

Anticonvulsant-induced toxic epidermal necrolysis: Monitoring the immunologic response

Laura Leyva, MD, PhD,^a María José Torres, MD, PhD,^a Sinfioriano Posadas, BS,^a Miguel Blanca, MD, PhD,^a Guillermo Besso, MD,^c Francisco O'Valle, MD, PhD,^e Raimundo García del Moral, MD, PhD,^e Luis F. Santamaría, PhD,^d and Carlos Juárez, MD, PhD^b *Malaga, Granada, and Barcelona, Spain*

Background: Toxic epidermal necrolysis is a severe reaction with skin involvement induced by different drugs and other agents. The mechanisms implicated in the induction of the reaction are poorly understood.

Objective: Our purpose was to study the involvement of T lymphocytes and other immunocompetent cells in the peripheral blood, blister fluid, and affected skin of 3 patients who had a severe reaction after receiving anticonvulsant medication.

Methods: Quantification of T lymphocytes expressing the skin-homing receptor (cutaneous lymphocyte-associated antigen [CLA]) in peripheral blood, skin, and skin blister fluid and assessment of other adhesion molecules, activation markers, and inflammatory interleukins by flow cytometry, immunohistochemistry, and reverse transcription-PCR.

Results: An increase in CD3⁺CLA⁺ cells paralleling the severity of the disease was observed in both peripheral blood and skin, tending to normalize as soon as patient's conditions improved. E-selectin was detected in endothelial vessels in parallel with CLA expression on lymphocytes. An overexpression of TNF α , IFN- γ , and IL-2 was also observed in PBMCs. The expression of the different markers changed over the course of the disease.

Conclusions: These data show an increase in activated T cells expressing the skin-homing receptor in both tissue and peripheral blood accompanying clinical symptoms, with a recruitment of macrophages and an overexpression of cytokines. All these results suggest an important role for T cells in the production of toxic epidermal necrolysis. (*J Allergy Clin Immunol* 2000;105:157-65.)

Key words: Toxic epidermal necrolysis, anticonvulsant, severe skin reactions, T lymphocytes, cutaneous lymphocyte-associated antigen, homing, cytokines

Toxic epidermal necrolysis (TEN) is a severe, sometimes life-threatening skin disorder often triggered by drugs. It is characterized by extensive shedding of skin in sheets, producing the appearance of scalded skin.¹ Mucosal involvement as well as severe constitutional symptoms are a common feature. TEN occurs at all ages, at an estimated incidence of 0.4 to 1.2 cases per million people per year,² and is associated with a mortality rate of about 30%. Drugs are considered to be the most frequent triggering factor of TEN. *Mycoplasma pneumoniae* or herpes simplex infections, immunization, and food additives have also been reported as inducers of this syndrome, but in some patients a causative agent of the reaction cannot be found.^{3,4} Drugs most frequently involved include antibiotics, sulfonamides, nonsteroidal anti-inflammatory drugs, allopurinol, and anticonvulsant agents.^{5,6} Qualitative defects in the oxidative metabolizing enzymes (cytochrome P450) of these drugs have been reported to lead to the generation of reactive metabolites with a higher cellular toxicity than the parent compounds, initiating immunologic responses.⁷

The pathophysiologic mechanisms leading to TEN are not well understood, although an immune reaction is suspected. Some authors suggest a delayed-type hypersensitivity reaction, with a crucial role for T lymphocytes, either for T-helper lymphocytes⁸ or for cytotoxic T cells.^{9,10} Others support monocyte-macrophage lineage cytotoxicity.¹¹ However, to our knowledge, there have been few studies providing the characterization of the cellular subsets during the evolution of the disease, both in the peripheral blood and the affected skin. We studied 3 patients with a severe reaction induced by phenytoin in 2 cases and carbamazepine in 1 case. Peripheral blood samples were monitored in parallel to the skin response in an attempt to elucidate the sequential steps of the immunologic response implicated in this reaction. Our results provide useful information about T-cell involvement and a better understanding of the pathogenic mechanisms leading to TEN.

From the ^aResearch Unit for Allergic Diseases, the ^bInternal Medicine Department, and the ^cUnit of Intensive Care, Carlos Haya Hospital, Malaga, the ^dAlmirall Prodesfarma SA, Centro de Investigación, Barcelona, and the ^ePathology Department, University Hospital, Granada, Spain.

Supported in part by grants No. CICYT SAF 96/0240 from the Consejería de Salud de la Junta de Andalucía and No. Fondo de Investigaciones Sanitarias 98/0861. L. L. has FIS fellowship No. 98/5061.

Received for publication Feb 24, 1999; revised Sept 20, 1999; accepted for publication Sept 30, 1999.

Reprint requests: Carlos Juárez, MD, PhD, Internal Medicine Department, "Carlos Haya" Hospital Complex, Avda Carlos Haya, Malaga 29010, Spain.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/1/103417

Abbreviations used

CLA:	Cutaneous lymphocyte-associated antigen
GC:	Glucocorticoids
GR:	Glucocorticoid receptor
ICAM-1:	Intercellular adhesion molecule-1
mRNA:	Messenger RNA
PE:	Phycoerythrin
PerCP:	Peridinin chlorophyll protein
RT-PCR:	Reverse transcription-PCR
SBAP:	Streptavidin-biotin-alkaline phosphatase
TEN:	Toxic epidermal necrolysis
VCAM-1:	Vascular cell adhesion molecule-1

PATIENTS AND METHODS**Case 1**

A 55-year-old man with glioblastoma multiforme started therapy with dexamethasone (24 mg/d) and phenytoin (1000 mg/d). Four weeks later, he had swelling of the tongue and lip edema. After 24 hours he had a pruritic maculopapular eruption spreading rapidly on the trunk, neck, and face with involvement of the mucous membranes. One day later, on day 3 of the disease, confluent blisters appeared on the trunk, mainly on the back, and phenytoin therapy was withdrawn. The patient had severe epidermal loss (50% of the body surface area) and erosions on conjunctival, oropharyngeal, and urogenital mucosae. After 2 weeks the skin lesions began to improve with a slow re-epithelialization that was almost complete 6 weeks after the onset of the symptoms.

