Targeting keratinocyte apoptosis in the treatment of atopic dermatitis and allergic contact dermatitis

Axel Trautmann, MD,[a] Mübeccel Akdis, MD,[a] Peter Schmid-Grendelmeier, MD,[a] Rainer Disch, MD,[b] Eva-B. Bröcker, MD,[c] Kurt Blaser, PhD,[a] and Cezmi A. Akdis, MD[a] *Davos, Switzerland, and Würzburg, Germany*

Background: Activation and skin-selective homing of T cells and effector functions in the skin represent sequential events in the pathogenesis of atopic dermatitis and allergic contact dermatitis.
Objective: T cell–mediated keratinocyte apoptosis plays a key pathogenetic role in the formation of eczematous dermatitis. IFN-γ released from activated T cells upregulates Fas on keratinocytes, which renders them susceptible to apoptosis. The lethal hit is given to keratinocytes by means of Fas ligand expressed on the T-cell surface or released to the inflammatory microenvironment. We sought to investigate whether drugs used for the treatment of eczematous disorders interfere with this pathogenic pathway.
Methods: T cell–mediated, Fas-induced keratinocyte apoptosis in a keratinocyte-T cell coculture system serves as an in vitro model of eczematous dermatitis. We tested, in this model, whether immunomodulatory agents (dexamethasone, cyclosporine A, rapamycine, tacrolimus/FK506, intravenous immunoglobulin [IVIG], and theophylline) are able to inhibit apoptosis of keratinocytes. Additionally, skin biopsy specimens from patients with untreated and successfully treated eczematous dermatitis were evaluated for keratinocyte apoptosis.
Results: Dexamethasone, cyclosporine A, FK506, rapamycine, and IVIG are inhibitors of keratinocyte apoptosis induced by activated T cells. This effect is mediated by 2 major mechanisms directed on T cells or keratinocytes. T-cell activation was mainly inhibited by dexamethasone, FK506, cyclosporine A, and rapamycine. Interestingly, high-dose dexamethasone and IVIG directly inhibited Fas-mediated keratinocyte apoptosis. In vivo keratinocyte apoptosis was significantly reduced after successful topical treatment of eczematous lesions.
Conclusion: These results demonstrate mechanisms of action of current treatment approaches and provide a future for more focused therapeutic applications. (J Allergy Clin Immunol 2001;108:839-46.)

From [a]the Swiss Institute of Allergy and Asthma Research (SIAF), Davos; [b]the Clinic of Dermatology and Allergy, Davos; and [c]the Department of Dermatology, University of Würzburg, Würzburg.

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Reprint requests: Axel Trautmann, MD, Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse 22, CH-7270 Davos, Switzerland.

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Eczematous dermatitis is a distinctive pattern of skin inflammation that can be induced or maintained by a variety of environmental or intrinsic factors (eg, contact allergens, irritants, infective agents, and atopy). In the acute stage both atopic dermatitis (AD) and allergic contact dermatitis (ACD) are characterized by redness, edema, and papules, possibly with the formation of vesicles. In the upper dermis a perivascular T-cell infiltrate consisting predominantly of activated memory-effector T cells bearing cutaneous lymphocyte-associated antigen and CD45RO is an integral part of the observed response.[1-3] ACD is regarded as a type 1 T cell–mediated phenomenon on the basis of a number of observations.[2] A polarized type 2 T-cell cytokine pattern was previously regarded as a specific feature reflecting immune dysregulation in AD, but current studies demonstrate that both type 1 and type 2 T-cell cytokines play important roles in the skin inflammatory response in AD.[4-7] Activated T cells infiltrating the skin in AD and ACD induce keratinocyte apoptosis.[7] Induction of keratinocyte apoptosis by skin-infiltrating T cells, subsequent cleavage of E-cadherin, and resisting demsosomal cadherins demonstrate molecular events in spongiosis formation.[8]

Avoidance of exacerbating factors, such as contact allergens, infectious agents, and irritants, in conjunction with topical skin care is essential for effective management of eczematous dermatitis. With an increased understanding of the immunopathogenesis, therapy directed at correcting specific immune abnormalities has been attempted.[5,9] Topical corticosteroids reduce inflammation and are widely used in controlling acute flares of eczema.[10,11] Several studies have demonstrated that patients with severe AD refractory to treatment with topical corticosteroids can benefit from oral cyclosporine A.[12,13] Topical tacrolimus/FK506 can effectively reduce the clinical symptoms of AD.[14,15] There may also be a rationale for considering human intravenous immunoglobulin (IVIG) in the treatment of severe AD.[9,16]

In this context we investigated the effects of different immunomodulatory agents in keratinocyte-T cell cocultures, which serve as an in vitro disease model of

Abbreviations used
ACD: Allergic contact dermatitis
AD: Atopic dermatitis
IVIG: Intravenous immunoglobulin
TUNEL: Terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling

eczematous dermatitis. Keratinocyte-T cell cocultures and subsequent T cell–induced, Fas-mediated keratinocyte apoptosis offers the possibility to investigate targets of various therapeutics in the skin.

