Successful Ultraviolet B Treatment of Psoriasis Is Accompanied by a Reversal of Keratinocyte Pathology and by Selective Depletion of Intraepidermal T Cells

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Summary

Skin irradiation with ultraviolet B (UVB) is a common and often durable treatment for psoriasis and other inflammatory skin disorders. We studied the effects of UVB on keratinocytes and leukocytes in psoriatic tissue and in culture. In nine patients treated repetitively, most of the cellular and molecular changes that typify the psoriatic epidermis reverted to normal. Keratinocyte hyperplasia, assessed by expression of the Ki-67 cell cycle antigen, decreased by 70%, and residual cell proliferation was appropriately confined to the basal layer. Epidermal thickening was reduced by 60%, and a granular layer reformed. Expression of keratin 16, as well as suprabasal integrin $\alpha3$ and insulin-like growth factor-1 receptors, was eliminated, whereas filaggrin increased markedly. UVB also depleted >90% of the $CD3^+$, $CD8^+$, and $CD25^+$ T cells from the psoriatic epidermis, whereas dermal T cells were only minimally depressed. The latter finding parallels the known inability of these doses of UVB to penetrate the dermis. In tissue culture, UVB was antiproliferative and cytotoxic toward T cells and keratinocytes, but the T cells were 10-fold more sensitive. Furthermore, low doses of UVB induced apoptosis in lymphocytes but not keratinocytes, as detected by the TUNEL (TdT-mediated dUTP–biotin nick end labeling) technique. The selective effects of UVB on intraepidermal T cells in situ and in culture support the hypothesis that epidermal alterations in psoriasis can be normalized by a depletion of activated intraepidermal T cells.

Psoriasis is a common skin disorder typified by hyperplasia and incomplete differentiation of epidermal keratinocytes. These epidermal changes parallel those seen in wound healing and are collectively termed the "regenerative phenotype." Molecular markers of this phenotype include the synthesis of such keratinocyte products as the ψ-3 protein and keratins 6 and 16, suprabasal expression of integrin $\alpha3$ and IGF receptors, and reduced levels of the filaggrin and involucrin proteins that mediate keratin cross-linking and stratum corneum formation (1–3 and Gottlieb, S. L., E. Hayes, P. Gilleaudeau, I. Cardinale, A. B. Gottlieb, and J. G. Krueger, manuscript submitted for publication).

Psoriasis is also accompanied by an infiltration of activated T cells. $CD25^+$ and $CD3^+$ cells are found in both the epidermis and dermis, the former being predominantly $CD8^+$ and the latter $CD4^+$ (4–6). Both types of T cells have been proposed to play a pathogenic role (6, 7). A role for $CD8^+$ cells is favored by the observed linkage of psoriasis to certain MHC I alleles, especially HLA Cw6 (8), and the skewing of $CD8^+$ TCR $V\beta$ usage to the $V\beta$ 3 and 13.1 subtypes (9). Other inflammatory dermatoses are not accompanied by a high density of $CD8^+$ T cells, especially activated $CD25^+$ cells in the epidermis (10).

Aberrant keratinocyte proliferation, regenerative epidermal differentiation, and infiltration of $CD8^+$ T cells in psoriatic epidermis were recently shown to be sensitive to a new therapeutic modality, the IL-2 diphtheria toxin conjugate DAB_{389} IL-2 (11). Since IL-2 directs the toxin to activated immune cells rather than to keratinocytes, a pathogenic role for T cells is indicated. The reciprocal also applies, i.e., psoriatic epidermal changes are only partly reversed by treatments (e.g., calcitriol, etretinate, or cyclosporine) that produce lesser reductions in infiltrating epidermal T cells (12, 13 and Lu, I., P. Gilleaudeau, J. A. McLane, N. Heftler, M. Kamber, S. Gottlieb, J. G. Krueger, and A. B. Gottlieb, manuscript submitted for publication).

Skin irradiation with ultraviolet light in the 280–320-nm range (ultraviolet B or UVB)[1] is one of the most common treatments for extensive psoriasis and can produce long-lived clinical remissions. UVB can be immunosuppressive, possibly at the level of antigen-presenting dendritic cells (14, 15). UVB also has direct effects on T cells, including

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[1]Abbreviations used in this paper: MED, minimum erythema dose; PUVA, psoralen + UVA; TUNEL, TdT-mediated dUTP–biotin nick end labeling; UVB, ultraviolet B.

