODSR were transplanted onto the NOG mice. After skin engraftment (approximately 12 days after transplantation), causative drug-stimulated PBMCs (2×10^6) from the same patient were injected intravenously into these mice, followed by oral administration of the causative drug. Changes in skin graft appearance, such as darkening, were observed. The skin grafts were investigated histopathologically.

RESULTS

Development of SJS/TEN mouse model using PBMCs from patients who had recovered from SJS/TEN

To develop the SJS/TEN mouse model, we used PBMCs from patients who had recovered from SJS/TEN. The PBMCs were injected intravenously into immunocompromised mice, followed by oral administration of the causative drug (Fig 1, A). There are several reports on the existence of drug-specific lymphocytes in patients who had recovered from drug allergies, and these lymphocytes were restimulated by the causative drug *in vitro*.^{17,18} Therefore if lymphocytes that specifically reacted to the drug remained in the peripheral blood, these lymphocytes would be restimulated by the causative drug, and identical immunologic reactions to those of patients with active SJS/TEN would occur in the mice.

First, we confirmed the presence of causative drug-specific lymphocytes in peripheral blood. ELISpot analysis of human IFN- γ was conducted to detect antigen-specific human cells. When the causative drug was added to cultured PBMCs from

patients who had recovered from SJS/TEN, causative drug-specific lymphocytes were detected (Fig 1, B). After *in vitro* restimulation of the causative drug, the number of drug-specific lymphocytes increased. To exclude the possibility of *in vitro* priming of naive T cells, we performed the ELISpot assay using PBMCs of naive healthy volunteers who had never experienced cutaneous adverse drug reactions. We stimulated the PBMCs of healthy volunteers ($n = 4$) with amoxicillin, one of the causative drugs in our study, and restimulated them after 5 days. In ELISpot data we were unable to detect drug-specific T cells, even on restimulation (see Fig E1 in this article's Online Repository at www.jacionline.org). Either a breakdown product or a drug metabolite might be the drug form that is responsible for drug reactions that are presumed to be immunologic in nature. On the other hand, in our experiments the addition of native drugs to PBMCs induced the activation of drug-specific lymphocytes, indicating that a breakdown product of the drug might be recognized as an antigen *in vitro*. These data reconfirm that even after resolution of SJS/TEN, drug-specific lymphocytes still circulate, as previously reported.¹⁸

We used NOG mice, which lack T cells, B cells, and natural killer (NK) cells, as immunocompromised mice.¹⁹ NOG mice are characterized by tolerance to human cells, which enables humanized mice to be established.²⁰ However, when human cells are applied to NOG mice, GVHRs can occur because engrafted human immune cells attack mouse tissues.²¹ The NOG mice showed GVHD symptoms at 46.3 ± 14.3 days after intravenous transplantation of 2.5×10^6 PBMCs.²¹ Indeed, at 40 days after human PBMCs were injected intravenously into the NOG mice, weight loss, skin erosion, and diarrhea were noticed as symptoms of GVHRs in our experiments. The skin lesions of patients with GVHRs were quite similar to those of patients with SJS/TEN. Skin erosion and hair loss were observed clinically, and epithelial cell death and epidermal detachment were observed histologically. In addition, human CD45⁺ cells were detected in mouse peripheral blood at 20 days after PBMC injection (see Fig E2 in this article's Online Repository at www.jacionline.org), showing that activation of injected human cells had occurred in the NOG mice.