METHODS

Subjects and skin specimens

Eleven patients with AD and 5 with ACD were included in the study. AD was diagnosed according to the standard criteria.[17] ACD was diagnosed by means of epicutaneous patch testing. Punch biopsy specimens were taken from acute eczematous skin lesions in untreated patients. Biopsy specimens were also taken from previously eczematous skin lesions that had successfully been treated with different topical corticosteroids (fluocinonide, 0.5%; hydrocortisone-butyrate, 0.1%; prednicarbate, 0.25%; or betamethasone, 0.5%; each in ointment form) and tacrolimus (Protopic ointment, 0.1%; Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan) for 6 to 10 days. Control skin was obtained from 3 healthy, nonatopic individuals. T cells were purified from 10 healthy and 4 atopic donors. Informed consent was obtained from all subjects, and the study was approved by the Ethical Committee of Davos.

Reagents and antibodies

Ethidium bromide was purchased from Sigma Chemical Co (St Louis, Mo). Apoptosis-inducing anti-Fas IgM mAb (CH-11) was from Immunotech (Marseilles, France); annexin V-FITC was from R&D Systems Europe, Ltd (Abingdon, United Kingdom); and neutralizing anti-IFN-γ (45-15) was provided by Novartis Pharma (Basel, Switzerland). Recombinant human IL-12 was from PharMingen (San Diego, Calif) and IFN-γ was from Novartis Pharma. Recombinant soluble Fas ligand and Fas-Fc protein were purchased from Alexis Corp (San Diego, Calif). Dexamethasone was from Sigma Chemical Co. FK506 was from Calbiochem-Novabiochem Corp (San Diego, Calif). Cyclosporine A, rapamycine, and theophylline were from Novartis Pharma. Human IVIG preparations were Sandoglobulin (Novartis Pharma), Gammagard S/D (Baxter Hyland, Glendale, Calif), and Globhuman Berna (Serum & Impfinstitut, Bern, Switzerland).

Keratinocyte cultures

Human keratinocytes were obtained from neonatal foreskins. The skin was split overnight in sucrose-trypsin solution (0.1% sucrose, 0.25% trypsin, and 1 mmol/L EDTA, all from Sigma Chemical Co) at 4°C. Epidermal sheets were removed from the dermis, and keratinocyte cell suspensions were cultured in a fully supplemented, low-calcium (0.15 mmol/L Ca^{2+}), serum-free keratinocyte growth medium (Clonetics Corp, San Diego, Calif).[7,8] Hydrocortisone and the antibiotics were removed from the culture medium during experiments.

Isolation of CD45RO+ memory-effector T cells and differentiation of CD4+ and CD8+ type 1 and type 2 T cells

Mononuclear cells were isolated by Ficoll (Biochrom KG, Berlin, Germany) density gradient centrifugation of peripheral venous blood. $CD45RO^+$ T cells were isolated with the MACS system, according to the instructions of the manufacturer (Miltenyi Biotec, Bergisch Gladbach, Germany).[7] $CD4^+$ and $CD8^+$ type 1 and type 2 T cells were generated from naive $CD45RA^+$ T cells isolated from cord blood, as previously described.[7,18]

Quantification of cytokines

The cytokine profile (IL-4, IL-5, IL-13, and IFN-γ) of in vitro differentiated type 1 and type 2 T cells was determined by means of ELISA, as previously described.[7,18] Soluble Fas ligand and IFN-γ in supernatants of stimulated (with a combination of soluble anti-CD2 mAb, 0.5 μg/mL; anti-CD3 mAb, 1.0 μg/mL; and anti-CD28 mAb, 0.5 μg/mL) $CD45RO^+$ T cells were detected with commercial ELISA kits (MBL Co, Nagoya, Japan, and Immunotech).

