

Toxic epidermal necrolysis: Effector cells are drug-specific cytotoxic T cells

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Background: Toxic epidermal necrolysis (TEN) is a very rare but extremely severe drug reaction characterized by widespread apoptosis of epidermis with extensive blisters. We previously found drug-specific cytotoxic CD8 T lymphocytes in the blisters of a single patient.

Objective: To confirm the role of drug specific cytotoxic lymphocytes in a larger series, to test the cytotoxicity on keratinocytes, and to look for cross-reactivity between chemically related drugs.

Methods: The phenotype of lymphocytes present in the blister fluids of 6 patients with TEN was analyzed by flow cytometry. Cytotoxic functions were tested by chromium release assay on a variety of target cells (autologous or MHC class I-matched EBV-transformed lymphocytes, autologous keratinocytes) after nonspecific (CD3 monoclonal antibody) or specific (suspected and potentially cross-reactive drugs) activation.

Results: Blister lymphocytes were CD8⁺HLA-DR⁺CLA⁺CD56⁺. In all 6 cases, they were cytotoxic after nonspecific activation. A drug-specific cytotoxicity was observed in 4 cases (3 related to cotrimoxazole and 1 to carbamazepine) toward lymphocytes. Blister cells also killed IFN- γ -activated autologous keratinocytes in the presence of drug in the 2 patients tested. Blister cells showed a strong immunoreactivity for granzyme B, and cytotoxicity was abolished by EGTA, but not by anti-Fas/CD95, suggesting perforin/granzyme-mediated killing.

By using several sulfonamides for testing the specificity of the drug T-cell receptor interaction, we observed cross-reactivity only between 4 structurally closely related medications.

Conclusion: These results strongly suggest that drug-specific, MHC class I-restricted, perforin/granzyme-mediated

cytotoxicity probably has a primary role in TEN. (J Allergy Clin Immunol 2004;114:1209-15.)

Key words: Toxic epidermal necrolysis, drug hypersensitivity, cytotoxic T lymphocytes, MHC, keratinocytes

Stevens-Johnson syndrome and toxic epidermal necrolysis (TEN), considered variants of a single disease, are among the most dramatic adverse reactions to drugs (especially anticonvulsants, antibacterial sulfonamides, oxycam nonsteroidal anti-inflammatory drugs, nevirapine, or allopurinol).¹⁻³ An acute rash progresses toward widespread blisters and erosions of skin and mucous membranes. The death rates average 25%, and many survivors have sequelae.

Blisters and erosions result from disseminated apoptosis of keratinocytes, possibly mediated by the death receptor Fas/CD95.^{4,5}

It has been suspected for years that TEN was immunologically mediated. Lesions contain a scarce infiltrate of T lymphocytes, mainly of CD8 phenotype, suggesting that apoptosis could be initiated by cytotoxic T lymphocytes (CTLs).⁶

In the last decade, several teams obtained medication-specific human T-cell clones from blood mononuclear cells of patients with history of drug reactions.⁷⁻¹² These clones were either of CD4 or CD8 phenotypes and proliferated in presence of the parent drug, reactive metabolite, or both. Some exhibited cytotoxicity against allogeneic targets.^{11,12}

In TEN, blisters that result from accumulation of interstitial fluid under the necrotic epidermis contain T lymphocytes with a phenotype of cytotoxic cells.¹³ In 1 patient with sulfamethoxazole-induced TEN, we reported the presence in the blister fluid of CD8 CTLs killing autologous lymphocytes in a drug-specific, class I-restricted, and perforin/granzyme-mediated pathway.¹⁴ Because this was a single case report, it remained to determine whether these findings could be generalized. We report here additional findings in 6 patients, suggesting that drug specific cytotoxicity is a key factor in the pathogenesis of TEN. We also demonstrate that the blister cells can kill autologous keratinocytes and study cross-reactivity of drug-specific CTL.