Case 2

A 61-year-old man with an olfactory groove meningioma started therapy with dexamethasone (24 mg/d) and phenytoin (1500 mg/d). Four weeks later a pruritic maculopapular eruption developed on the trunk, face, and neck with involvement of the mucous membranes, and phenytoin treatment was stopped. On day 3 of the disease blisters appeared on the abdomen and back. The patient had severe epidermal loss (60% of the body surface area). On day 16 the skin lesions began to heal and were almost completely healed 30 days after the onset of the symptoms.

Case 3

A 19-year-old woman started carbamazepine therapy (600 mg/d) because of partial seizures. Fourteen days later a pruritic maculopapular eruption developed on the trunk, neck, and face. The eruption spread rapidly with involvement of the mucous membranes. Carbamazepine therapy was discontinued and prednisone (80 mg/d) was prescribed. Three days later, on day 5 of the disease, generalized and confluent blisters appeared. The following day the patient had severe epidermal loss (80% of the body surface area) and painful erosions on the mucosae. Because no improvement was observed, corticosteroids were withdrawn. On day 17 a slow re-epithelialization began and was almost complete by day 40. Cutaneous residual hyperpigmentation, corneal erosions, and neovascularization of the conjunctiva were the only sequelae.

Serologic studies for HIV were negative in all cases. Written informed consent for all the diagnostic procedures was obtained from the patients or their families.

mAbs

Leu4 (CD3, peridinin chlorophyll protein (PerCP), FITC), Leu8 (L-selectin, FITC), HECA-452 (a rat IgM against cutaneous lymphocyte-associated antigen [CLA], FITC), Leu-23 (CD69, phyco-

erythrin [PE]), G1CL (mouse IgG1 control, FITC, PE, PerCP), and G2G1 (mouse IgG2 control, FITC, PE) were obtained from Becton Dickinson (San Jose, Calif). The rat IgMk control (FITC, PE) was purchased from PharMingen, Becton Dickinson (San Diego, Calif). mAb Leu3a (CD4, FITC, PE), Leu2a (CD8, FITC, PE), anti-IL-2 receptor (CD25, PE), and anti-HLA-DR (PE) were purchased from Ortho Diagnostic Systems (Raritan, NJ). Unconjugated HECA-452 was kindly provided by Dr Louis J. Picker and goat antirat IgM (PE) was obtained from Jackson Immunoresearch Laboratories (West Grove, Pa).

Phenotypic immunofluorescence analysis

Mononuclear cells from heparinized blood and from blister fluid were isolated by density gradient centrifugation. Blister fluid was obtained from tense blisters of sufficient size (>1 mL) by withdrawal into a syringe. Five-parameter analysis was performed on a Cytoron absolute flow cytometer equipped with Immunocount System software (Ortho) with use of FITC, PE, and PerCP as the 3 fluorescent parameters. Immunofluorescence staining for this multiparameter analysis was performed as described previously.¹² Negative isotype controls were used to verify the staining specificity of the antibodies used.

Lymphocyte transformation test

The lymphocyte transformation test was performed as described,¹³ with minor modifications, in the presence of several concentrations (10-200 µg/mL) of carbamazepine, phenytoin, phenobarbital, and valproic acid. Tetanus toxoid (kindly provided by Instituto Berna, Madrid, Spain) at 10 limit flocculation/mL was used as positive control antigen.

Immunohistochemical studies

Punch biopsy specimens were obtained 5, 12, and 90 days after admission. On day 90, a biopsy specimen of apparently healthy skin at least 10 cm away from any lesion was also taken in cases 1 and 2. The specimens were snap-frozen in isopentane chilled with liquid nitrogen and stored at -70°C until processed. Cryostat sections (4 µm) were cut, air-dried, fixed with acetone, and postfixed in chloroform. Sections were incubated for 30 minutes at room temperature, with an appropriate dilution of the following primary mAbs to identify the leukocyte subsets (anti-CD45, anti-CD3, anti-CD4, anti-CD8, anti-CD20, anti-CD57, and anti-CD68 [Master Diagnostica, Granada, Spain]) and the adhesion molecules (vascular cell adhesion molecule-1 [VCAM-1] [anti-CD106], E-selectin [anti-CD62E] [all from Master Diagnostica], and CLA [HECA-452]) with use of the 3-step streptavidin-biotin-alkaline phosphatase (SBAP) method. A purified rat IgM was used as an isotype control for CLA (PharMingen). Sections were counterstained with Fast Red (Master Diagnostica). The dermoepidermal junction cellularity was quantified with use of a 1-cm² grid divided into 100 1-mm² squares placed in the eyepiece of a microscope. Positive cells in 20 randomly selected squares were scored with the ×40 lens, and final counts for cell subsets were expressed as the number of positive cells per square millimeter. The adhesion molecules were scored semiquantitatively. Endothelial cells were evaluated as NS (no stain), + (10%-25%), ++ (25%-50%), and +++ (>50%) in the stained vascular vessels and epidermal dendritic cells and macrophages were evaluated as + (mild), ++ (moderate), and +++ (intense stain).