Keratinocyte-T cell cocultures

$CD45RO^+$, $CD4^+$, and $CD8^+$ type 1 and type 2 T cells grown in RPMI-1640 (supplemented as described)[7] were stimulated with a combination of soluble anti-CD2, anti-CD3, and anti-CD28 mAb for 1 day, washed, and then cocultured with keratinocytes for 3 days in 6-well Transwell plates (3×10^5 keratinocytes and 1×10^5 T cells) or 96-well plates (3×10^4 keratinocytes and 1×10^4 T cells; Corning-Costar Corp, Cambridge, United Kingdom). The donors of keratinocytes and T cells were not identical, and therefore we used the Transwell plates, allowing the diffusion of soluble mediators but preventing cell-cell contact.[7] In some experiments type 2 T cells were stimulated in the presence of IL-12 (10 ng/mL). Dexamethasone, cyclosporine A, rapamycine, FK506, IVIG, and theophylline were added to the keratinocyte-T cell coculture in increasing concentrations from the start of coculture (day 0).

Viability and apoptosis detection

Keratinocyte viability was evaluated with ethidium bromide (1 μmol/L) uptake and flow cytometry (EPICS XL-MCL flow cytometer; Beckmann Coulter Int SA, Nyon, Switzerland). Apoptotic cells were identified in situ by staining double-strand DNA breaks. Terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) was used, as previously described.[7,19] HOECHST and annexin V staining of cultured keratinocytes was done as previously described.[7,8,20] Stained tissue sections and keratinocyte cultures were evaluated with an inverted microscope equipped with interference contrast and UV light (Axiovert 405M; Carl Zeiss AG, Feldbach, Switzerland).

Demonstration of apoptosis by means of DNA gel electrophoresis

Isolation of total genomic DNA of keratinocytes was done with the DNeasy kit according to the protocol of the manufacturer (Qiagen AG, Basel, Switzerland). The ApoAlert LM-PCR ladder assay kit (Clontech Laboratories AG, Basel, Switzerland) was used for the detection of nucleosomal ladders in apoptotic keratinocytes.[21] The resulting ladder was separated on an agarose-ethidium bromide gel and visualized with an imaging system (FLA-3000; Raytest Schweiz AG, Urdorf, Switzerland).

Statistical analysis

Results are shown as means ± SD. The paired Student *t* test was used for comparison of paired conditions.

RESULTS

T cell–mediated, Fas-induced keratinocyte apoptosis in eczematous dermatitis

Features of T cell–induced keratinocyte apoptosis

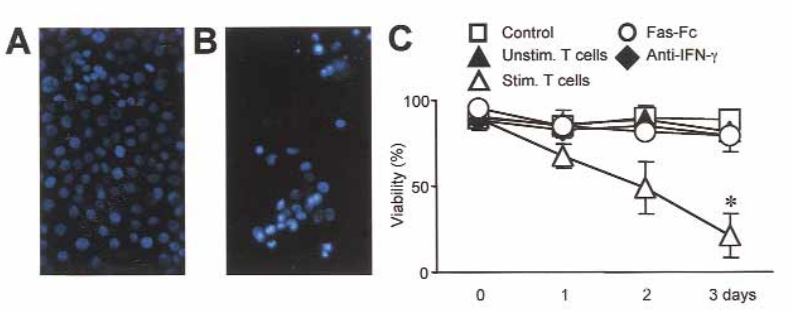

FIG 1. T cell–induced keratinocyte apoptosis is mediated by IFN-γ and Fas triggering. Keratinocytes were cultured in Transwell plates with unstimulated **(A)** and stimulated **(B)** CD45RO+ T cells for 3 days (HOECHST staining; original magnification, ×200). **C**, Coculture of keratinocytes and CD45RO+ T cells for 3 days. Keratinocyte viability was determined with ethidium bromide exclusion and flow cytometry. *Control*, Isotype control antibody. $*P < .05$. Results shown represent 3 experiments.

were recently reported in detail.[7,8] HOECHST staining and fluorescence microscopy showed that keratinocytes cultured with stimulated T cells had bright, condensed, and fragmented nuclei, which are typical characteristics of apoptosis (Fig 1, *A* and *B*). To eliminate that the apoptotic bodies or the fragmented DNA are derived from T cells, keratinocytes and T cells were separated in Transwell plates. In keratinocyte-T cell cocultures, we observed killing of keratinocytes by stimulated CD45RO+ memory-effector T cells (Fig 1, *C*). Keratinocyte apoptosis could be inhibited by pretreatment with either neutralizing anti-IFN-γ mAb (10 μg/mL) or with the Fas-Fc protein (10 μg/mL), a competitive inhibitor of Fas ligand-Fas interactions.