METHODS

Cells from patients

The study was performed on the blister fluid lymphocytes of patients hospitalized for TEN in a specialized unit. Among 25

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Abbreviations used

CLA:	Cutaneous lymphocyte-associated antigen
CTL:	Cytotoxic T lymphocyte
EGTA:	Ethyleneglycol-bis(β -aminoethyleter)-N,N,N',N'-tetraacetic acid
E/T:	Effector/target
SMX:	Sulfamethoxazole
SMX-NO:	Nitroso of sulfamethoxazole
SMX-NOH:	Hydroxylamine of sulfamethoxazole
TCR:	T-cell receptor
TEN:	Toxic epidermal necrolysis
TMP:	Trimethoprim
TMP-SMX:	Cotrimoxazole (combination of trimethoprim and sulfamethoxazole)

consecutive patients with biopsy-proven TEN, we studied all 6 with enough blister fluid cells ($n = 15$), a single drug suspected as responsible (8/15), and availability of a soluble form of this drug (6/8). An ethical committee (Comité Consultatif pour la Protection des Personnes se prêtant à une Recherche Biomédicale Hôpital Pitié-Salpêtrière, Paris, France) approved the study, and patients provided written informed consent. Only 1 patient (#1) received high doses of intravenous immunoglobulins. In this patient, blister fluid was obtained before the onset of infusion.

Blister fluid was obtained on admission through puncture of several blisters. After centrifugation, cells were resuspended, washed twice, and frozen in culture medium consisting of RPMI 1640 (GIBCO, Paisley, United Kingdom), 2 mmol/L L-glutamine, penicillin (100 U/mL), streptomycin (100 mg/mL), 20% heat-inactivated human serum, and 10% DMSO. The frozen vials were stored in liquid nitrogen.

Blood lymphocytes were isolated by Ficoll-Isopaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation. The cells were washed twice and frozen as described.

Autologous PHA-stimulated PBMCs (patient 4) or EBV-transformed B-cell lines generated by usual procedures¹⁵ were used as target cells.

Keratinocytes were released by trypsinization of hair follicles plucked from the scalp of 2 patients after recovery. They were seeded at a density of 2 to 3×10^3 cells/cm² on an irradiated mouse cell line (3T3) according to Limat and Noser.¹⁶ After 2 weeks in primary culture, keratinocytes were detached by trypsin and replated in culture medium at a density of 10^4 cells/cm² on a fresh 3T3 feeder layer.

Antibodies and reagents

Anti-CD8, anti-CD4, anti-CD56, anti-HLA-DR, anti-CD95 (Fas) monoclonal antibodies were purchased from Beckman-Coulter (Marseille, France) and antigranzyme B antibody from Caltag Laboratories (San Francisco, Calif). Anti-CLA (HECA 442) was purchased from BD Biosciences (Le Pont de Claix, France). All other monoclonal antibodies were produced locally.

Phenotypic analysis was performed on thawed cells (aliquots of 2×10^5 cells) incubated for 15 minutes at 4°C with the corresponding fluorescent antibodies. For indirect immunofluorescence staining, we used as second reagent a fluorescein isothiocyanate-conjugated goat antimouse immunoglobulin from Caltag Laboratories. Controls were treated with unrelated antibodies of the same isotype.

Immunostaining for granzyme B used 5 μ L phycoerythrin-labeled antigranzyme B monoclonal antibody. The cells were fixed for 20 minutes with 2% formaldehyde before labeling with antigranzyme

B in permeabilization buffer (0.1% saponin, 1% human serum AB, 0.1% NaN₃) at 4°C.

Stained cells were analyzed by using a single argon flow cytometer analyzer XL Epics (Coulter, Hialeah, Fla).

Determination of drug-specific cytotoxic activity *in vitro*

Assays at various effector to target ratios were performed in triplicate and replicated twice to 5 times when enough effector cells were available (patients 1, 2, and 4). Cytotoxicity assays were performed according to the standard ⁵¹Cr-release method. Target cells were incubated with 100 μ Ci sodium 51 chromate (CEA, Saclay, France) for 90 minutes at 37°C, washed in medium containing 10% human serum, and plated at 1000 cells/well by using 96-well V-bottomed microtiter plates. For blocking experiments, anti-Fas/CD95 antagonist monoclonal antibody (ZB4) was preincubated with target cells for 1 hour 30 minutes at 37°C. Ethyleneglycol-bis(β -aminoethyleter)-N,N,N',N'-tetraacetic acid (EGTA; 4 mmol/L) and MgCl₂ (3 mmol/L) were added during the assay to inhibit Ca⁺⁺-dependent perforin/granzyme-mediated lysis.