Messenger RNA expression of cytokines

Semiquantitative determinations of IL-2, IL-4, IFN-γ, and TNF-α messenger RNA (mRNA) expression in PBMCs were performed by reverse transcription-PCR (RT-PCR) on days 1, 3, 5, 15, and 24 after admission. Briefly, total RNA was isolated from PBMCs by

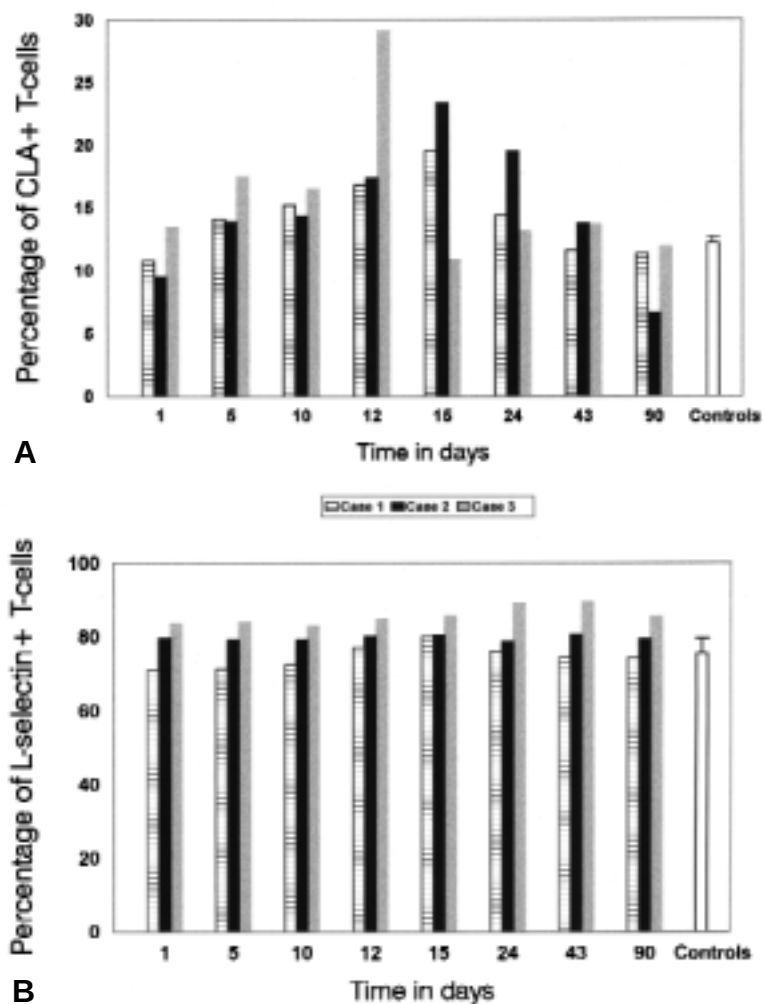


FIG 1. Serial expression of homing receptors in T lymphocytes (CD3⁺) from PBMCs at different time intervals after admission in 3 patients. **A**, Expression of skin homing receptor CLA. **B**, Expression of secondary lymphoid tissue homing receptor L-selectin.

the method of Chomczynski and Sacchi¹⁴ and was reverse transcribed (1 μ g per sample) in a standard reaction mixture with avian myeloblastosis virus. Equal loading of RNA (1 μ g) was quantified by spectrophotometry and confirmed by competitive PCR with housekeeping β -actin as control. Test complementary DNA was introduced in constant volumes into tubes containing serial 2-fold dilutions of competitor from v-erb plasmid and amplified by specific primers.¹⁵ PCR was performed for 35 cycles consisting of 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 2 minutes. The sequences of cytokine-specific primer pairs were provided by Clontech Laboratories (Palo Alto, Calif).

RESULTS

The serial expression of CLA in peripheral blood T lymphocytes in the 3 cases is shown in Fig 1, A. All 3 demonstrated a progressive increase in peripheral CD3⁺CLA⁺ T cells in parallel with the severity of the clinical symptoms. The highest increase (from 13.85% on day 1 to 29.09% on day 12 after admission) was found in the patient with the most severe skin involvement (case

3). In contrast, no significant up- or down-regulation of L-selectin expression on peripheral blood T cells was observed at any time, as can be seen in Fig 1, B.

CLA expression on skin blister T cells was around 4-fold higher than on peripheral blood T cells collected the same day in all cases, except in the first determinations where the levels were similar or higher in peripheral blood (Table I). CLA expression was also assessed in T-cell subsets, and higher values were found for the helper-inducer subpopulation than for the suppressor-cytotoxic subpopulation in all cases (Fig 2).

To investigate whether both CD4⁺ and CD8⁺ T-cell subsets were activated, we assessed the expression of the activation markers CD25, CD69, and HLA-DR compared with controls processed the same day (Fig 3). There was a moderate increase in CD25 expression in CD4 subsets in all patients at each determination. CD69 expression in the CD8 subsets in both cases 1 and 3 was also increased initially. In fact, the highest expression of activation markers occurred in case 3, the most severe