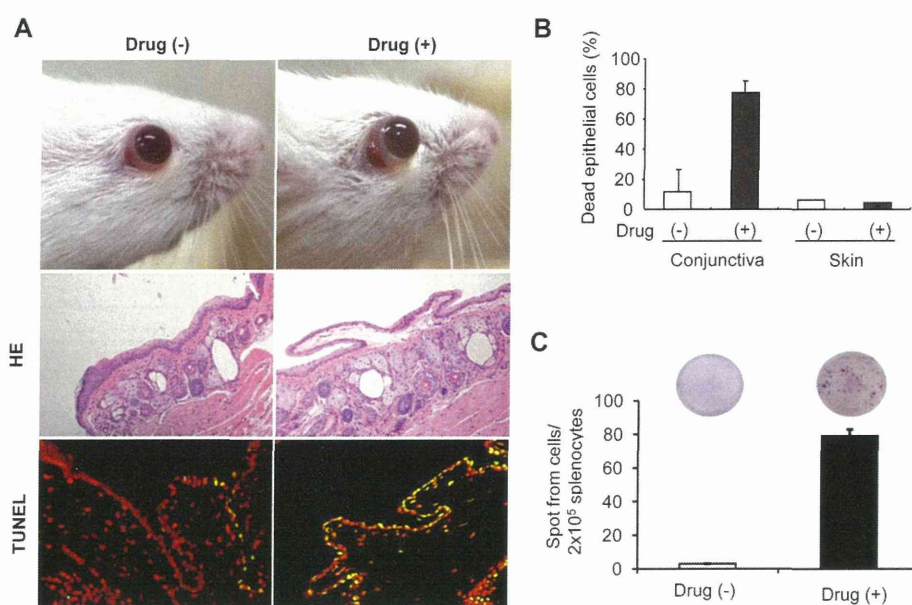


FIG 2. PBMCs from patients with SJS/TEN and causative drug-induced ocular manifestations similar to those of SJS/TEN in NOG mice. **A and B,** At 12 days after PBMC injection and causative drug intake, significant conjunctival congestion and conjunctival chemosis are noticed. The PBMC-injected mice without causative drug intake showed no such symptoms. Histologic analysis showed marked edema of the conjunctival subepithelia in the SJS/TEN-PBMC⁺drug⁺ mice. The TUNEL assay detected numerous dead epithelial cells in the SJS/TEN-PBMC⁺drug⁺ mice (78% of cells) but not in the SJS/TEN-PBMC⁺drug⁻ mice (5% of cells). There was no increase in keratinocyte death in the skin in either group. *HE*, Hematoxylin and eosin. **C,** Human IFN- γ ELISpot with mice splenocytes was performed to confirm that causative drug-specific immune reaction occurred in those mice. At 12 days after PBMC injection, the number of causative drug-specific lymphocytes in the SJS/TEN-PBMC⁺drug⁺ mice was significantly increased compared with that of the SJS/TEN-PBMC⁺drug⁻ mice. Samples from patients 1, 2, 4, and 5 with SJS/TEN and patient 1 with an ODSR were used, and representative data (patient 2) are shown.

In contrast, within 20 days after PBMC injection, no GVHR symptoms appeared ($n = 5$). Therefore to distinguish clearly between GVHRs and SJS/TEN reactions, we examined mouse model experiments up to 14 days after PBMC administration.

At 12 days after PBMC injection and causative drug intake, marked conjunctival congestion and conjunctival chemosis were noticed, whereas PBMC-injected mice without causative drug intake showed no such symptoms (Fig 2, A). Histologic analysis showed marked edema of the conjunctival subepithelia and vasodilation in mice receiving PBMCs and causative drug administration (SJS/TEN-PBMC⁺drug⁺ mice). We made 20 model mice, all of which showed similar manifestations. Furthermore, the TUNEL assay found numerous dead epithelial cells in the SJS/TEN-PBMC⁺drug⁺ mice (78% of cells; Fig 2, B) but not in the PBMC-injected, non-drug-administered (SJS/TEN-PBMC⁺drug⁻) mice (5% of cells). Unexpectedly, there were no skin manifestations in either group and no difference in percentages of dead keratinocytes between the groups. At 40 days after PBMC injection, weight loss, skin erosion, hair loss, and diarrhea were noticed. Prominent epithelial cell death was observed histologically (see Fig E3 in this article's Online Repository at www.jacionline.org). These data were virtually identical to those of the GVHD model.

To analyze the occurrence mechanism of SJS/TEN in the model mice, we investigated various conditions that might elicit ocular manifestations similar to those of patients with SJS/TEN (Table I). We observed conjunctival congestion and conjunctival chemosis in our model mice.

TABLE I. Occurrence of ocular manifestations in NOG mice in various conditions

Injected cells	Causative drug intake	Ocular manifestations
PBMCs from patients with SJS/TEN	+	+ (Day 12)
PBMCs from patients with SJS/TEN	-	-
Restimulated PBMCs from patients with SJS/TEN	+	+ (Day 6)
PBMC-depleted CD4 ⁺ cells from patients with TEN	+	+ (Day 14)
PBMC-depleted CD4 ⁺ cells from patients with TEN	+	-
PBMCs from patients with ODSRs	+	-
PBMCs from patients with ODSRs	-	-
Healthy control PBMCs	+	-
Healthy control PBMCs	-	-
None	+	-

Samples from patients 1 and 2 with SJS/TEN and patient 1 with an ODSR were analyzed, and representative data (patient 2 with SJS/TEN and patient 1 with an ODSR) are shown.