For anti-CD3-induced redirected cytotoxicity against P815 cells (mouse mastocytoma cell line), blister fluid cells were resuspended and incubated for 15 minutes at room temperature with anti-CD3 (mAb ascites fluid diluted at 1/1000). The final culture volume was 200 μ L.

After 4 hours of culture, plates were spun, and 100 μ L of supernatant was removed from each well and counted in a γ -counter for determination of ⁵¹Cr release.

The percentage of specific lysis was calculated as $100 \times (\text{experimental release [cpm]} - \text{spontaneous release [cpm]}) / (\text{maximum release [cpm]} - \text{spontaneous release [cpm]})$.

The percentages of specific chromium release in the presence of both effector cells and drug were calculated as $(\text{release with both effector cells and drug on targets}) - (\text{release with effector cells alone on targets}) / (\text{maximum release}) - (\text{release with effector cells alone on targets})$.

Drugs used in the assays

Sulfamethoxazole and metabolites were used as follows:

Combination of trimethoprim and sulfamethoxazole (cotrimoxazole, TMP-SMX) as marketed (Bactrim, Roche, Neuilly sur Seine, France) for intravenous injection

Sulfamethoxazole (SMX) and purified hydroxylamine (SMX-NOH) were obtained from Françoise Camus-Baud, PharmD, Hôpital Bichat Paris

Purified nitroso-sulfamethoxazole (SMX-NO) was kindly provided by Dean Naisbitt, MD, Liverpool, United Kingdom

Final dilutions of 80 mg/L SMX used associated to 16 mg/L trimethoprim (TMP) when TMP-SMX was tested; at these concentrations, ⁵¹Cr release was similar to spontaneous release

Other antibacterial sulfonamides: sulfadiazine, sulfapyridine, sulfamethizole, sulfisoxazole, sulfasalazine, or related drugs (tolbutamide, chlorpropamide, glyburide, hydrochlorothiazide), purchased from Sigma (St Quentin Sallavie, France), were tested at concentrations within the range of therapeutic values (ie, from 100 μ g/L for glyburide to 60 mg/L for sulfapyridine)

Carbamazepine, purchased from Sigma, used at a concentration of 9 mg/L

Tetrazepam, provided by Sanofi-Synthelabo (Gentilly, France), used at a concentration of 1 μ g/mL

Piroxicam used at 15 μ g/mL from vials marketed for intravenous injection (Feldene, Pfizer, Paris, France)

TABLE I. Phenotypic analysis of T lymphocytes present in blister fluid from 6 patients with TEN*

Patient	Drug inducing	CD3	CD8	CD4	TCR $\alpha\beta$	CLA	HLA-DR	CD56
1	TMP-SMX	99	90	9	89	83	85	72
2	TMP-SMX	89	81	20	80	36	67	18
3	TMP-SMX	83	70	14	79	77	67	12
4	Carbamazepine	77	72	10	71	70	23	21
5	Tetrazepam	91	86	6	73	39	76	5
6	Piroxicam	91	93	21	87	88	69	39

*Fluorescence-activated cell sorting analysis of gated lymphocytes from the blister fluid. Numbers are percentages of positive cells.

Molecular modeling of sulfonamides

The simulations were performed with the software Sybyl version 6.9 (Tripos, St Louis, Mo) with the MMFF94s force field by using a 10-Å nonbonded interaction cutoff and a distance-dependent dielectric constant of 4R.

The structure of each molecule was built manually with the sketcher. Then, the structure was energy-minimized, with 100 steps of steepest descent and 500 steps of conjugate gradient. Next, a 40-picosecond molecular dynamics run was performed. The final structure of molecular dynamics served to search the low energy conformer of each molecule. The conformer search was realized by simulated annealing. Each of the 10 conformers generated was then energy-minimized, with 100 steps of steepest descent and 1000 steps of conjugate gradient. The lowest energy conformer, considered the most stable, was chosen as the best conformer for each molecule.¹⁷ The figure was generated with the software Viewer Lite (Accelrys, Orsay, France) by superimposition of these best conformers.