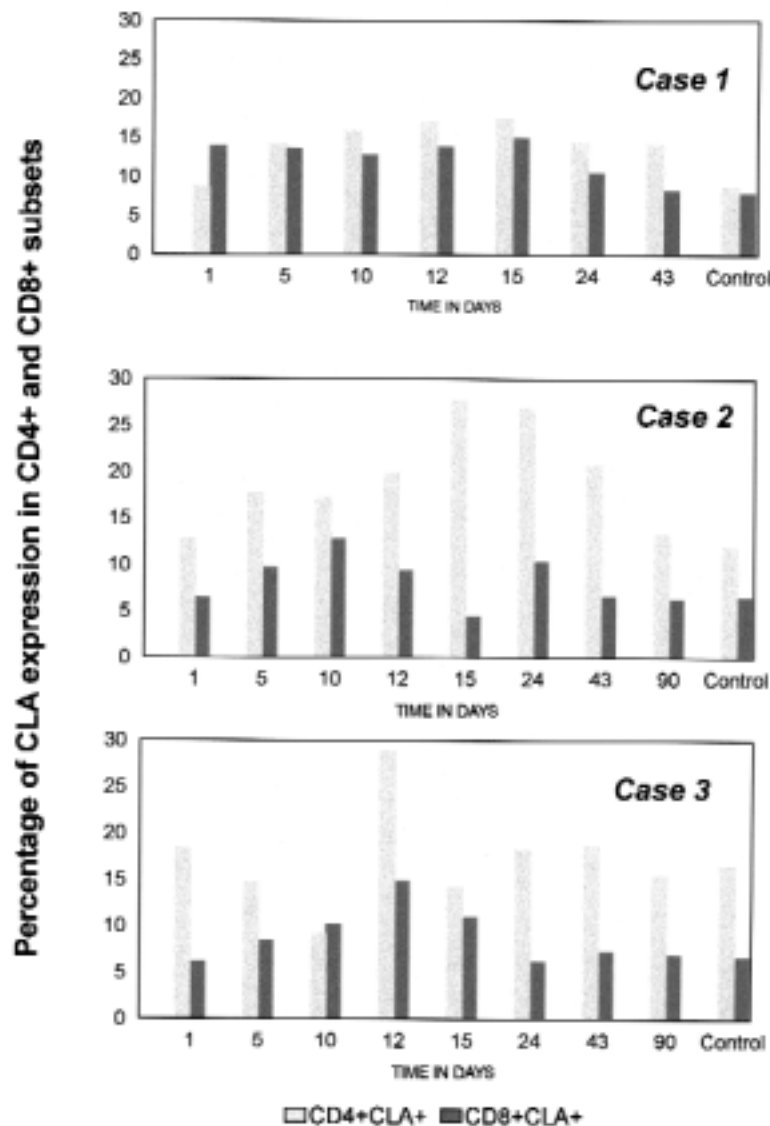


FIG 2. Serial expression of skin homing receptor CLA in T-lymphocyte subsets (CD4⁺ and CD8⁺) from PBMCs at different time intervals after admission in 3 patients.

TABLE I. Serial comparison of CD3⁺CLA⁺ percentages in peripheral blood and blister fluid in 3 cases of TEN

CD3 ⁺ CLA ⁺	Case 1			Case 2		Case 3	
Day	3	5	12	3	7	3	15
Peripheral blood	12.3	14.1	15.2	11.8	16.1	15.6	10.08
Blister fluid	2.4	55.7	58.3	5.3	64.4	14.2	44.62

clinically. In this case, apart from the increase in CD25 and CD69 expression mentioned above, HLA-DR expression was also enhanced in both subpopulations (higher for the CD4⁺ subset) in the early stages of the disease.

In the lymphocyte transformation test, the response to tetanus toxoid was similar for the patients and for 10 healthy subjects. Lymphocyte proliferation was not

induced by native carbamazepine, phenytoin, or any of the other anticonvulsants.

Histopathologic examination of the skin biopsy specimens of three patients on day 5 showed subepidermal blisters with confluent epidermal necrosis of varied extension and a moderate to severe lymphohistiocytic infiltrate around the dilated blood vessels of the superficial vascular plexus and in the blister fluid. On day 12 similar lesions were found in the biopsy specimens, whereas the last skin specimens, taken on day 90, showed complete re-epithelialization with an evident dermoepidermic lymphocytic and macrophagic infiltrate.

The cell subset values during the course of the reaction in the 3 cases are shown in Table II. The skin biopsy specimens taken on days 5 and 12 showed an infiltrate in the dermoepidermal junction composed predominantly of T lymphocytes (CD3⁺) and macrophages (CD68⁺). T

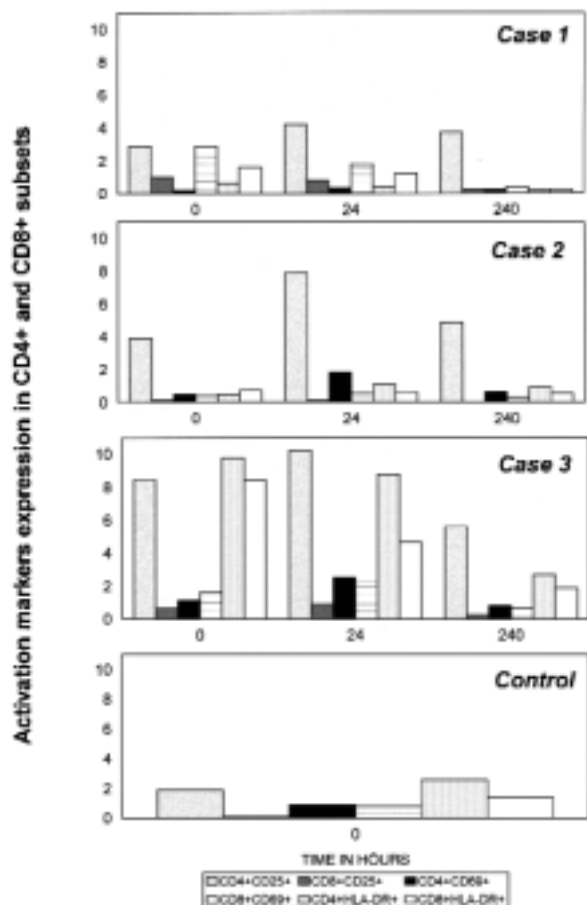


FIG 3. Serial expression of activation markers (CD25, CD69, and HLA-DR) in T-lymphocyte subsets (CD4⁺ and CD8⁺) from PBMCs at different time intervals after admission in 3 patients. Values are expressed as percentages.

cells were mainly CD4⁺, outnumbering CD8⁺ cells, especially in the biopsy specimen taken on day 12 in cases 2 and 3, by a ratio of 4:1 and 13:1, respectively. Deep dermal infiltrate was very mild but with the same cell distribution. In the last biopsy specimen, taken on day 90, most of the infiltrate was located in the papillary dermis. A weak decrease in all lymphocyte subpopulations and macrophages was observed but always above the corresponding control biopsy specimens (*normal skin*) by ratios of 3:1 (CD45⁺), 9:1 (CD3⁺, CD4⁺), 23:1 (CD8⁺), 29:1 (CD20⁺), 3:1 (CD57⁺), and 1.6:1 (CD68⁺). All the epidermal layers had already recovered by this time, although with less thickness and with an increase in the basal layer pigmentation. Pigment incontinence with melanin-loaded macrophages and regenerative features in the pilosebaceous follicles were observed in case 3.