Causative drug intake alone did not induce ocular manifestations in the NOG mice. Healthy control PBMCs and acetaminophen, amoxicillin, or phenytoin, which were the causative drugs in the patients with SJS/TEN described in this article, also did not induce ocular manifestations. Importantly, PBMCs from patients who recovered from ODSRs (ie, nonsevere drug-induced skin reactions) and causative drug intake did not elicit ocular

manifestations. However, we were able to find drug-specific T cells in the spleens of NOG mice after ODSR PBMC transfer and orally administered causative drug by using the same ELISpot assay (see Fig E4 in this article's Online Repository at www.jacionline.org). These data showed the ocular manifestations to be a phenomenon specific to PBMCs from patients with SJS/TEN. In addition, causative drug–restimulated PBMCs from patients with SJS/TEN accelerated the onset of ocular manifestations. CD4⁺ or CD8⁺ T lymphocytes were depleted from the PBMCs to identify which lymphocyte subtype is critical in inducing ocular manifestations. CD4⁺ T lymphocyte–depleted PBMCs from patients with SJS/TEN were able to induce ocular manifestations, whereas CD8⁺ T lymphocyte–depleted PBMCs from patients with SJS/TEN were not (see Fig E5 in this article's Online Repository at www.jacionline.org).

These data demonstrate that we have succeeded in establishing model mice showing ocular manifestations (ie, SJS/TEN model mice). In addition, the ocular manifestations similar to those of patients with SJS/TEN in NOG mice were found to be dependent on the causative drug–specific lymphocytes of patients with SJS/TEN, and CD8⁺ T cells are essential to this phenomenon.

Soluble factors, such as granulysin, were not mediators of conjunctival cell death in the SJS/TEN mouse model

In our novel SJS/TEN mouse model, there were almost no infiltrating human cells (human CD45⁺ cells) in the conjunctiva, whereas numerous human cells were detected in the conjunctiva of the mice with GVHRs (see Fig E6 in this article's Online Repository at www.jacionline.org). Therefore it might be that conjunctival cell death is partially induced by soluble factors in addition to direct lymphocyte–epithelium interaction. As mentioned above, we and others have shown that granulysin might contribute to SJS/TEN occurrence. We examined granulysin levels in the supernatants of causative drug–stimulated PBMCs. Causative drug–stimulated PBMCs from patients with SJS/TEN secreted granulysin at levels less than (0.2 ± 0.3 ng/mL) than the serum levels of patients with acute-phase SJS/TEN (24.8 ± 21.2 ng/mL) and those of healthy control subjects (1.6 ± 0.6 ng/mL).¹¹ In addition, granulysin immunohistostaining in the conjunctiva of SJS/TEN model mice and in grafted skin from patients with SJS-TEN showed almost no granulysin expression in these tissues (data not shown). Furthermore, human granulysin and sFasL levels in the sera of mice were measured at days 8 and 12. Serum levels of human granulysin and sFasL were undetectable at these time points. Although ELISA for murine granulysin was not available, we measured murine sFasL levels in these samples. We did not detect an increase in murine sFasL levels in sera from SJS/TEN model mice (data not shown). Therefore granulysin was unlikely to be a candidate for mediating conjunctival cell death in SJS/TEN in our model mice.

Development of an SJS/TEN mouse model using PBMCs and the skin of patients who had recovered from SJS/TEN

In our novel SJS/TEN mouse model skin manifestations did not appear; these model mice do not mimic human SJS/TEN

completely because the target epithelium was murine in origin. We tried to generate another mouse model to simulate skin manifestations.

First, skin from a patient who had recovered from SJS/TEN was grafted onto the backs of NOG mice. After engraftment was confirmed, PBMCs from the same patient were administered intravenously, followed by oral administration of the causative drug (Fig 3, A). In this model both the effector cells and the target cells originate from the same patient with SJS/TEN.

Darkening appeared in the skin graft at 4 days after PBMC injection (Fig 3, B). The darkened area increased at 8 days after PBMC injection (Fig 3, C). In contrast, we observed no color changes and histologic findings showed few keratinocyte deaths in skin-grafted areas at 8 days after PBMC injection without causative drug intake (see Fig E7 in this article's Online Repository at www.jacionline.org). With PBMCs and skin from the same healthy control subject, no necrotic area appeared. Detection of caspase-3 proves that apoptosis has occurred because it is either partially or totally responsible for the proteolytic cleavage of many key proteins, such as the nuclear enzyme poly (ADPribose) polymerase. Using cleaved caspase-3 staining, we confirmed the increase in keratinocyte death in the SJS/TEN skin graft compared with that seen in the healthy control animals (Fig 3, D). In addition, we transplanted skin from patients with ODSRs onto NOG mice and injected the same patients' PBMCs, followed by administration of the causative drug or the vehicle. These mice showed no changes in the appearance of the skin-grafted areas. Histopathologically, the number of apoptotic keratinocytes in ODSR model mice did not differ between the causative drug group and the vehicle group (see Fig E8 in this article's Online Repository at www.jacionline.org).