RESULTS

Immunophenotype of blister fluid cells

Phenotypic analysis of T lymphocytes present in blister fluids from 6 patients with TEN is presented in Table I. The majority of the lymphocytes were CD3⁺T-cell receptor (TCR) $\alpha\beta$ ⁺, with a huge predominance of the CD8⁺ subset. In most patients, these lymphocytes were activated as they expressed HLA-DR and had been targeted to home in the skin because they were stained by anti-cutaneous lymphocyte-associated antigen (CLA) monoclonal antibody. In 5 of the 6 patients, there was a significant expression of CD56. By multiple labeling, the CD56⁺ cells were shown to belong to the lymphocyte subset expressing CD3, TCR $\alpha\beta$, and CD8.

Blister fluid cells were cytotoxic

A CD3-induced redirected cytotoxic assay was performed with blister fluid cells from all 6 patients, without any *ex vivo* stimulation, to determine whether these cells exhibited CTL activity. Unstimulated blister fluid cells incubated with anti-CD3 mAb were able to kill mouse mastocytoma cells (P815) in all 6 cases (Table II). The percentage of specific ⁵¹Cr release increased with higher effector/target (E/T) ratios and when effector cells were preincubated overnight with IL-2 (200 UI/mL; Fig 1). In the absence of anti-CD3 mAb, the blister fluid cells were unable to lyse P815. This confirmed that the cytotoxic potential of the CD8 T cells

TABLE II. Redirected cytotoxicity of blister fluid cells*

Patient	% Cytotoxicity: E/T ratio 50/1	
	P 815	P 815 + anti-CD3
1	3 ± 1	20 ± 1.5
2	1 ± 0.4	22 ± 1
3	4 ± 1.8	14 ± 1.4
4	1 ± 1.3	30 ± 2.7
5	1 ± 1	16 ± 1
6	0.2 ± 0.9	12 ± 1.6

*Blister fluid cells were functionally cytotoxic after activation of the TCR by anti-CD3.

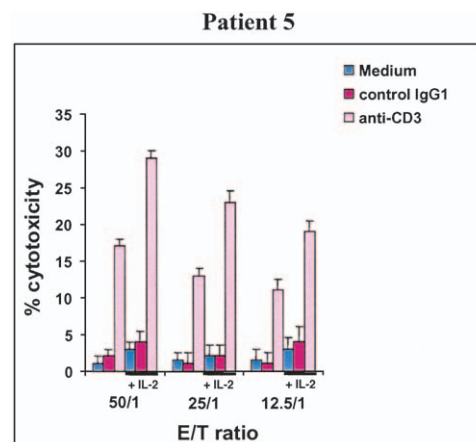


FIG 1. Redirected cytotoxicity of blister fluid cells. Blister fluid cells were lytic for murine mastocytoma (P815) after activation of the TCR with anti-CD3 mAb. Cytotoxicity was increased by IL-2.

present in the blister fluid was dependent on CD3/TCR engagement.

Blister fluid T cells displayed drug-specific cytotoxic activity against autologous B-lymphocyte cell lines

In the absence of drug as well as in the presence of a nonrelevant drug, the cells from blister fluid revealed no cytotoxic effect toward autologous EBV-derived B-cell lines (patients 1, 2, 4-6) or EBV-derived cell line from a HLA-matched allogeneic donor (patient 3: HLA-B35, HLA-Cw4). A low but significant and reproducible specific cytotoxic activity was observed in 4 cases when

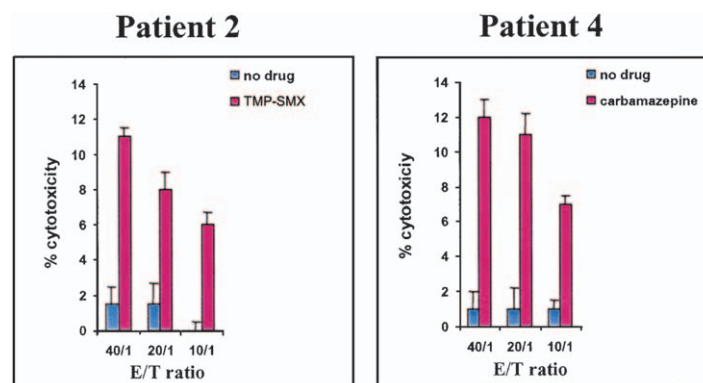


FIG 2. Drug-dependent cytotoxicity of blister fluid cells on autologous lymphocytes. Blister fluid cells were lytic for autologous EBV-transformed lymphocytes preincubated with TMP-SMX for patient 2 and with carbamazepine for patient 4. Controls in the absence of drug.