The adhesion molecule distribution is shown in Table III. The distribution of these molecules was very similar in all cases at the same interval of time so we present data as the mean values of the 3 patients. The CLA was not present on skin lymphocytes at the first biopsy (5 days) but was 265.3 ± 85.7 cells/mm² on day 12 and 237.0 ± 81.9

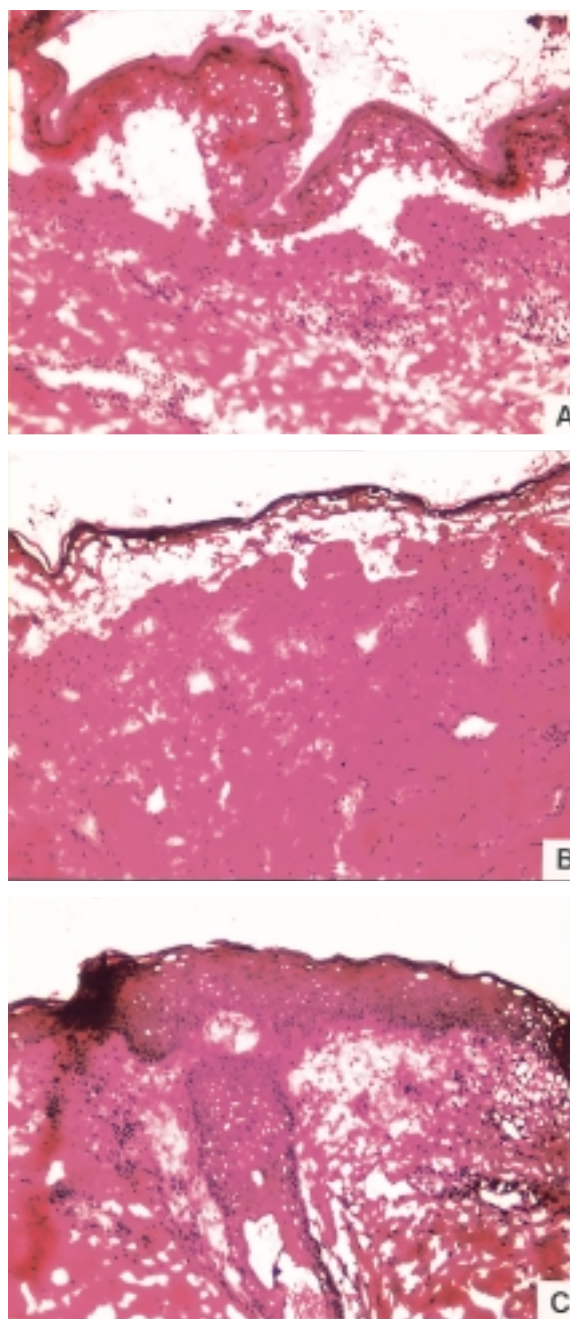


FIG 4. Histopathologic evolution of skin lesion in case 2. (Hematoxylin-eosin stain, original magnification $\times 200$.) **A**, Subepidermal blister with inflammatory infiltrate in dermoepidermal junction on day 5. **B**, Epithelial confluent necrosis of blister roof on day 12. **C**, Complete re-epithelialization with evident dermoepidermal lymphocytic and macrophagic infiltrate on day 90.

cells/mm² on day 90. Its ligand, E-selectin, had a mild expression on endothelial cells on day 5 and increased remarkably on day 12, becoming less intense on day 90. VCAM-1 expression was strong on endothelial cells in all biopsy specimens and moderate on epidermal dendritic cells in the first (5 days) and third biopsy specimens (90

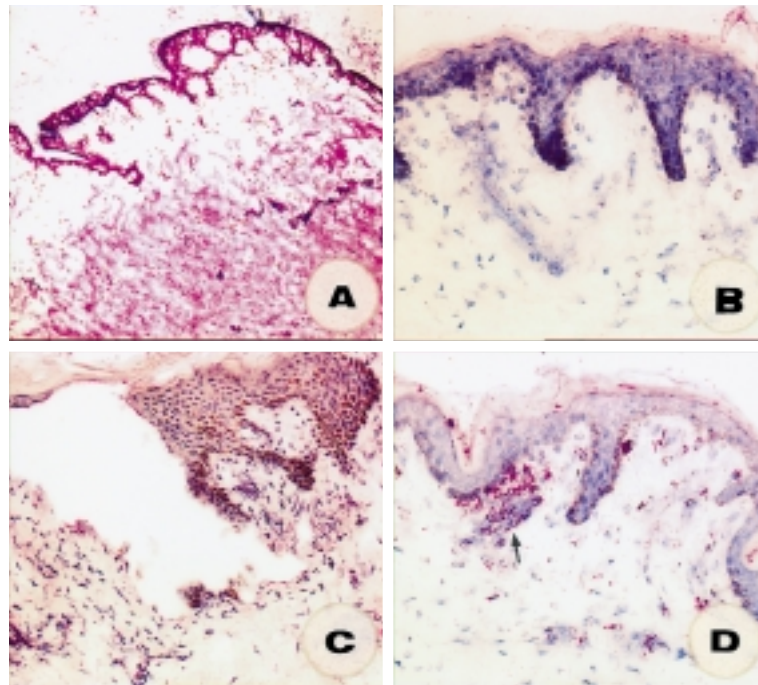


FIG 5. **A**, Skin biopsy specimen of case 3 on day 5. (Mayer's hematoxylin, original magnification $\times 20$). **B**, Iso-type control for CLA on day 5. (SBAP, original magnification $\times 100$.) **C**, No positive staining for CLA can be observed on day 5. (SBAP, original magnification $\times 100$.) **D**, Day 12. Presence of CD3⁺CLA⁺ cells is marked by arrow. (SBAP, $\times 100$.)