To investigate the infiltrated cell types, we performed skin graft staining. Both human CD4⁺ and CD8⁺ T cells were infiltrated in the transplanted skin areas. However, there were no differences in the numbers of cells between the patient with SJS/TEN and the control (Fig 4). These findings indicate that infiltrating lymphocytes are not critical in this model. The manifestations of these models were quite similar to those in skin lesions of patients with SJS/TEN.

DISCUSSION

The present study aimed to develop a mouse model to mimic human SJS/TEN. We succeeded in reproducing SJS/TEN-like manifestations in the mice. Our results provide an SJS/TEN animal model that promises to be useful in experiments involving SJS/TEN.

To date, investigations to reveal the pathomechanism of SJS/TEN have been carried out with human samples. In previous reports reactions in the acute phase of SJS/TEN in peripheral blood and skin lesions were analyzed, and these investigations have shown that inflammatory mediators, proapoptotic mediators, or infiltrated cells in the skin lesions might be linked to the occurrence of SJS/TEN.

Chung et al¹⁰ attempted to identify key molecules in skin lesions (bullae), and they focused on the most highly expressed proapoptotic molecule: granulysin. Granulysin was found to induce cultured keratinocyte death. Furthermore, recombinant granulysin injection into the murine skin elicited skin necrosis. In addition, we detected higher concentrations of serum granulysin in the acute phase of SJS/TEN than in ODSRs.¹¹ In contrast,

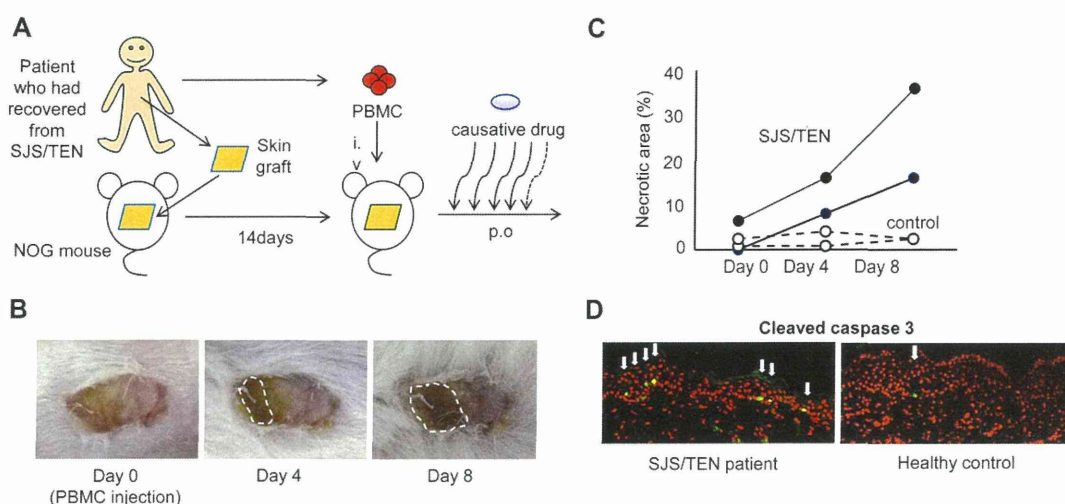


FIG 3. Development of the SJS/TEN mouse model using PBMCs and skin grafts of patients who had recovered from SJS/TEN. **A**, Scheme of mouse model development. Skin grafts from a patient who had recovered from SJS/TEN were grafted onto the backs of NOG mice. After engraftment was confirmed, PBMCs from the same patient were applied intravenously, followed by oral administration of the causative drug. **B** and **C**, Darkened areas appear in the skin graft at 4 days after PBMC injection. These areas were increased at 8 days after PBMC injection. In contrast, the darkened area did not appear in skin grafts of mice when using PBMCs and skin from the healthy control subject. **D**, By using cleaved caspase-3 staining, a great increase in keratinocyte death in the SJS/TEN skin graft was detected in comparison with the keratinocyte death seen in the healthy control specimens. Samples from patient 6 with SJS/TEN and patient 2 with an ODSR were used. *i.v.*, Intravenous; *p.o.*, by mouth.