target cells were incubated with the appropriate drug (TMP-SMX for patients 1-3; carbamazepine for patient 4). The representative results obtained in patients 2 and 4 are presented in Fig 2. The specific CTL activity occurred at all E/T ratios tested but increased at higher ratios. The specific CTL activity was increased up to 70% when effector cells were preincubated overnight with IL-2. In 2 cases (patients 5 and 6), we did not observe any significant chromium release in the presence of the medication that had been suspected (tetrazepam and piroxicam, respectively).

Blister fluid T cells displayed drug-specific cytotoxic activity against autologous keratinocytes

Culture of keratinocytes could be obtained in 2 patients (1 and 2) after recovery. In both cases, keratinocytes were lysed by autologous blister fluid cells in the presence of the culprit drug (TMP-SMX in both cases). Fig 3, A, shows representative experiments obtained in patient 1. This drug-specific cytotoxicity occurred only after activation of keratinocytes by IFN- γ , which strongly increased the expression of MHC class I molecules and induced the expression of MHC class II (Fig 3, B). Further, we tested the cytotoxicity of effector cells from patient 1 (A2, A2, B18, B35, Cw4, Cw7) against keratinocytes obtained from 2 allogeneic donors (one A1, A2, B13, B14, Cw6, Cw8, and the other A1, A3, B49, B35, Cw4, Cw7). A drug-specific cytotoxicity was obtained on keratinocytes of the second control, suggesting a restriction by HLA-B35, HLA-Cw4, or HLA-Cw7 alleles, and not by HLA-A2. This was in accordance with previous restriction studies performed in patient 1 on lymphocyte targets, which suggested a restriction by Cw4 more probably than by B35.¹⁴

Drug-specific cytotoxicity was mediated by perforin/granzyme

The mechanisms of cytotoxicity of cotrimoxazole-specific lymphocytes were evaluated in 2 patients. We

used as target cells IFN- γ -activated autologous keratinocytes (patient 1) and EBV-transformed autologous B lymphocytes (patients 1 and 2). In all instances, the specific chromium release was totally abolished by incubation of target cells with EGTA and MgCl₂, but not modified by an antagonist anti-CD95/Fas mAb (Fig 4, A). This result strongly suggests a perforin/granzyme-mediated apoptosis of target cells. In all 5 tested cases, blister fluid lymphocytes were highly positive for intracellular granzyme B (Fig 4, B).

Cross-reactivity of cotrimoxazole-specific blister fluid cells

Cotrimoxazole is an association of TMP and a sulfonamide: SMX. It is generally assumed that SMX itself or 1 of its metabolites is responsible for the reactions. Because TMP induced a nonspecific chromium release from target lymphocytes, any specific cytotoxicity related to TMP was not evaluable. On the other hand, the cell-mediated cytotoxicity observed with 100 μ g/mL SMX was of the same magnitude as that with 100 μ g/mL SMX plus 20 μ g/mL TMP (Fig 5).

We also tested the cytotoxicity of blister fluid cells from 2 patients with cotrimoxazole-induced TEN in the presence of 2 main reactive metabolites of sulfamethoxazole: hydroxylamine and nitroso-sulfamethoxazole. Cytotoxicity was observed in both cases at high concentrations (5-20 μ g/mL) of the nitroso metabolite and in 1 case with the hydroxylamine metabolite. At lower and more physiologic concentration (1 μ g/L), no cross-reaction was observed. To investigate whether the response of SMX-specific cells was directed to a common structure of sulfonamides, we tested in the cytotoxicity assay the blister fluid cells from patient 1 with other sulfonamide medications: medications used for antibacterial purposes (sulfapyridine, sulfasalazine, sulfamethizole, sulfadiazine, and sulfisoxazole), medications used in diabetes (glyburide, chlorpropamide, and tolbutamide), and medications used as diuretics (hydrochlorothiazide). A significant cytotoxicity was observed exclusively with 3 other antibacterial sulfonamides (sulfamethizole, sulfapyridine,