Untreated psoriasis
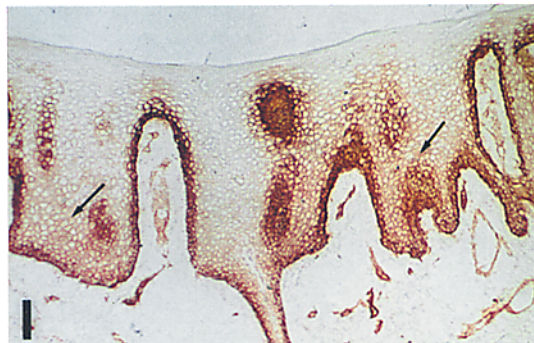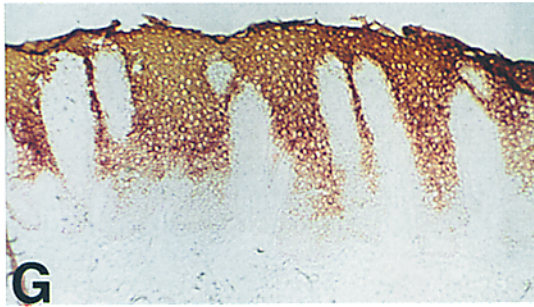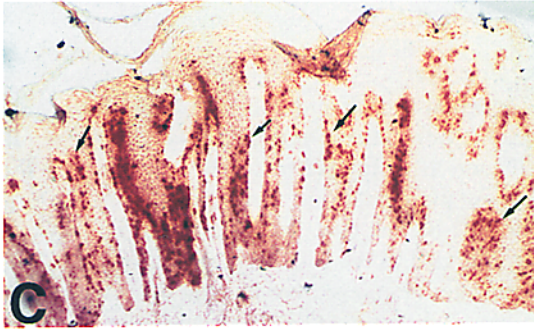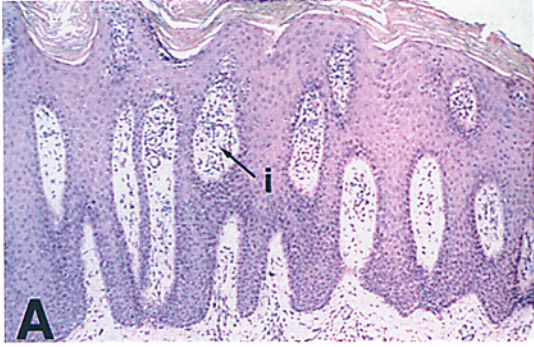
After UVB treatment
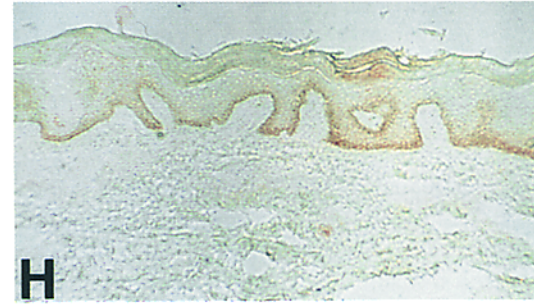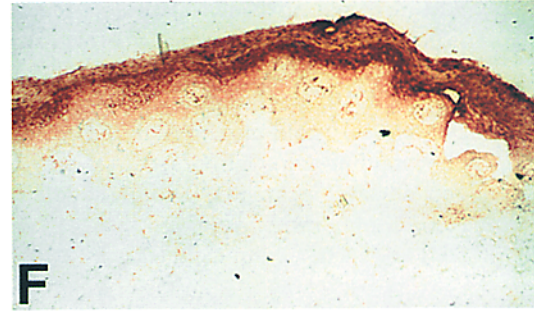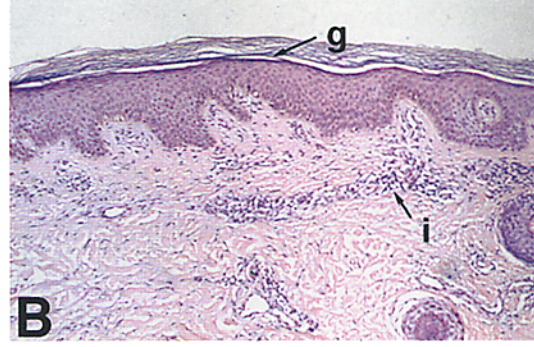
decreased TCR signaling, decreased lymphokine synthesis, and direct cytotoxicity (14–17). These UVB effects have been previously studied, chiefly in the context of the sensitization phase of the immune response. Much less information is available on the ability of UVB to modulate ongoing lymphocyte function in chronic disorders, especially in a human disease like psoriasis.

Here we describe findings on the effects of repetitive UVB therapy in nine consecutive patients with extensive psoriasis. UVB normalized a large group of cellular and molecular disease markers, but primarily reversed the infiltrate of epidermal, rather than dermal, T cells. Additional tissue culture studies showed that UVB, in doses that might be expected to penetrate the epidermis, inhibited proliferation and induced cytotoxicity or apoptosis in T cells to a much greater degree than it did in keratinocytes. Coupling our results with earlier data showing that the epidermal pathology of psoriasis can be reversed by an IL-2 toxin, we conclude that UVB exerts its therapeutic effects principally at the level of activated epidermal T cells.