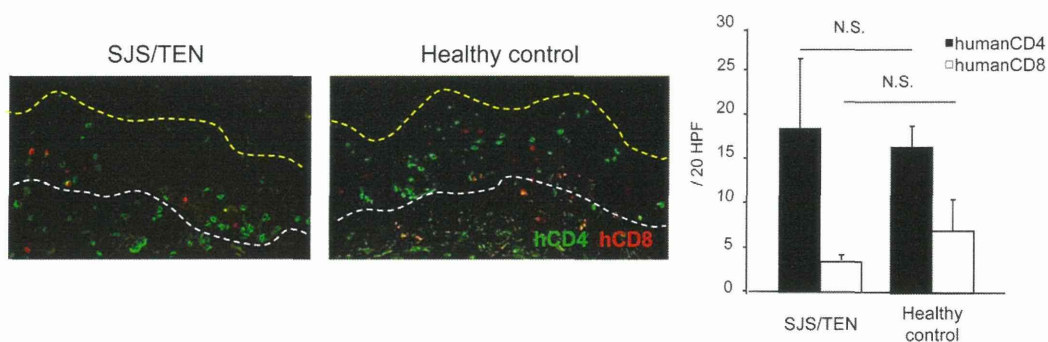


FIG 4. Human CD4 and CD8 staining was performed. Both human CD4⁺ and CD8⁺ T cells infiltrated the transplanted skin areas. There were no differences in the numbers of cells between patients with SJS/TEN and controls. Samples from patient 6 with SJS/TEN were used. *HPF*, High-powered field; *N.S.*, not significant.

French's group reported that Fas and Fas ligand interaction is critical in SJS/TEN pathogenesis.⁷ They reported that keratinocyte apoptosis in patients with SJS/TEN is mediated by Fas and Fas ligand, which are both expressed on keratinocytes. We also reported that sFasL was able to induce keratinocyte death⁸ and that the serum level of sFasL was increased in the acute phase of SJS/TEN, as was the serum level of granulysin.²² Other candidates, such as α -defensin, have also been reported.²³

A recent article reported that NK-cytolytic T lymphocyte (CTLs) might be a cell component that contributes to SJS/TEN occurrence. Activated CTLs gain NK cell-like function; these are called NK-CTLs, and they specifically express CD94/NKG2C. NK-CTLs accumulate in skin lesions of patients with SJS/TEN, where they induce keratinocyte death through an interaction between CD94/NKG2C and HLA-E that is expressed specifically on keratinocytes of SJS/TEN lesions.²⁴

Even though knowledge regarding the mechanism of SJS/TEN has been accumulated, these findings have never been confirmed in an *in vivo* model. If some molecules were highly expressed in skin lesions or blood during the acute phase of SJS/TEN, there would be no way to confirm whether these molecules are key players. Regarding proapoptotic molecules, several candidates have been reported, as described above. However, most reports focused on molecules that researchers anticipated to contribute to SJS/TEN pathogenesis. Granulysin was identified by means of DNA microarray of cells in bullae; however, not only did the data show that the mRNA level of granulysin was increased, they also showed that the mRNA levels of other proapoptotic molecules, such as Fas ligand, perforin, and granzyme B, were increased.¹⁰ This suggests that several pathways are activated in the apoptotic phenomenon *in vivo*, and there is no solid evidence of an exclusive key player. Animal model experiments involving

depletion or inhibition of specific molecules were essential in proving their uniqueness. Previous experiments with patients' specimens provided fragmentary information on disease etiology. Our model mice open the possibility of recreating the pathologic conditions of SJS/TEN in mice and conducting circumstantial *in vivo* examinations, such as on the benefits of treatments. In our study CD8⁺ lymphocyte-depleted PBMCs did not produce SJS-like symptoms, strongly demonstrating that CD8⁺ lymphocytes are necessary to SJS/TEN pathogenesis. Previous reports showed that CD4⁺ T cells were the predominant population that infiltrated into "maculopapular rash" skin lesions and that most drug-specific T cells were CD4⁺ T cells.²⁵ However, in severe cutaneous adverse drug reactions CD8⁺ T cells were the predominant population that infiltrated into the epidermis of skin lesions of patients with SJS/TEN,¹⁰ and HLA-B*1502 was found to be associated with carbamazepine-induced SJS in all cases.²⁶ In addition, drug-specific CD8⁺ T cells were found to predominantly proliferate during the acute stages of SJS.²⁷ Although drug-specific CD4⁺ T cells are essential in drug-mediated immune reactions, CD8⁺ T cells are critical to the development of SJS/TEN. Furthermore, in the skin lesions of model mice, the numbers of CD4⁺ and CD8⁺ lymphocytes did not differ between patients with SJS/TEN and control subjects. These findings indicate that infiltrating lymphocytes are not required to generate SJS/TEN skin lesions. Given the findings of ocular lesions, we made the supposition that soluble factors might be involved in keratinocyte death. Although granulysin and sFasL were not specific mediators of this phenomenon, we speculated that causative drug-stimulated PBMCs from patients with SJS/TEN secreted certain cell-death mediators.