Dexamethasone, cyclosporine A, rapamycine, FK506, and IVIG inhibit T cell–mediated keratinocyte apoptosis

T cell–mediated, Fas-induced keratinocyte apoptosis in a keratinocyte-T cell coculture system serves as an in vitro model of eczematous dermatitis.[7] We tested, in this model, whether immunomodulatory agents are able to inhibit apoptosis of keratinocytes. Dexamethasone, cyclosporine A, rapamycine, and FK506 significantly inhibited apoptosis of keratinocytes (Fig 2, *A*). The effects were dose-dependent with the effective concentrations in the 100 nmol/L to 1 μmol/L range. IVIG also inhibited keratinocyte apoptosis but was not as potent as the immunosuppressants. At all concentrations used, theophylline had no effect on keratinocyte apoptosis. Electrophoresis of DNA extracted and amplified detect the banding of a fragmented DNA ladder comprised of bands at intervals of approximately 200 bp, which is a widely recognized hallmark of apoptosis (Fig 2, *B*). The inhibitory effect of dexamethasone, cyclosporine A, rapamycine, and FK506 on keratinocyte apoptosis could be shown (Fig 2, *B*). Because of its high sensitivity, the LM-PCR assay reveals apoptosis that may be undetectable by other methods. As seen in Fig 2, *A*, keratinocyte viability is higher with IVIG, and a smaller fraction of keratinocytes are apoptotic; however, the LM-PCR–amplified ladder was visible at day 3 (Fig 2, *B*). Membrane phosphatidylserine redistribution from the inner to the outer membrane leaflet takes place in early apoptotic cells. Annexin V is a phosphatidylserine-binding protein and is used to detect apoptotic cells.[7] The dose-dependent inhibitory effect of IVIG on keratinocyte apoptosis was demonstrated by annexin V staining at day 1. The percentage of annexin V–stained cells decreased from 73.6% ± 9.0% to 22.6% ± 7.1% in the 40 mg/mL IVIG dose (Fig 2, *C*). Stimulated CD45RO+ T cells secrete soluble Fas ligand and IFN-γ. Addition of dexamethasone, cyclosporine A, rapamycine, and FK506 ablated secretion of soluble Fas ligand and IFN-γ (Fig 2, *D*).

Prevention of keratinocyte apoptosis mediated by type 1 and IL-12–stimulated type 2 CD4 and CD8 T cells

As a more disease-related model for keratinocyte apoptosis in eczematous disorders, we studied the effect of different phenotypes of T cells on keratinocytes. Type 1 CD4 and CD8 T cells predominantly contain and secrete IFN-γ, but only low amounts of IL-4 and IL-5. In contrast, the CD4 and CD8 type 2 T cells contain and secrete IL-4, IL-5, and IL-13, but only low IFN-γ.[7] Both CD4 and CD8 type 1 T cells were able to induce keratinocyte death (Fig 3, *A* and *C*). In contrast, type 2 T cells had no effect on keratinocytes after differentiation. However, stimulation of type 2 T cells in the presence of 10 ng/mL IL-12 enabled the induction of keratinocyte apoptosis (Fig 3, *A* and *C*). Again, dexamethasone, cyclosporine A, rapamycine, and FK506 significantly inhibited apoptosis of keratinocytes under these conditions (Fig 3, *B* and *D*). Addition of Sandoglobulin also inhibited keratinocyte apoptosis but was not as potent as the immunosuppressants.

Direct effect of dexamethasone and IVIG on keratinocyte apoptosis

One of our early findings in evaluating keratinocyte apoptosis, described in Fig 1, *C*, was that keratinocytes pretreated with IFN-γ (10 ng/mL) were efficiently killed by cross-linking of Fas with anti-Fas mAb (1 μg/mL) and soluble Fas ligand (10 ng/mL; Fig 4, *A*). We therefore