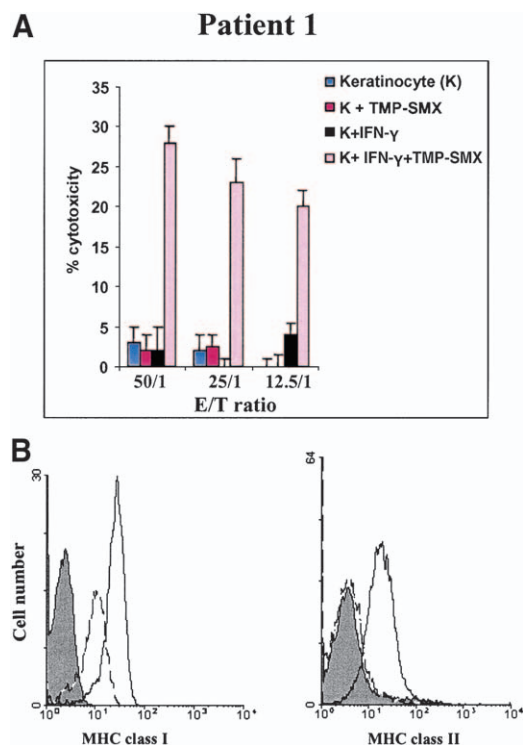


FIG 3. A, Drug-dependent cytotoxicity of blister fluid cells on autologous keratinocytes. Blister fluid cells were cytotoxic against autologous keratinocytes pretreated with IFN- γ and TMP-SMX. Controls: effector cells with keratinocytes untreated, treated with IFN- γ alone, or treated with TMP-SMX alone. **B,** Flow-cytometric analysis for MHC class I and MHC class II expression on keratinocytes. Staining with control IgG1 (gray histogram), untreated (white histogram, discontinuous line), and IFN- γ -treated keratinocytes (white histogram, plain line).

and sulfadiazine) (Fig 5). Interestingly, according to the best conformer (Fig 6) and the predominant conformation in molecular dynamics run (data not shown), the 3 drugs with a potential for cross-reactivity appeared very similar to sulfamethoxazole, with an elongated conformation. The 2 other antibacterial sulfonamides (and the 4 nonantibacterial sulfonamides), which did not interact with cytotoxic cells, had a folded conformation, and the side chain pointed in the opposite direction compared with the recognized molecules.

DISCUSSION

We had previously found CD8⁺ T lymphocytes exhibiting drug-specific cytotoxicity in the blister fluid of a single patient with TMP-SMX-related TEN.¹⁴ These specific CTLs were MHC class I-restricted and used a perforin/granzyme-mediated pathway to exert their killing.

The current data extend these initial findings in a total of 6 patients, demonstrate that the blister fluid cells can also kill autologous keratinocytes in the presence of the drug, and suggest cross-reactivity of sulfonamide-specific CTL.