Our data using this model also suggest that drug presentation and recognition in the skin might be less necessary than expected because some symptoms develop with little or no recruitment of drug-specific T cells. In our model mice it seems that drug-specific cells were presented and activated by APCs at the spleen and not the skin. Therefore we consider that human APCs presented drug/antigen to T cells in the spleen or peripheral blood. Taken together, we conclude that soluble factors from CD8⁺ lymphocytes are critical for SJS/TEN development. Our model mice are useful experimental tools to reveal the SJS/TEN pathomechanism.

It is possible that PBMCs from patients with SJS/TEN, which are activated by the causative drug, are highly proliferative and lead to human PBMC reconstitution at day 12, resulting in accelerated GVHRs. However, GVHRs are mediated by human cell infiltration into murine tissue. Indeed, in the GVHR model we detected numerous human cells in the conjunctiva of the mice, whereas in the SJS/TEN-PBMC model, we detected few human cells in conjunctiva and peripheral blood at day 12. These data indicated that at least the ocular manifestations of mice receiving SJS/TEN-PBMC/drug at day 12 were not GVHRs. If PBMCs are highly proliferative at day 12, ocular manifestations can be regarded as a drug-specific phenomenon because mice receiving only PBMCs from patients with SJS/TEN never show such symptoms.

In our model mice it is speculated that the APCs of mice presented drug/antigens to human drug-specific lymphocytes. However the NOG mice used in our experiments have no T, B, and NK cells. In addition, the dendritic cells (DCs) of NOG mice are deficient in antigen presentation.¹⁹ Indeed, dysfunction of DCs allowed engraftment by human cells in NOG mice (ie, DCs of NOG

were unable to present xenoantigen to human lymphocytes sufficiently). Therefore we assume that donor human APCs present drug antigens to T cells. Furthermore, by using human IFN- γ ELISpot, the number of drug-specific lymphocytes increased in the spleens of the SJS/TEN-PBMC model mice.

From the clinical aspect, issues have included the difficulty of early diagnosis of SJS/TEN and unresponsiveness to treatment. In the early stage SJS/TEN presents clinically as edematous papules or erythema multiforme-like target rashes that are very similar to those of ODSRs. Such a clinical course makes it difficult to reach a diagnosis of SJS/TEN in the early stage, which results in high mortality rates. Furthermore, the majority of SJS/TEN cases progress rapidly within a few days; therefore methods of early diagnosis are urgently required. We previously analyzed serum samples from 5 patients with SJS/TEN in the early stage (before skin erosions or mucous lesions appeared) and showed that serum levels of sFasL and granulysin are predictors of SJS/TEN diagnosis.^{11,22} However, collecting samples at the early stage was quite difficult because of the rarity of the diseases and the intractability of the diagnosis in the early stage, as mentioned above. The present mouse model might allow assessment of changes over time and provide other predictors of early diagnosis and severity of SJS/TEN. Furthermore, treatment interventions are able to be implemented in the early phase in our model, contributing to the prediction of disease onset and prognosis.

We thank Ms Ayumi Moriya for her technical expertise.

Clinical implications: We report a novel mouse model of SJS/TEN that was developed by using PBMCs and skin from patients who had recovered from SJS/TEN. The model promises to promote diagnostic and therapeutic approaches.

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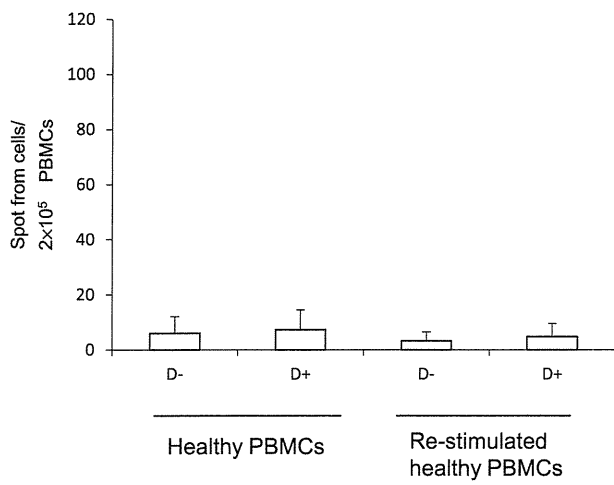


FIG E1. ELISpot assays with PBMCs of naive healthy volunteers who had never experienced cutaneous adverse drug reaction were performed. PBMCs of healthy volunteers ($n = 4$) were stimulated with amoxicillin, one of the causative drugs in our study, and restimulated after 5 days. ELISpot analysis did not detect drug-specific T cells, even in restimulation. *D+*, Drug addition; *D-*, no drug addition.