TABLE II. Distribution of cell subsets (cells per square millimeter) during course of TEN in 3 patients

Biopsy specimen		CD45	CD3	CD4	CD8	CD20	CD57	CD68
Case 1								
	Day 5	1540	951.5	693	714	201.6	16.1	467.7
	Day 12	870.9	459	453	458	16.1	16.1	524.1
	Day 90	1008.0	919	419	64.5	80.6	24.2	475.8
	Day 90 (normal skin)	338	96.7	41	8.06	0	16	395
Case 2								
	Day 5	1112	669.3	693.5	241.9	137.1	8.1	903.2
	Day 12	588.2	467	225.8	48.3	16.1	24.2	217.7
	Day 90	879.0	256	225.8	48.3	0	16.1	379
	Day 90 (normal skin)	100	8.06	40.3	0	0	8	121
Case 3								
	Day 5	120.9	193.5	80.6	40.3	97.7	24.2	516.1
	Day 12	467.0	161.5	330.6	24.2	153.2	16.1	733.8
	Day 90	645.0	185.4	459.0	169.3	8.1	56.4	435.4
	Day 90 (normal skin)	ND	ND	ND	ND	ND	ND	ND

ND, Not done.

TABLE III. Distribution of adhesion molecules during course of TEN

Biopsy specimen	CLA (cells/mm ²)	E-selectin	VCAM-1
Day 5	0 $\pm$ 0	+	+++ (E), ++ (EDC)
Day 12	265.3 $\pm$ 85.7	+++ (E)	+++ (E), +/- (M)
Day 90	237.0 $\pm$ 81.9	++ (E)	++ (E), ++ (EDC), +/- (M)

Mean values of 3 patients. E, Endothelium; EDC, epidermal dendritic cells; M, macrophages.

days) but could not be identified in the second (12 days) because the roof of the blister showed confluent necrosis (Fig 4). Macrophages stained weakly for VCAM-1 in the last 2 biopsy specimens. Expression of adhesion molecules was also distributed in the dermoepidermal junction, being very scattered in the dermis (Fig 5).

All patients expressed TNF- α and IFN- γ mRNA during the whole course of the disease, although expression tended to decrease with time. IL-2 was only expressed in the first sample in all cases and IL-4 could only be

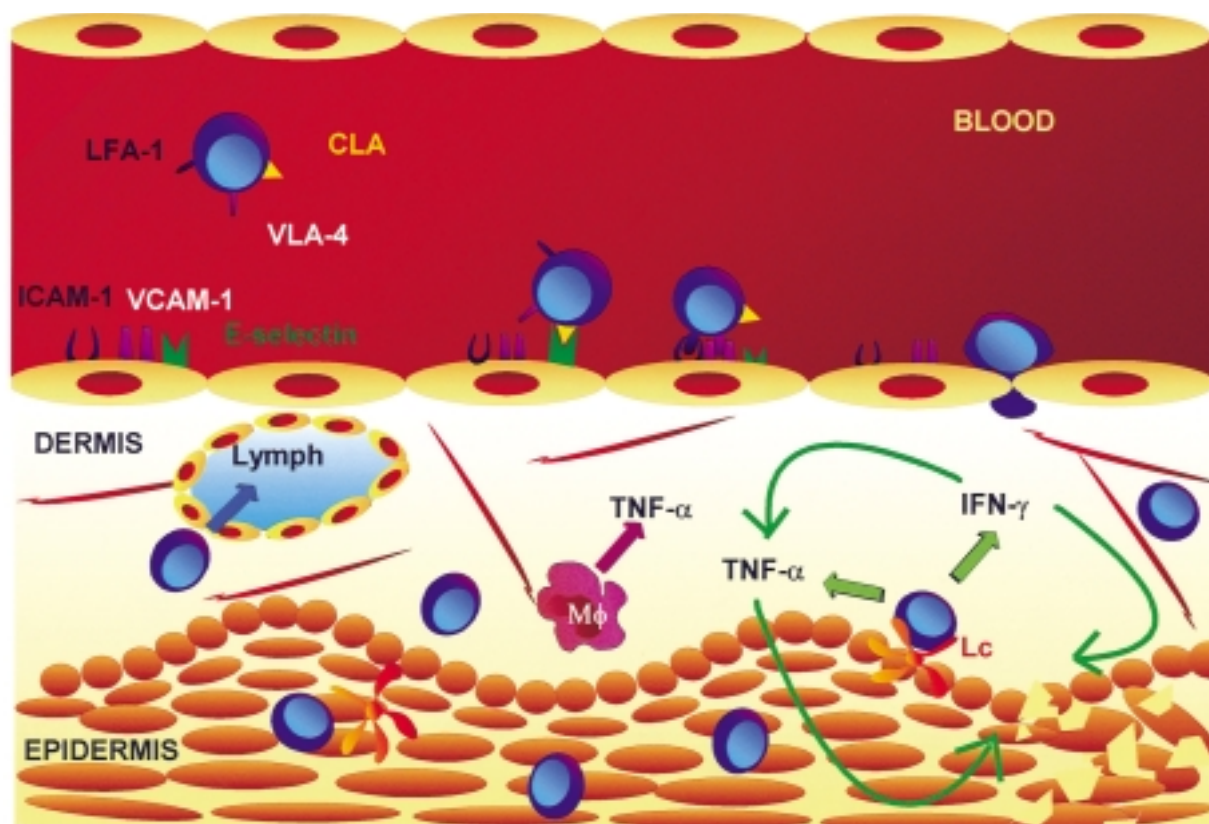


FIG 6. Proposed mechanism for anticonvulsant-induced TEN. An immunologic reaction originating in skin enables CLA⁺ T cells to migrate toward cutaneous areas by a multistep adhesion mechanism involving CLA/E-selectin, very late activation antigen-4 (VLA-4)/VCAM-1 and lymphocyte function-associated antigen-1 (LFA-1)/ICAM-1 interactions. After activation by Langerhans cells (Lc) in the skin, T cells can release TNF- α and IFN- γ , leading to the necrosis of epidermal cells.