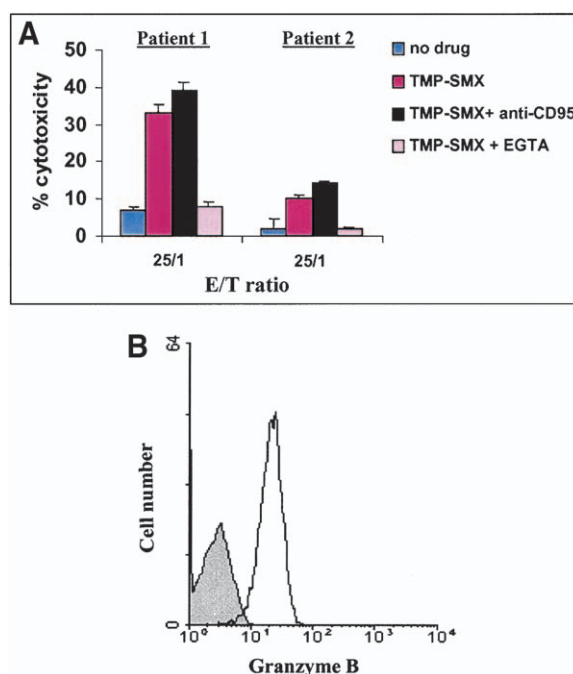


FIG 4. Mediators of cytotoxicity. A, The drug-dependent lysis on keratinocytes (patient 1) or B-cell line (patient 2) was totally abolished by EGTA but not by mAb anti-CD95/Fas. **B,** Blister fluid lymphocytes contain intracellular granzyme B. Staining with control IgG1 (gray histogram) or with antigranzyme B (white histogram).

Because the experiments could be performed in only 6 of 25 consecutive patients, our results do not provide a definite explanation for the mechanisms of all cases of TEN. We have no reason to suspect a major selection bias, because blisters are so easily broken that the availability of enough fluid depends principally on cautious handling of the skin before admission. It is also usual that approximately 1/2 of patients have been exposed to many drugs.

CD8 T cells exhibiting a very homogeneous phenotype were found in most patients. The expression of CD56 was increased in these blister fluid lymphocytes, indicating that they phenotypically corresponded with lymphocytes with cytotoxic functions.¹⁸

In 4 of 6, cases these cells induced without any stimulation a drug-specific cytotoxicity toward autologous lymphocytes in the presence of the parent form of the suspected medication. This specific CTL activity was enhanced by short-term culture with IL-2.

That we did not find a drug-specific reaction in 2 patients should not be interpreted too quickly as suggesting the intervention of other mechanisms. The phenotype of blister fluid lymphocytes from these 2 patients was almost identical to those of the 4 others, with the exception of lower CD56 expression in 1. Blister fluid lymphocytes of these 2 patients were actually cytotoxic after non-specific activation of the TCR by anti-CD3. Therefore, we consider that our inability to demonstrate drug specificity in these 2 cases may be explained by uncertainty about the

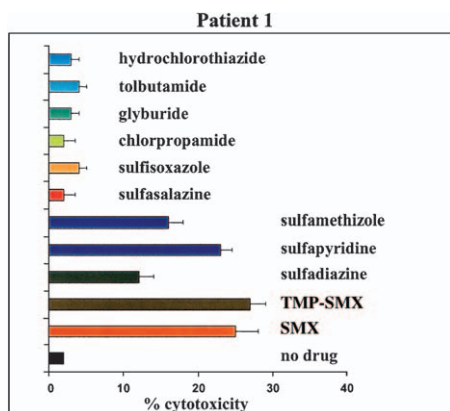


FIG 5. Cross-reaction of TMP-SMX and other sulfonamides. Blister fluid cells were lytic for autologous lymphocytes preincubated with TMP-SMX, SMX, sulfapyridine, sulfamethizole, and sulfadiazine, but not in the presence of hydrochlorothiazide, tolbutamide, sulfisoxazole, glyburide, chlorpropamide, and sulfasalazine, or in the absence of drug.

causative drugs or by a reaction directed against metabolites.

Overall, our findings support that drug-specific CTLs are often the initiators of the widespread apoptosis of epidermal cells in TEN.

We also demonstrated that autologous keratinocytes, the actual *in vivo* targets in TEN, were killed as well as lymphocytes in the presence of the culprit drug. Some previous publications reported that clones derived from lymphocytes extracted from skin lesions of patients with drug reactions could be cytotoxic for keratinocytes.¹² Because these clones had been obtained after several stimulations *in vitro* with the medication, the relevance of these findings remained questionable. To the best of our knowledge, it is the first time that lymphocytes present in skin lesions have been shown to kill autologous keratinocytes in the presence of the culprit drug without any restimulation.

In agreement with most publications, we found that keratinocytes must be activated by IFN- γ before being susceptible to lysis. Because CD8 CTLs are restricted by MHC class I, this is probably a result of the enhanced expression by activated keratinocytes of MHC class I and also of costimulatory molecules. This could be also explained by other phenotypic alterations that increase sensitivity to apoptosis.