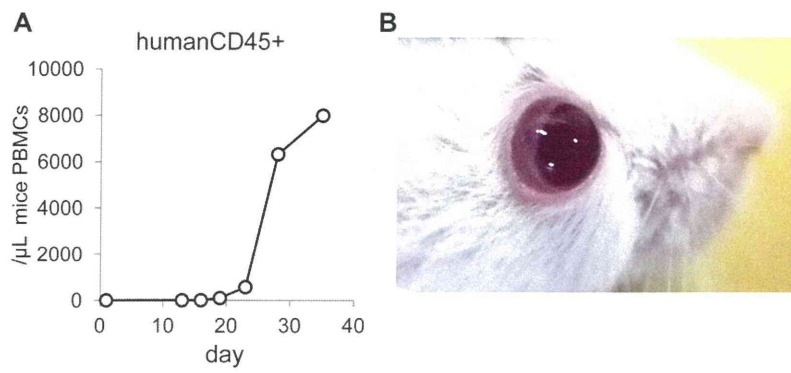


FIG E2. A, Human CD45⁺ cells were detected in peripheral blood of NOG mice 20 days after PBMC injection. B, Ocular manifestations 14 days after PBMC injection.

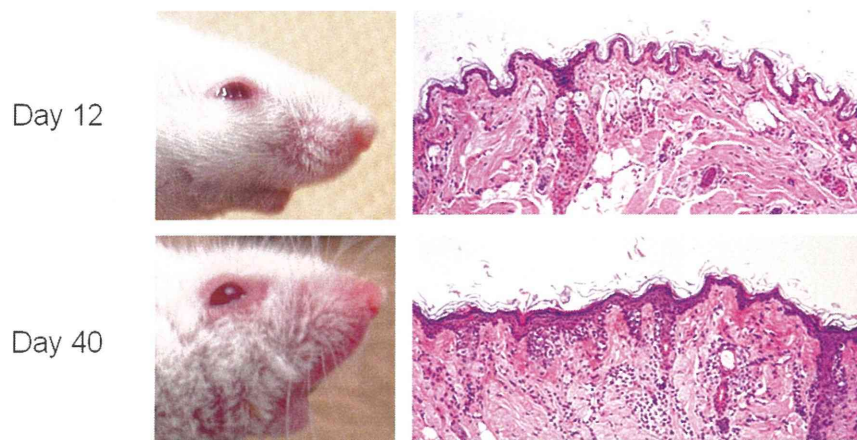


FIG E3. At 12 days after injection of PBMCs from patients with SJS/TEN, NOG mice receiving causative drug showed no significant skin manifestation, and histologic findings indicated intact skin. At 40 days after injection of PBMCs from patients with SJS/TEN, these NOG mice showed weight loss, skin erosion, hair loss, and diarrhea. Prominent epithelial cell death was observed histologically. Samples from patients 1 and 2 with SJS/TEN were analyzed, and representative data (patient 2 with SJS/TEN) are shown.

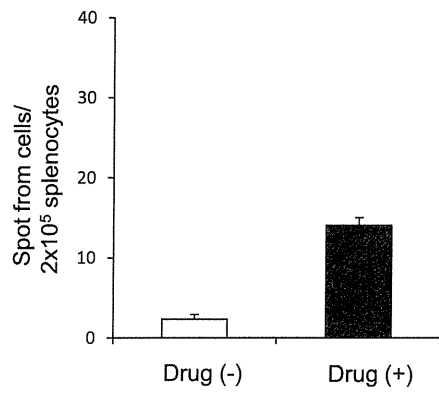


FIG E4. Increase in drug-specific T-cell numbers in the spleens of NOG mice at 12 days after transfer of PBMCs from patients with ODSRs orally given causative drug is detected by using the ELISpot assay. These results are comparable with those for patients with SJS/TEN. Samples from patient 1 with an ODSR were analyzed.