Materials and Methods

UVB Treatment and Measurement. Patients with extensive psoriasis (>10% body surface area affected) were hospitalized for inpatient UVB treatment on a modified "Goeckerman" protocol. Upon entry into the study, the patients had not been treated with systemic antipsoriatic medications for a minimum of 4 wk and had not been treated with topical agents for a minimum of 2 wk. For each patient, a minimum erythema dose (MED) was established before whole body irradiation, which was begun with a UVB dose 10–20% lower than the MED. For each subsequent day, the UVB dose was increased by 10% unless marked erythema developed. Treatment was terminated upon attaining clinical resolution of psoriatic lesions, typically after 4–5 wk of daily treatment. (4 of 13 patients sequentially entered into this study did not respond to UVB treatment, and posttreatment effects were not studied in these nonresponders.) UVB treatment was given in conjunction with skin lubrication using petrolatum containing 3% crude coal tar, but the tar-containing preparation was removed from the skin before each irradiation. Skin was irradiated in a unit (model 57000; Psoralite Corp., Columbia, SC) containing LS-FS72T12-UVB-HO fluorescent bulbs, which emit in a broad peak of 285–345 nm except for a spike of increased irradiance at 313 nm. UVB output was metered with a radiometer (model IL1700; International Light, Newburyport, MA) and calibrated UVB detector (model SED 240; International Light). Irradiation of cultured cells was performed using a panel of smaller (24 in.) fluorescent bulbs with identical spectral output and metering with the same UVB detector.

Histopathological Assessment. 6-μm Cryostat sections were made on biopsies of unaffected skin and of lesional psoriatic skin taken immediately before the start of UVB treatment and again upon

completion. All methods have been described (5). Briefly, immunohistochemical staining of tissue was performed as described (5) using 3-amino-9-ethylcarbazol as the chromagen, since its red reaction product could easily be distinguished from melanin. Mouse mAbs to α3-integrin were a gift from Dr. Anthony Albino (Memorial Sloan-Kettering Cancer Center, New York). Mouse mAbs to ICAM-1, clone RR1/1, were as previously described (18). Epidermal thickness was measured with a calibrated microscope ocular (5). Hematoxylin and eosin–stained tissue sections were prepared by a commercial laboratory from formalin-fixed, paraffin-embedded tissue.

Cell Culture Analysis. Keratinocytes were cultured from neonatal human skin using serum-free keratinocyte growth medium (Clonetics Corp., San Diego, CA) (5). Peripheral blood mononuclear cells were cultured in PHA-containing RPMI 1640 medium (5). A transformed T lymphocyte cell line (HUT 102) was maintained in RPMI 1640 medium containing 10% FCS. For UVB irradiation, cells were briefly switched to PBS and were irradiated in open-topped tissue culture plates, followed by replacement with the usual growth medium. Keratinocyte proliferation was measured by direct cell counting, and lymphocyte proliferation was determined by the incorporation of [³H]thymidine (5). Assessment of cell viability was performed by incubating cells for 90 min at room temperature in buffered saline containing a mixture of 2 mM calceinAM (viable cells) and 4 mM ethidium homodimer (dead cells) (both dyes were obtained from Molecular Probes, Inc., Eugene, OR). Fluorescence of calcein or ethidium was visualized using a Photomicroscope III (Carl Zeiss, Inc., Thornwood, NY) equipped with excitation and barrier filters for green and red fluorescence. Induction of DNA fragmentation or apoptosis was detected by the TUNEL (TdT-mediated dUTP–biotin nick end labeling) reaction as described (21). The TUNEL procedure was modified to include a 30-min wash with 0.01 SSC buffer and a brief wash with 10× Tris-buffered saline (pH 7.6) after incubation of cells with biotin–dUTP (Boehringer Mannheim Biochemicals, Indianapolis, IN) and TdT enzyme (Promega, Madison, WI). Controls included reactions in which TdT was omitted. Positive staining reactions were confirmed with a second detection system (digoxigenin-conjugated dUTP followed by peroxidase-conjugated sheep antidigoxigenin antibodies).

Results

The effects of phototherapy on epidermal and immunological activation in lesional psoriatic plaques were studied in nine patients, who responded to this form of therapy by clinical clearing of psoriatic lesions. For consistent delivery of phototherapy, all patients were treated as inpatients in our General Clinical Research Center and were closely observed for daily application of topical tar ointment and for development of phototoxicity on therapy. Increasing (suberythogenic) doses of UVB were delivered each day for an average treatment of 27 d (the cumulative exposure to UVB averaged 4.7 J/cm² per patient, which is equivalent