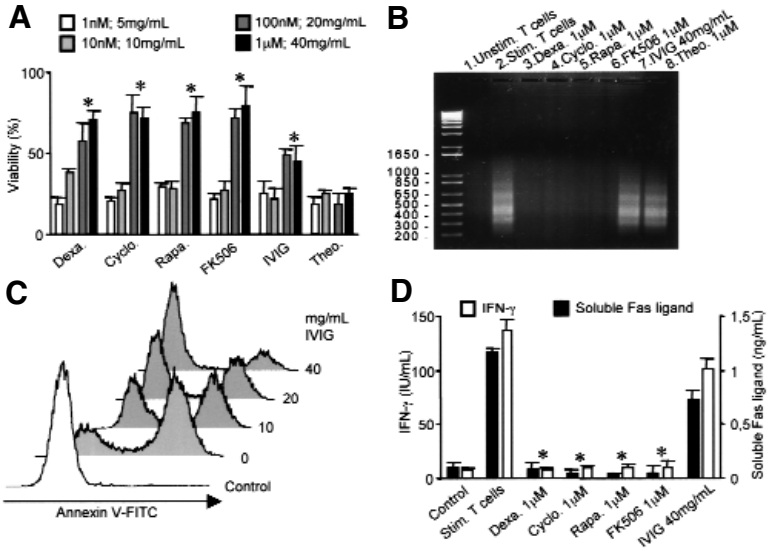

FIG 2. Effect of immunomodulatory agents on T cell–induced keratinocyte apoptosis. **A**, Coculture of keratinocytes and stimulated CD45RO+ T cells at day 3. *$P < .05$. **B**, Electrophoresis of keratinocyte DNA. Keratinocytes were cultured in Transwell plates with unstimulated *(lane 1)* and stimulated (*lanes 2* to *8*) CD45RO+ T cells. At day 3, the keratinocytes were lysed, and the DNA was isolated. **C**, Coculture of keratinocytes and stimulated CD45RO+ T cells in Transwell plates at day 1. Keratinocytes were stained with annexin V-FITC and subjected to flow cytometry. Histograms show fluorescence intensity *(x-axis)* versus cell number *(y-axis)*. *Control*, Keratinocytes alone. **D**, Levels of soluble Fas ligand and IFN-γ in supernatants of stimulated CD45RO+ T cells at day 3, as determined with ELISA. *$P < .05$. Results shown represent 3 (*A-C*) and 2 (*D*) experiments.

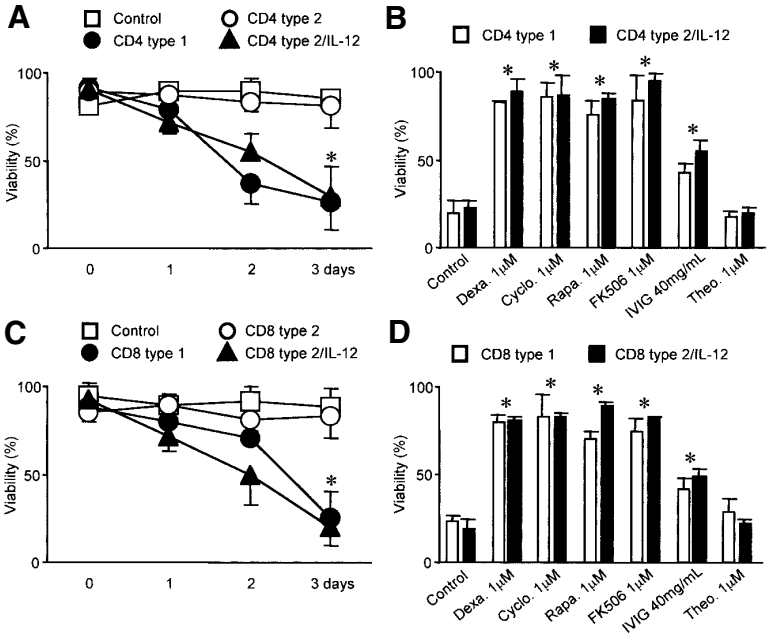

FIG 3. Keratinocyte apoptosis induced by CD4 and CD8 T cells is inhibited by immunomodulatory agents. **A** and **C**, Stimulated type 1, but not type 2, CD4+/CD8+ T cells induced keratinocyte death. Stimulation of type 2 T cells in the presence of IL-12 enabled the induction of keratinocyte apoptosis. *Control*, Keratinocytes alone. *$P < .05$. **B** and **D**, Coculture of keratinocytes and stimulated CD4+/CD8+ T cells at day 3. *Control*, Keratinocytes without immunosuppressants. *$P < .05$. Results shown represent 2 experiments.