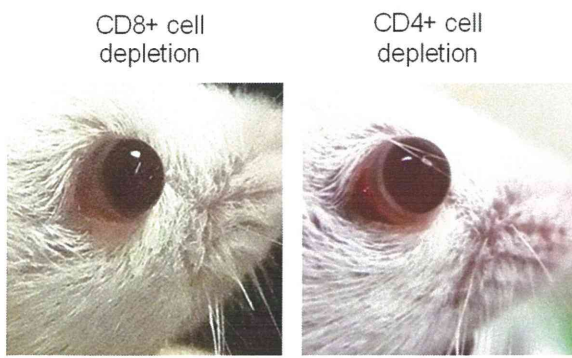


FIG E5. At 12 days after injection of CD4⁺ T lymphocyte-depleted PBMCs from patients with SJS/TEN and causative drug intake, significant conjunctival congestion and conjunctival chemosis were noticed, whereas this was not the case with CD8⁺ T lymphocyte-depleted PBMCs from patients with SJS/TEN. Samples from patient 2 with SJS/TEN were analyzed.

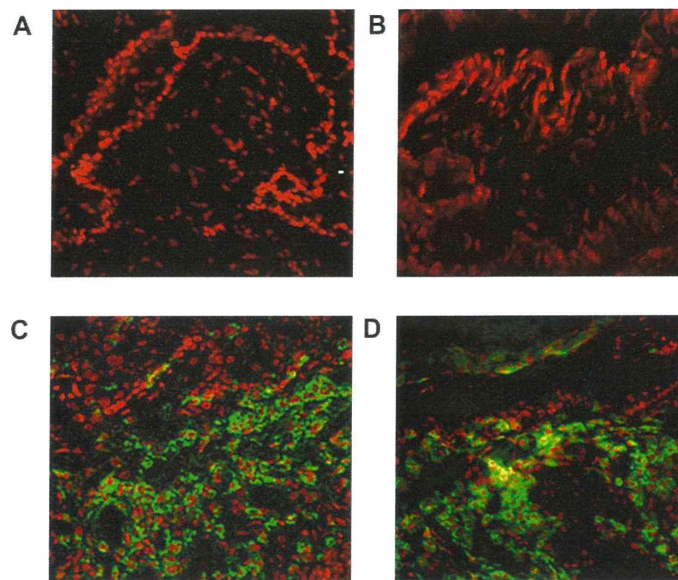


FIG E6. The conjunctiva of SJS/TEN model mice or mice with GVHRs were stained with anti-human CD45 antibodies. FITC-conjugated goat anti-mouse IgG was used as the secondary antibody. The nuclei were counterstained with propidium iodide. **A**, Day 12 for PBMCs from patients with SJS/TEN with causative drug intake. **B**, Day 12 for the GVHD model. **C**, Day 40 for PBMCs from patients with SJS/TEN with causative drug intake. **D**, Day 40 for the GVHD model. In the conjunctiva of both SJS/TEN model and GVHR model mice, there are almost no infiltrating human CD45⁺ cells at day 12 after PBMC injection, whereas numerous human cells are detected in the conjunctiva of both SJS/TEN model and GVHR model mice. Samples from patients 1 and 2 with SJS/TEN were analyzed, and representative data (patient 2 with SJS/TEN) are shown.

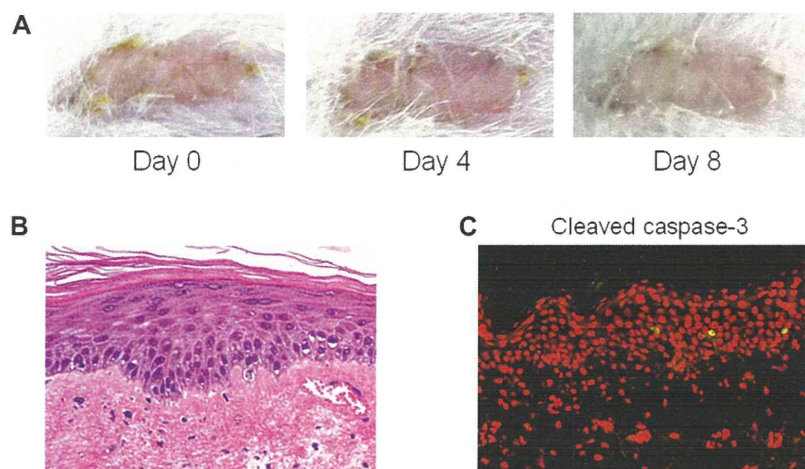


FIG E7. Skin from a patient who had recovered from SJS/TEN was grafted onto the back of NOG mice. After engraftment was confirmed, PBMCs from the same patient were administered intravenously without causative drug administration. Histopathologically, there were few apoptotic keratinocytes in the skin-grafted area. Cleaved caspase-3 staining showed almost no positive cells in the skin-grafted area. Samples from patient 6 with SJS/TEN were analyzed.