Figure 1. Pathological and immunohistochemical assessment of psoriatic epidermis before and after UVB treatment. The left column displays micrographs from untreated psoriasis, whereas the right column shows paired micrographs from UVB-treated psoriasis. Sections have been hematoxylin and eosin stained (*A* and *B*) or reacted with antibodies to the Ki67 nuclear protein (*C* and *D*), to filaggrin (*E* and *F*), to keratin 16 (*G* and *H*), or to α3-integrin (*I* and *J*). In (*A* and *B*), "i" points to chronic inflammatory dermal cells and "g" points to a granular layer; in (*C* and *D*), arrows point to some Ki67-positive nuclei; in (*E*), arrows point to focal areas of epidermis expressing filaggrin; in (*I*), arrows point to suprabasal keratinocytes expressing cell surface α3-integrin. All micrographs are shown at the same magnification (×80).
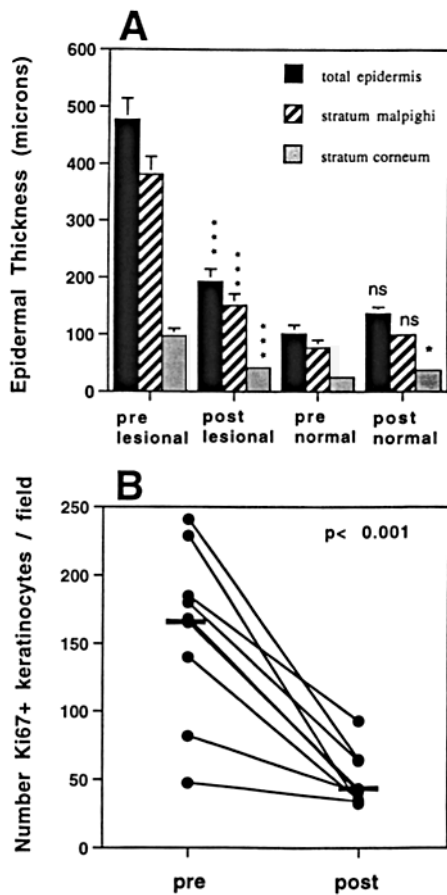
Figure 2. Quantitative analysis of epidermal acanthosis or keratinocyte Ki67 reactivity in psoriatic skin before and after UVB treatment. (*A*) Bar graphs show mean epidermal thickness for psoriatic lesional and unaffected skin in patients before and after UVB treatment (standard error is shown for each mean). *P* values for differences between untreated and UVB-treated epidermis were either $P < 0.001$ (***), $P < 0.05$ (*), or $P > 0.05$ (*ns*). (*B*) The number of Ki67$^+$ nuclei per microscope field for lesional psoriatic epidermis before and after UVB treatment is shown for individual patients. Solid bars indicate mean values for each group.

keratinocytes. In comparison with normal skin (or unaffected skin in psoriatics), lesional psoriasis is characterized by marked keratinocyte hyperplasia, by profound thickening of the epidermis (acanthosis), by incomplete terminal differentiation of keratinocytes (absent granular layer and nuclear retention in cornified epidermis [parakeratosis]), and by parakeratosis, an "alternate" or "regenerative" differentiation program that alters expression of epidermal proteins and cytokines (1, 2). A number of inflammatory alterations, considered below, also occur in active psoriatic lesions.

Upon the conclusion of phototherapy, as illustrated in Fig. 1, *A* and *B*, lesional psoriatic epidermis had markedly reduced acanthosis, a granular layer was typically restored and parakeratosis in the stratum corneum was resolved, although this layer was thicker than normal (Fig. 1 *D*). Nuclear retention (parakeratosis) was not observed in the stratum corneum of psoriatic lesions after phototherapy. Of note, however, were numerous mononuclear inflammatory cells that continued to be present in the dermis of UVB-treated psoriatic lesions (Fig. 1 *B*). Keratinocyte proliferation, as judged by expression of the Ki67 nuclear protein confined in cycling cells, was markedly reduced such that residual proliferating keratinocytes were confined to the basal layer (compare Fig. 1, *C* and *D*) as in normal skin. Filaggrin, a protein normally produced by keratinocytes undergoing terminal differentiation in the granular layer, was minimally expressed by lesional epidermis (Fig. 1 *E*) but highly expressed by psoriatic keratinocytes following UVB treatment (Fig. 1 *F*). Because a visible granular layer is produced by the action of filaggrin to aggregate keratin filaments, the restored granular layer after phototherapy probably results from increased production of filaggrin by differentiating keratinocytes.

Fig. 2 presents quantitative analyses of these changes. The thickness of the epidermis was quantified separately for viable layers (stratum Malpighi) and cornified layers. Phototherapy reduced epidermal thickness by 60%, with reductions evident in both the stratum Malpighi and the stratum corneum ($P < 0.001$). We noted that epidermal thickness of nonlesional skin was somewhat greater after phototherapy (101 versus 137 μm, $P = 0.07$) and that a statistically significant increase in thickness of the statum corneum (25 versus 37 μm, $P = 0.03$) also could be measured (Fig. 2 *A*). The stratum corneum of treated plaques (Fig. 1 *D*), as well as the unaffected epidermis (not shown), appeared visibly thicker than unaffected skin before phototherapy. Keratinocyte proliferation was also consistently reduced by phototherapy, with an average reduction of 69% ($P < 0.001$) (Fig. 2 *B*).