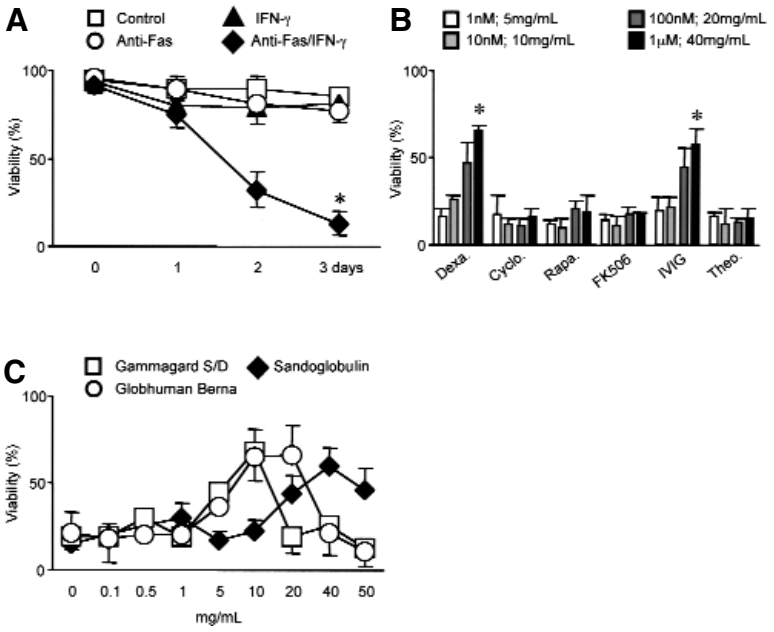

FIG 4. Direct effects of dexamethasone and IVIG on Fas-mediated keratinocyte apoptosis. **A,** IFN-γ– and Fas-induced keratinocyte apoptosis. *Control,* Keratinocytes alone. **P* < .05. **B,** Keratinocyte culture at day 3. **P* < .05. **C,** Different IVIG preparations (Sandoglobulin, Gammagard S/D, and Globhuman Berna) protect keratinocytes from IFN-γ– and Fas-induced apoptosis at different concentrations. Results shown represent 4 (**A** and **B**) and 2 (**C**) experiments.

evaluated the direct effects of immunomodulatory agents on keratinocyte apoptosis induced by IFN-γ and Fas triggering. We found that keratinocyte apoptosis decreased only in the presence of higher doses of dexamethasone and IVIG (Fig 4, *B*). The effects were dose-dependent with the effective concentrations in the 100 nmol/L to 1 μmol/L and 20 to 40 mg/mL range, respectively. We used 2 different batches of Sandoglobulin but observed no significant variations between them. By using IVIG preparations from 3 different manufacturers, we found that the protective effect is observed at different concentrations (Fig 4, *C*).

Diminished keratinocyte apoptosis after successful topical treatment of acute eczematous dermatitis

Skin biopsy specimens from acute AD and acute ACD showing dense cellular infiltration in the upper dermis and epidermal spongiosis were analyzed by using the TUNEL technique. In the stratum basale and spinosum of normal healthy skin, no TUNEL-positive stained cells were observed.[7,8] An expression of TUNEL-positive keratinocyte nuclei was visible, mainly in the stratum spinosum of acute eczematous lesions (Fig 5, *A* and *D*). The condensation and fragmentation of intense red-stained DNA was clearly visible. After successful topical treatment with corticosteroids and tacrolimus/FK506, the previously eczematous skin lesions still showed a varying degree of infiltration, scaling, and lichenification, but no signs of acute inflammation. TUNEL staining of the epidermis was negative or significantly reduced after successful treatment for 6 to 10 days (Fig 5, *B*, *C*, and *E*). In parallel, after topical treatment of AD and ACD, epidermal spongiosis disappeared, and the cellular infiltration in the upper dermis was significantly diminished.

DISCUSSION

In AD and ACD the skin is infiltrated by T cells, and T cell–driven inflammation plays a crucial role in pathogenesis.[22] Clinical improvement associated with a reduction in the numbers of skin-infiltrating T cells after therapy with immunosuppressants supports the view that eczematous dermatitis is based on T cell–mediated skin inflammation. The results of our study suggest that dexamethasone, cyclosporine A, rapamycine, FK506, and IVIG can inhibit T cell–induced apoptosis of cultured keratinocytes. However, the anti-inflammatory activity of these drugs is based on their ability to inhibit the functions of T cells. On the other hand, the inability of cyclosporine A, rapamycine, and FK506 to directly inhibit keratinocyte apoptosis induced by IFN-γ and Fas triggering was expected. Interestingly, only high doses of dexamethasone and IVIG exerted an inhibitory effect on apoptosis directly on keratinocytes.