TABLE IV. Cytokine mRNA expression in PBMCs by semiquantitative RT-PCR at different time intervals after admission

		Day 1	Day 3	Day 5	Day 15	Day 24
Case 1	TNF- α	+++	++	++	+	+
	IFN- γ	+++	+++	+++	++	—
	IL-2	+++	—	—	—	—
	IL-4	—	—	—	—	+
Case 2	TNF- α	+++	++	++	+	+
	IFN- γ	+++	++	++	++	—
	IL-2	++	—	—	—	—
	IL-4	—	—	—	—	+
Case 3	TNF- α	+++	+++	+++	++	++
	IFN- γ	+++	+++	+++	+++	++
	IL-2	++	+	—	—	—
	IL-4	—	—	—	—	—

detected in the last determination (patients 1 and 2) (Table IV). Expression of cytokine mRNA in 10 nonatopic healthy control subjects was also assessed. There was IL-4 and TNF- α expression in all controls but the values were never higher than mild (+). No expression of IL-2 and IFN- γ was found in any of the controls.

DISCUSSION

In the transmigration of skin-homing CLA⁺ T cells toward the skin, CLA mediates initial lymphocyte-endothelial cell contact and E-selectin¹⁶; intercellular adhesion molecule-1 (ICAM-1) and VCAM-1 are responsible for the extravasation in skin^{17,18} (Fig 6).

Follow-up of peripheral blood and cutaneous CLA⁺ T

cells allowed us to monitor cell trafficking in parallel with the disease evolution. An increased activation of peripheral T lymphocytes and the enhanced expression of CLA may determine T-cell recruitment into the skin and might explain the increased number of these T cells in the epidermis. A parallel increase in E-selectin expression on cutaneous endothelial cells also supports this hypothesis.

When sequential values of the peripheral CLA⁺ T cells were assessed, the higher levels of CD3⁺CLA⁺ lymphocytes coincided with the worsening of the cutaneous symptoms, but the percentages returned to control values as soon as the patients' skin condition improved. CLA expression on the skin T-cell infiltrate remained for a longer period, probably because of a continuous trafficking toward the cutaneous compartment of activated CLA⁺ T cells and their soluble mediators. We believe that these cells play an important role not only in those allergic reactions in which the antigen penetrates via the skin¹⁹ but also in those induced by oral intake.²⁰

Our results seem complementary to those of previous studies. Roujeau et al¹¹ identified most of the mononuclear cells in the epidermis as macrophages, whereas others^{10,21} provide evidence that most of these cells were mature T lymphocytes, mainly cytotoxic/suppressor. On the other hand, Villada et al⁸ found a skin infiltrate of activated T lymphocytes of the helper-inducer subset. The infiltrates found in our cases consisted of macrophages and T lymphocytes, mainly CD4⁺ cells, throughout the whole disease process, although cytotoxic/suppressor T cells, B lymphocytes, and natural killer cells were also present but in lower numbers.

One possible explanation for the negative response in lymphocyte proliferation tests is that the antigens eliciting the reactions were not the anticonvulsant themselves but metabolites that could not be generated in vitro by mononuclear cells.^{21,22}

T lymphocytes involved in allergic cutaneous reactions to drugs may have a T_H1 or T_H2 pattern.^{23,24} In our cases IFN- γ had a marked mRNA expression from the early stages of the disease that can induce the aberrant expression on the keratinocyte membrane of HLA-DR and *Fas*, leading to keratinocyte death.^{8,25} In addition, IFN- γ increases the production of TNF- α (markedly enhanced in our patients) and these TNF- α excesses can also lead to necrosis of the epidermis.²⁶ This cytokine microenvironment may contribute to the regulation of the homing receptors but, so far, IFN- γ and TNF- α are not known to up-regulate CLA activity.¹²

The response of our patients did not seem to be influenced by the administration of glucocorticoids (GC) either clinically or immunologically. Although cases 1 and 2 had been taking high doses of GC for more than 4 weeks, GC did not prevent the onset of the allergic reactions. Furthermore, case 3 had no clinical improvement with corticoid therapy. None of the changes expected in the immunologic response arising from GC therapy were seen (development of a T_H2 cytokine profile and blocking of T_H1 function)²⁷ because IFN- γ and TNF- α expres-

sion were markedly increased. The mechanisms for this unresponsiveness to GC are poorly understood²⁸⁻³⁰ and most hypotheses concern either abnormalities in glucocorticoid receptor (GR) binding affinity or a reduced number of GRs³¹ because of the synergic effects of IL-2 and IL-4. All these studies seem quite contradictory to our findings because not only was no IL-4 expression detected in any of our cases but TNF- α expression was markedly increased, which is known to enhance GR number and transcriptional activity.³²

We propose that an immunologic reaction originating in the skin, triggered by a culprit metabolite, develops a specific mechanism activating immunocompetent CLA⁺ T cells, which migrate toward the skin and contribute to the development of TEN. The presence in peripheral blood of high numbers of activated T cells expressing the skin homing receptor, followed by their increase and persistence in the skin, support this hypothesis. This report therefore provides information of value for future immunologic interventions attempting to control lymphocyte trafficking and reduce disease severity.

We thank Ian Johnstone for help with the final English language version of this manuscript.