Regenerative differentiation of the epidermis is the term used to describe a transient phenotype associated with a

to a mean dose of 174 mJ/cm^2 per d). Upon the conclusion of treatment, psoriatic plaques were converted to flat, hyperpigmented patches without residual scale or erythema in skin areas exposed to phototherapy. Markers of cellular activation in the epidermis and infiltrating leukocytes were studied in skin biopsies obtained from lesional plaques at the conclusion of phototherapy, and the findings were compared to biopsies obtained from psoriatic lesions immediately before starting phototherapy.

Effects of Phototherapy on Epidermal Pathology. Psoriatic pathology is largely defined by a series of changes in the proliferation and differentiation characteristics of epidermal

Figure 3. Immunohistochemical assessment of T lymphocyte infiltration and inflammatory activation in psoriatic skin before and after UVB treatment. The left column displays micrographs from untreated psoriasis, while the right column shows paired micrographs from UVB-treated psoriasis. Cryostat skin sections have been reacted with antibodies to CD8 (*A* and *B*), to CD4 (*C* and *D*), to CD1 (*E* and *F*), to HLA-DR (*G* and *H*) or to ICAM-1 (*I* and *J*). In (*A*), arrows identify CD8$^+$ cells in epidermis; in (*G*), arrows point to keratinocytes expressing HLA-DR; in (*I*), arrows point to keratinocytes expressing ICAM-1; in (*I*) and (*J*), ICAM-1 expression by vascular endothelium is indicated by v.