Topical corticosteroids represent the most common treatment of eczematous dermatitis.[10,11,23] Our study demonstrated the efficacy of corticosteroids in inhibiting T-cell activation in the skin. However, because corticosteroids are reported to regulate the gene expression of chemotactic factors, it is conceivable that chemokines

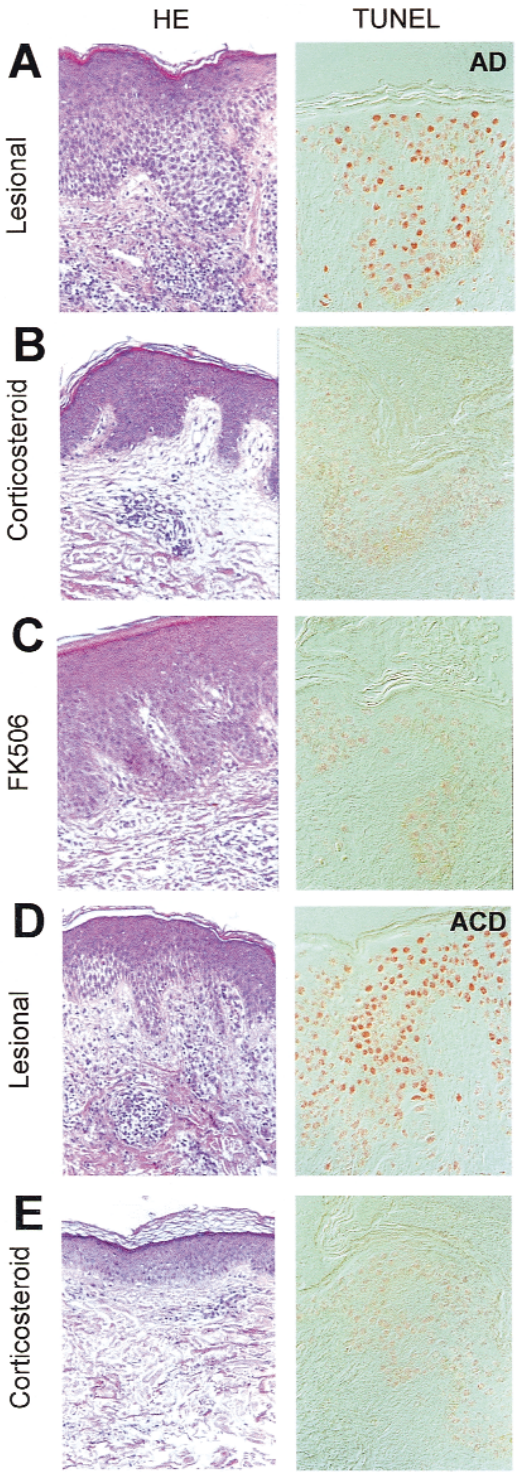

FIG 5. Demonstration of apoptotic keratinocytes in lesional and treated AD and ACD. Representative histologic findings of acute eczematous dermatitis demonstrated with hematoxylin-eosin *(HE)* staining (original magnification, ×200). Skin sections were subjected to TUNEL (original magnification, ×400). **A** and **D**, Lesional skin of acute AD and ACD. **B**, **C**, and **E**, Previously eczematous AD and ACD skin lesions successfully treated topically with 0.25% prednicarbate and 0.1% tacrolimus. Results are representative of 11 specimens of AD (5 untreated and 6 treated) and 5 with ACD (3 untreated and 2 treated).

involved in the recruitment of T cells might be responsible for the control of skin inflammation in vivo.[22] The most potent inhibitor of keratinocyte apoptosis directly induced by IFN-γ and Fas triggering was dexamethasone. It was shown that dexamethasone protects epithelial cells from IFN-γ and anti-Fas–induced cell death through the induction of human inhibitor of apoptosis.[24] Dexamethasone inhibits caspase 3 and caspase 7 activation and may directly block apoptosis as a result of inhibition of caspase activation.[24] Therefore the present data suggest that the direct inhibition of keratinocyte apoptosis by potent corticosteroids may be an additional mechanism to the blocking of T cell–mediated effector functions. Supporting these results, corticosteroids have been reported to be effective in suppressing irritant reactions, which primarily damage keratinocytes.[11]