REFERENCES

1. Lyell A. Toxic epidermal necrolysis (the scalded skin syndrome): a reappraisal. *Br J Dermatol* 1979;100:69-86.
2. Roujeau JC, Stern RS. Severe cutaneous adverse reactions to drugs. *N Engl J Med* 1994;331:1272-85.
3. Rzany B, Hering O, Mockenhaupt M, Schröder W, Goerttler E, Ring J, et al. Histopathological and epidemiological characteristics of patients with erythema exudativum multiforme major, Stevens-Johnson syndrome and toxic epidermal necrolysis. *Br J Dermatol* 1996;135:6-11.
4. Revuz J, Penso D, Roujeau JC, Guillaume JC, Payne CR, Weschler J. Toxic epidermal necrolysis: clinical findings and prognosis factors in 87 patients. *Arch Dermatol* 1987;123:1160-5.
5. Guillaume JC, Roujeau JC, Revuz J, Penso D, Touraine R. The culprit drugs in 87 cases of toxic epidermal necrolysis (Lyell syndrome). *Arch Dermatol* 1987;123:1166-70.
6. Roujeau JC, Guillaume JC, Fabre JP, Penso D, Fléchet ML, Girre JP. Toxic epidermal necrolysis (Lyell syndrome): incidence and drug etiology in France, 1981-1985. *Arch Dermatol* 1990;126:37-42.
7. Shear NH, Spielberg SP. Anticonvulsant hypersensitivity syndrome: in vitro assessment of risk. *J Clin Invest* 1988;82:1826-32.
8. Villada G, Roujeau JC, Clérici T, Bourgalet I, Revuz J. Immunopathology of toxic epidermal necrolysis, keratinocytes, HLA-DR expression, Langerhans cells, and mononuclear cells: an immunopathologic study of five cases. *Arch Dermatol* 1992;128:50-3.
9. Correia O, Delgado L, Ramos JP, Resende C, Fleming Torrinhá JA. Cutaneous T-cell recruitment in toxic epidermal necrolysis: further evidence of CD8⁺ lymphocyte involvement. *Arch Dermatol* 1993;129:466-8.
10. Miyauchi H, Hosokawa H, Akaeda T, Iba H, Asada Y. T cell subsets in drug-induced toxic epidermal necrolysis: possible pathogenic mechanism induced by CD8-positive cells. *Arch Dermatol* 1991;127:851-5.
11. Roujeau JC, Dubertret L, Moritz S, Jouault H, Heslan M, Revuz J, et al. Involvement of macrophages and pathology of toxic epidermal necrolysis. *Br J Dermatol* 1985;113:425-30.
12. Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Bergstresser PR, Terstappen LWM. Control of lymphocyte recirculation in man, II: differential regulation of the cutaneous lymphocyte-associated antigen, a tissue selective homing receptor for skin-homing T cell. *J Immunol* 1993;150:1122-36.
13. Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ. Activation of drug-specific CD4⁺ and CD8⁺ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepine. *J Immunol* 1995;155:462-72.

14. Chomczynsky P, Sacchi N. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
15. Siebert PD, Larnick JW. Competitive PCR. *Nature* 1992;359:557-8.
16. Santamaría Babi LF, Moser R, Perez-Soler MT, Picker LJ, Blaser K, Hauser C. Migration of skin-homing T cells across cytokine-activated human endothelial cell layers involves interaction of the cutaneous lymphocyte-associated antigen (CLA), the very late antigen-4 (VLA-4), and the lymphocyte function-associated antigen-1 (LFA-1). *J Immunol* 1995;154:1543-50.
17. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol* 1990;136:1053-68.
18. Picker LJ, Treer JR, Kishimoto TK, Smith CV, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for the skin homing T cells. *Nature* 1991;349:796-9.
19. Santamaría Babi LF, Picker LJ, Perez-Soler MT, Drzimalla K, Flohr P, Blaser K, et al. Circulating allergen-reactive T cells from patients with atopic dermatitis and allergic contact dermatitis express the skin-selective homing receptor, the cutaneous lymphocyte associated antigen. *J Exp Med* 1995;181:1935-40.
20. González FJ, Carvajal MJ, del Pozo V, Lahoz C, Santamaría L, Blanca M, et al. Erythema multiforme to phenobarbital: involvement of eosinophils and T cells expressing the skin homing receptor. *J Allergy Clin Immunol* 1997;113:345-7.
21. Friedmann PS, Strickland I, Pirmohamed M, Park K. Investigation of mechanisms in toxic epidermal necrolysis induced by carbamazepine. *Arch Dermatol* 1994;130:598-604.
22. Roujeau JC, Chosidow O, Saiag P, Guillaume JC. Toxic epidermal necrolysis (Lyell syndrome). *J Am Acad Dermatol* 1990;23:1039-58.
23. Leung DYM. Immunological basis of chronic allergic diseases: clinical messages from the laboratory bench. *Pediatr Res* 1997;42:559-68.
24. Hatfield SM, Rodgers GH, Marder P, Roehm NW. Patterns of lymphokine production by primary antigen-specific/MHC restricted murine helper T cell clones. *Immunol Invest* 1991;20:55-73.
25. Sayama K, Yonehara S, Watanabe Y, Miki Y. Expression of Fas antigen on keratinocytes in vivo and induction of apoptosis in cultured keratinocytes. *J Invest Dermatol* 1994;103:330-4.
26. Paquet P, Nikkels A, Arrese JA, Vanderkelen A, Pierard GE. Macrophages and tumor necrosis factor α in toxic epidermal necrolysis. *Arch Dermatol* 1994;130:605-8.
27. Costas MA, Kovalovsky D, Artz E. Mechanisms of glucocorticoid sensitivity modulation by cytokines. *Medicina* 1997;51:75-80.
28. Roujeau JC, Kelly JP, Naldi L, Rzany B, Stern RS, Anderson T, et al. Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. *N Engl J Med* 1995;333:1600-7.
29. Gonzalez FJ, Carvajal MJ, Leyva L, Juárez C, Blanca M, Santamaría LF. Expression of the cutaneous lymphocyte-associated antigen in circulating T cells in drug allergic reactions. *Int Arch Allergy Immunol* 1997;113:345-7.
30. Corrigan CJ, Brown PH, Barnes NC, Tsai J-J, Frew AJ, Kay AB. Glucocorticoid resistance in chronic asthma. *Am Rev Respir Dis* 1991;144:1026-32.
31. Alvarez J, Surs W, Leung DYM, Iklé D, Gelfand EW, Szeffler SJ. Steroid-resistant asthma: immunologic and pharmacologic features. *J Allergy Clin Immunol* 1992;89:714-21.
32. Brattsand R, Linden M. Cytokine modulation by glucocorticoids: mechanisms and actions in cellular studies. *Aliment Pharmacol Ther* 1996;10(2 Suppl):81-92.