untreated psoriasis　　　**UVB treated**
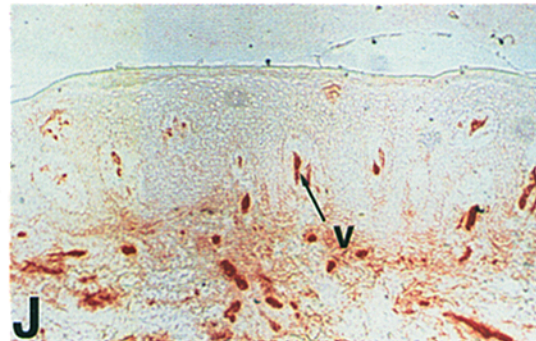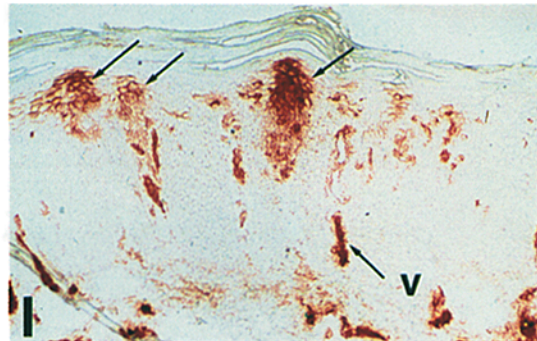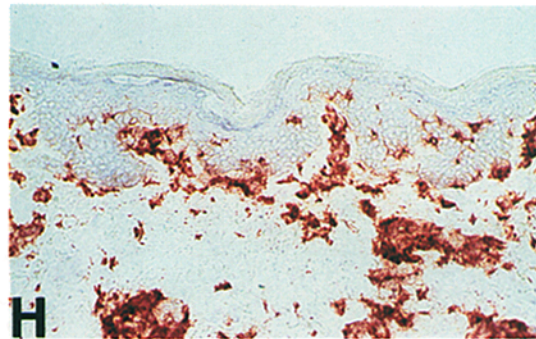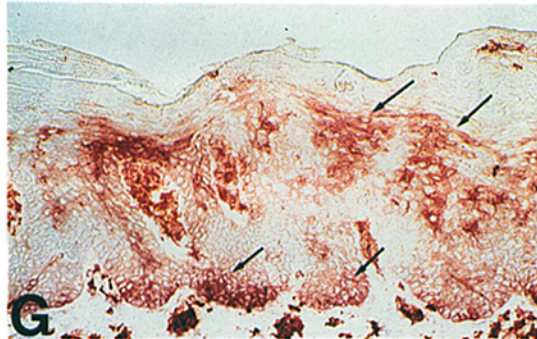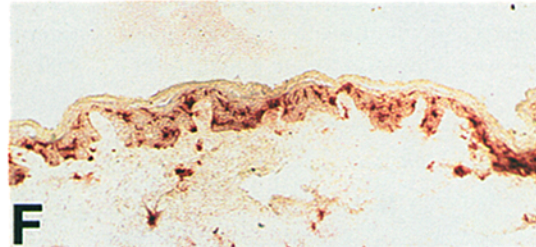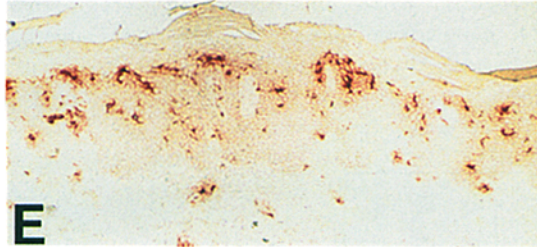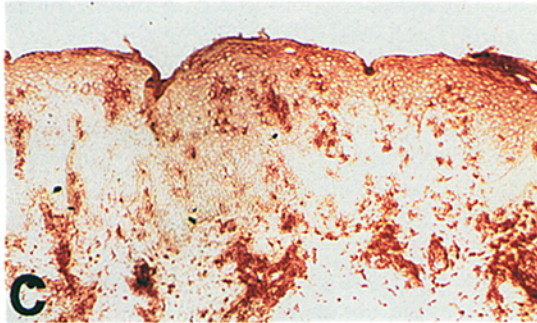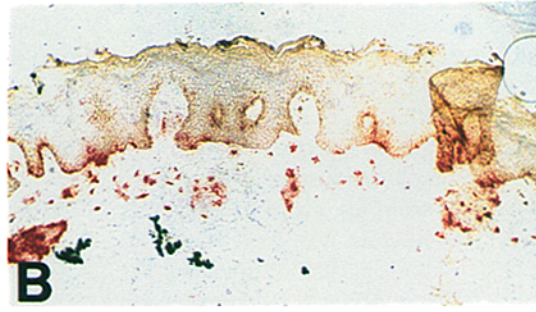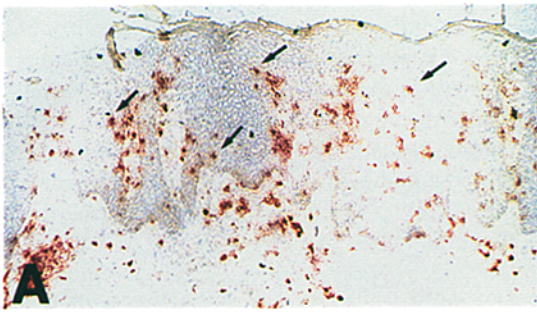
wound-repair response (1, 2). Lesional psoriatic epidermis consistently displays this phenotype, which is marked by altered expression of keratin 16, α3-integrin, IGF-1 receptors, and the differentiation-associated proteins involucrin and filaggrin (2, 20, 21). In nine out of nine cases, we observed that UVB reversed the regenerative epidermal growth. Keratin 16, for example, was expressed by suprabasal keratinocytes in all psoriatic lesions before treatment (Fig. 1 G), and it was absent from lesional epidermis after phototherapy (Fig. 1 H). α3-Integrin was expressed in lesional epidermis (Fig. 1 I) by both basal and suprabasal keratinocytes, but after phototherapy (Fig. 1 J) its expression was predominantly basal, as in normal epidermis. Expression of involucrin and of IGF-1 receptors was also normalized to the suprabasal and basal layers, respectively (not shown). In sum, all the known changes in the regenerative epidermal growth pattern were coordinately reduced by phototherapy of active psoriatic plaques.