Recent studies have shown that cyclosporine A is a highly effective treatment for AD and hand eczema.[12,13,23] We have explored the possible mechanism of cyclosporine A treatment by examining the effect on T cell–induced keratinocyte apoptosis. As expected, we demonstrated that the suppressive action of 1 μmol/L cyclosporine A (corresponding to 1.2 μg/mL) is primarily directed toward T-cell functions. Determinations of the skin levels of cyclosporine A in patients with psoriasis have shown that tissue concentrations of 1.0 to 2.9 μg/mL are reached by using therapeutic doses of 3 to 5 mg/kg per day.[25] Cyclosporine A is able to directly inhibit keratinocyte proliferation; at 1 to 10 μg/mL there is a cytostatic effect on keratinocytes.[26] Cyclosporine A exerts its pharmacologic effects by binding to immunophilins (FK506-binding proteins), forming complexes that interfere with calcineurin and nuclear factor of activated T cells, which are important for the clonal expansion of T cells.[5,9] The inhibitory effect on T-cell activation is not specific for cyclosporine A and is achieved by FK506 and rapamycine as well. Similar to cyclosporine A, FK506 binds to immunophilins (FK506 binding proteins), and the resulting complex functionally blocks calcineurin, resulting in inhibition of nuclear factor of activated T cell translocation to the nucleus.[27,28] Our results and those of previous studies underline that the beneficial effects of FK506 could be attributed mainly to the inhibition of T-cell activation.

There are several reports of IVIG therapy used in patients with AD.[5,9,16] There are 6 main nonexclusive mechanisms that have been proposed for IVIG[29]: (1) functional blockade of Fc receptors on macrophages; (2) inhibition of complement-mediated damage; (3) modulation of the production of cytokines; (4) neutralization of circulating autoantibody by anti-idiotypic antibody; (5) neutralization of pathogens; and (6) blockade of Fas by anti-Fas antibody.[30] In the present study IVIG (efficient doses between 10 and 40 mg/mL; 30 mg/mL is the calculated equivalent of the daily dose used for treating a 60-kg individual) significantly inhibited keratinocyte apoptosis induced by IFN-γ and Fas triggering. It was shown that IVIG inhibits Fas-mediated cell death by blocking Fas rather than interacting with Fas ligand.[30] In our study IVIG inhibited keratinocyte apoptosis induced

by stimulated T cells but was not as potent as the immunosuppressants. The keratinocyte apoptosis-blocking effect of IVIG in the viability assay was not visible in the DNA fragmentation assay at day 3 after induction of apoptosis. However, the efficiency of IVIG was confirmed by using annexin V staining at an earlier time point (ie, day 1). Moreover, we observed remarkable differences between IVIG from different manufacturers regarding the effective dose for inhibition of keratinocyte apoptosis. This observation points to the difficulties in standardization of IVIG preparations. A number of in vitro studies have demonstrated that IVIG reduces T-cell proliferation and cytokine production.[31,32] We observed only a partial decrease of IFN-γ and soluble Fas ligand secretion by $CD45RO^+$ T cells with IVIG.

UV light is known for its immunosuppressive properties, and AD and ACD respond well to phototherapy with UVB radiation. It was shown that UVB blocks the effects of IFN-γ by inhibiting the phosphorylation of signal transducer and activator of transcription 1.[33] Inhibition of cytokine-induced nuclear transcription factors may be a mechanism for the therapeutic effect of UVB radiation on eczematous dermatitis and fit in our model of the pathogenesis. Recent observations in asthmatic patients demonstrated anti-inflammatory and immunomodulatory activities of theophylline in addition to the bronchodilating effect.[34] However, in our experimental system we were not able to demonstrate an inhibitory effect of theophylline on T cell–induced keratinocyte apoptosis.

Recent studies focused on the ability of T cells to damage epidermal keratinocytes after reaching the skin and releasing inflammatory mediators.[7,8,35,36] Interestingly, after appropriate stimulation of T cells, we could not elaborate significant differences concerning the killing ability of T cells between healthy and atopic donors.[7,8] Therefore T cell–induced keratinocyte apoptosis is not specific for AD and may operate in other forms of eczematous dermatitis as well.

The present study demonstrates that keratinocyte apoptosis induced by activated T cells appears to be a useful parameter to evaluate the activity of eczematous dermatitis. The T-cell infiltrate is not the best estimation to determine the severity of eczematous dermatitis, and the number of T cells has poor sensitivity as a marker in monitoring the skin inflammation. In contrast, the pathologic changes in the epidermis represent a more reliable indication of the severity of the eczema. In particular, apoptosis of keratinocytes is remarkably higher in acute eczematous dermatitis, suggesting that the presence of apoptotic keratinocytes at the local level reflects the effector function of T cells after they have been attracted to the upper dermis by chemotactic factors. Monitoring keratinocyte apoptosis can therefore indicate the severity of the lesion and the effectiveness of any treatment.

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