We obtained some evidence of allogeneic restriction by MHC class I. In patient 3, in the absence of available autologous lymphocytes, the cytotoxicity of blister fluid cells was evidenced on allogeneic lymphocytes sharing 3 HLA antigens with the patient. Effector lymphocytes from patient 1 killed autologous EBV-transformed B cells sharing HLA-B35 and HLA-Cw4.¹⁴ They did not kill keratinocytes from 1 allogeneic control who expressed none of these 2 class I molecules, but killed efficiently keratinocytes from another control expressing HLA-B35 and HLA-Cw4.

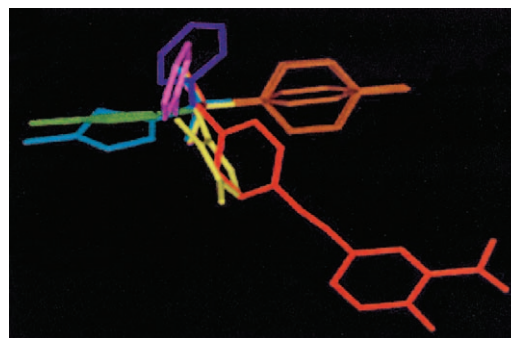


FIG 6. Molecular modeling of the 6 anti-infectious sulfonamides tested for cross-reactivity. The 4 cross-reacting drugs—SMX (green), sulfamethizole (blue), sulfapyridine (pink), and sulfadiazine (purple)—exhibited a close conformation. For the 2 non-reacting drugs, sulfisoxazole (yellow) and sulfasalazine (red), the side chain pointed in the opposite direction.

It has been proposed that the activation of the death receptor Fas/CD95 played a key role in the disseminated apoptosis of keratinocytes in TEN.⁵ Our results showed that the drug-dependent cytotoxicity by lymphocytes was not mediated by CD95. Actually, an antagonist mAb had no effect, whereas the inhibition of perforin activity abolished the specific chromium release. In addition, we found that the effector lymphocytes were strongly positive for intracellular granzyme. By RT-PCR analysis of cytokines produced by blister fluid cells in TEN, other authors observed overexpression only of perforin and granzyme B.¹⁹ Taken together, these results demonstrate that perforin/granzyme is the main pathway used by CTL for initiating the apoptosis of keratinocytes in TEN. That does not exclude further amplification by an overexpression of CD95 and its ligand by keratinocytes. Intravenous immunoglobulins had been shown to inhibit CD95-mediated apoptosis to some extent, but are not expected to inhibit CTL activity through perforin and granzyme. That may explain why the clinical benefits of this treatment in TEN remain so controversial.

Cross-reactivity between sulfonamides was evaluated in 1 patient by comparing specific chromium release in the presence of the culprit drug (SMX) and of 9 other sulfonamides. Reactivity was observed only with 3 structurally related antibacterial sulfonamides. Nothing was observed for diuretic or hypoglycemic sulfonamides with different structures. Two of the 6 antibacterial sulfonamides, sulfasalazine and sulfisoxazole, were not recognized. Comparing the structures of the different compounds suggested that 3-dimensional structure of the drug is essential for allowing MHC drug complex to be accessible for the interacting TCR.

In 2 patients tested, some cytotoxicity was obtained in the presence of high concentrations of the 2 main metabolites of SMX. This was abolished with lower concentrations, closer to the expected *in vivo* concentrations. This clearly shows that the parent compound often has the primary role, even if it does not exclude a role for reactive metabolites in other patients. T-cell clones

derived from the blood of patients who reacted to SMX also demonstrated specificity against SMX rather than against metabolites in more than 90% of cases.²⁰

In conclusion, we consider that the present results confirm our initial findings in a single patient. They strongly support that CTLs are inducing the widespread apoptosis of epidermal cells in TEN by using a perforin/granzyme B pathway. In most instances, these CTLs are specific of the medication that is clinically suspected to be responsible for the reaction. The key role of CTL in TEN was recently confirmed in an animal model in which where ovalbumin-specific CTLs were shown to induce TEN-like lesions in transgenic mice expressing a membrane-bound form of ovalbumin in the epidermal keratinocytes.²¹ These findings should prompt clinical trials of therapeutic agents capable of inhibiting the action of CTLs.

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