Effects of Phototherapy on Tissue Infiltration by T Lymphocyte Subsets. The appearance of CD8+, CD4+, and CD1+ cells in lesional psoriatic tissue before and after phototherapy is illustrated in Fig. 3. These micrographs illustrate numerous CD8+ lymphocytes in diseased epidermis and dermis before treatment (Fig. 3 A) but only a marked reduction of CD8+ lymphocytes in the epidermis after phototherapy (Fig. 3 B). CD4+ cells were common in the reticular dermis and in the papillary dermis, near the dermal–epidermal interface in lesional skin (Fig. 3 C). Some reduction in CD4+ cells was evident in the epidermal–dermal interface and in the upper papillary dermis after UVB treatment (Fig. 3 D). Fig. 4 presents a quantitative summary of T lymphocytes infiltrating the epidermis and dermis of lesional psoriatic tissue before and after phototherapy. As in earlier studies (4, 5), the T lymphocytes infiltrating the epidermis were predominantly CD8+, whereas those infiltrating the dermis were chiefly of the CD4+ phenotype (Fig. 4). Total (CD3+) T cells in the epidermis were reduced by an average of 91% (11-fold) by phototherapy (P <0.001), whereas these cells were reduced by a mean of only 19% (1.2-fold) in the dermis. The major T cell subset responding to phototherapy in the epidermis was CD8+, showing a mean reduction of 85% (P <0.001), whereas CD4+ cells were reduced by 49% (P <0.01). Expression of the IL-2 receptor α chain (CD25) was reduced to an even greater extent than was expression of T cells in the epidermis, with a mean reduction of 95% (21-fold, P <0.001). In the dermis, a 36% reduction in CD4+ cells and a 37% reduction in CD25+ cells were measured (Fig. 4). Interestingly, epidermal CD1+ (Langerhans) cells did not appear to be depleted, and even may have been increased, in epidermis of psoriatic lesions after phototherapy (Fig. 3, E and F). Although Langerhans cells were not strictly quantified after CD1 staining, neither
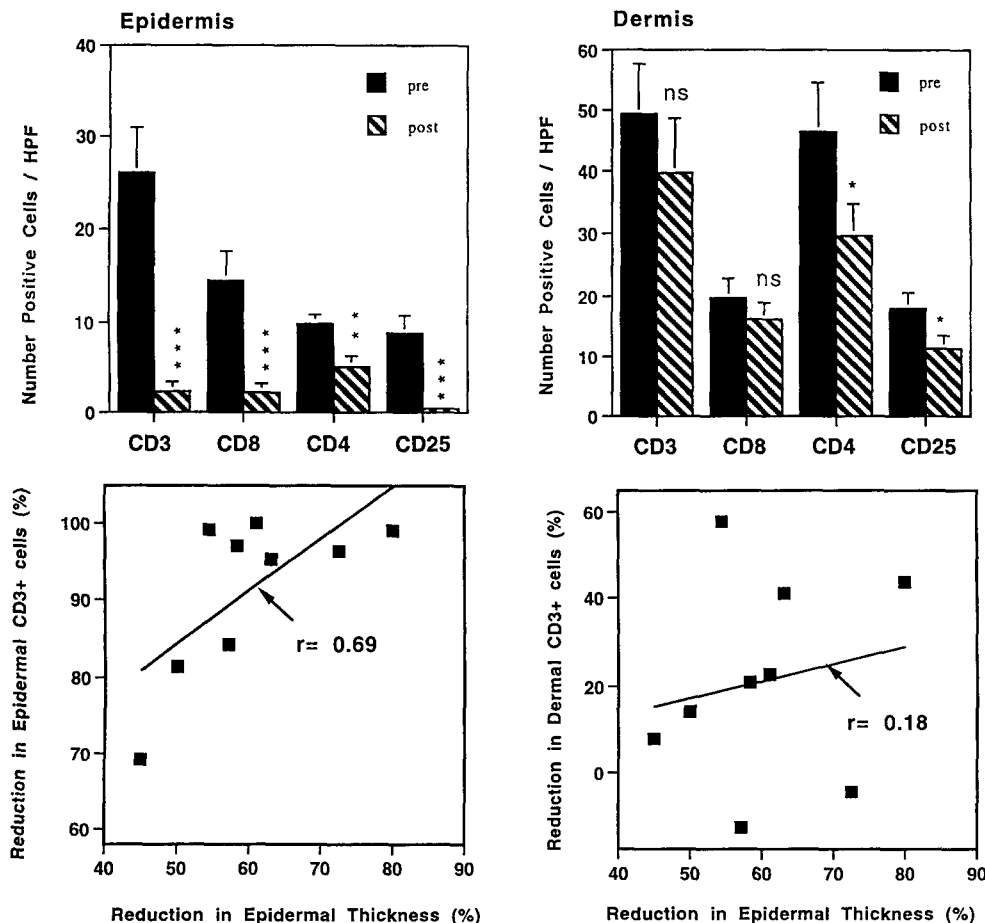
Figure 4. Quantitative analysis of T cell infiltration in psoriatic lesional tissue before and after UVB treatment. Top panels show mean numbers of CD3+, CD8+, CD4+, or CD25+ cells in the epidermis or dermis of psoriatic lesional tissue before treatment (*solid bars*) or after UVB treatment (*hatched bars*); standard error is shown for each mean value. P values for differences between untreated and UVB-treated tissue were P <0.001 (***), P <0.01 (**), P <0.05 (*), or P >0.05 (*ns*). Bottom panels display the relationship between reduction in epidermal acanthosis (thickness) and reduction in the number of total T lymphocytes (CD3+) in either the epidermis or dermis of biopsies analyzed for individual patients.

psoriatic lesional epidermis nor unaffected epidermis showed marked reductions in Langerhans cells at the conclusion of UVB treatment. In fact, the distribution of bone marrow–derived cells in psoriatic epidermis after phototherapy (few T lymphocytes, numerous Langerhans cells) is similar to that observed in normal skin or the unaffected skin of psoriatics. The presence of numerous residual T lymphocytes in psoriatic lesional skin after phototherapy, however, contrasts sharply with unaffected or normal skin, both of which contain only scant T lymphocytes outside the vasculature (5).

To illustrate further the relationship between epidermal activation and epidermal T cells, the lower panels of Fig. 4 graph the relationships between the reduction in epidermal or dermal CD3+ cells and the reduction in epidermal acanthosis produced by phototherapy. In individual cases, phototherapy reductions in epidermal thickness range from 43 to 80%, and reductions in epidermal CD3+ T cells range from 70 to 100%. The correlation coefficient of these reductions was 0.69 in relation to the magnitude in reduction of epidermal CD3+ cells (Fig. 4, *lower left*). In contrast, dermal CD3+ cells were reduced by a maximum of 58% or increased by up to 20% in individual cases, and these changes were poorly related to reductions in epidermal thickness ($r = 0.18$; Fig. 4, *lower right*).

The effect of phototherapy on expression of HLA-DR and ICAM-1 by keratinocytes in lesional psoriasis was also examined (Fig. 3, *G–J*). Although both of these proteins were produced by keratinocytes in active lesions (Fig. 3, *G* and *I*), little or no expression could be detected after phototherapy (Fig. 3, *H* and *J*). HLA-DR continued to be expressed by Langerhans cells in the epidermis after treatment (Fig. 3 *H*), and ICAM-1 was still produced by endothelium of papillary blood vessels and other dermal cells (Fig. 3 *J*).

Effects of UVB on Keratinocytes and Lymphocytes In Vitro. To distinguish among the possible cellular targets of phototherapy, we compared the ability of UVB to directly suppress the growth of keratinocytes and leukocytes. We used the same broad-band UVB fluorescent source (bulbs emit radiation over a broad band from 285 to 345 nm, with a sharp peak at 313 nm) with identical metering of output intensity. Cells were mock-irradiated or treated with 1–20 mJ/cm² UVB, and proliferation was measured after 1, 2, 3, or 4 d. Keratinocyte growth was minimally inhibited by 4 mJ/cm², moderately inhibited by 8–12 mJ/cm², and completely inhibited by 16–20 mJ/cm² (Fig. 5 *A*). In PHA-activated peripheral blood mononuclear leukocytes, 2 mJ/cm² of UVB produced detectable inhibition of lymphocyte growth 1–2 d after exposure, 4 mJ/cm² produced 65% inhibition 3 d after exposure, and complete inhibition was produced by ≥8 mJ/cm² (Fig. 5 *B*). A transformed T-lymphocyte cell line (HUT 102) was similarly sensitive to the antiproliferative effects of UVB (Fig. 5 *C*). Thus, lymphocytes appeared more sensitive than keratinocytes to the antiproliferative effects of UVB irradiation.

The inhibition of proliferation in lymphocytes and keratinocytes was associated with increased cellular cytotoxicity as assessed by fluorescent probes that distinguish viable from nonviable cells (Fig. 6). This assay scores for live cells
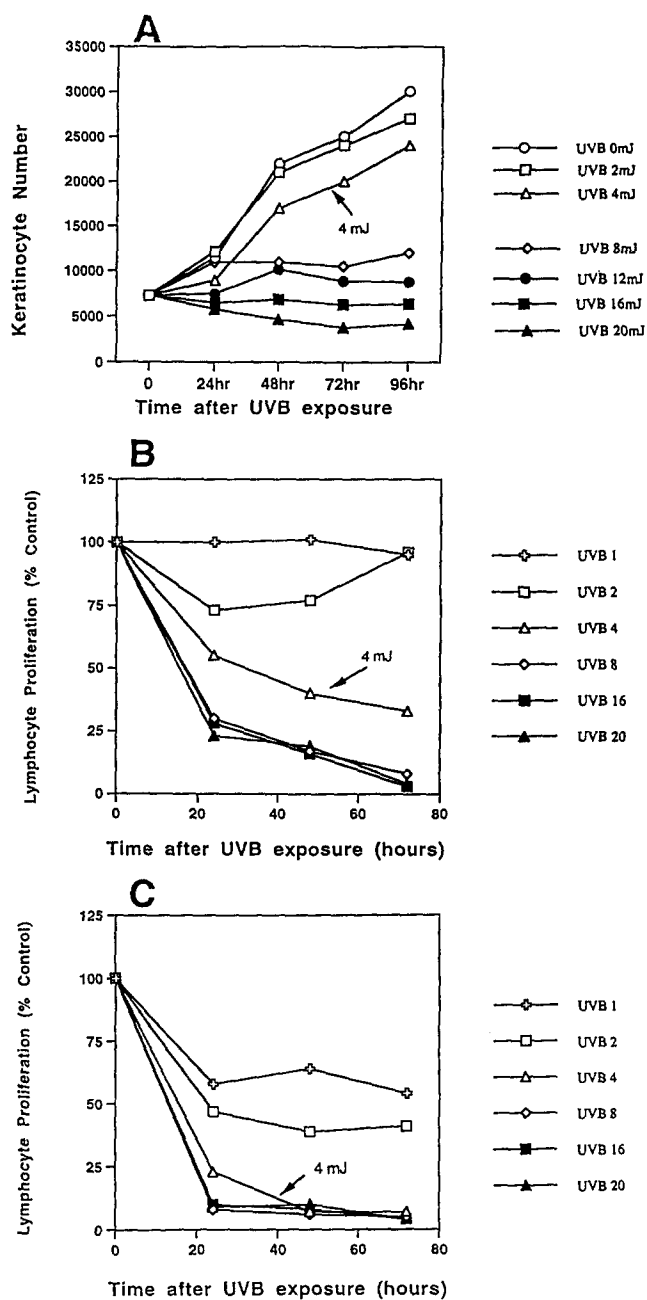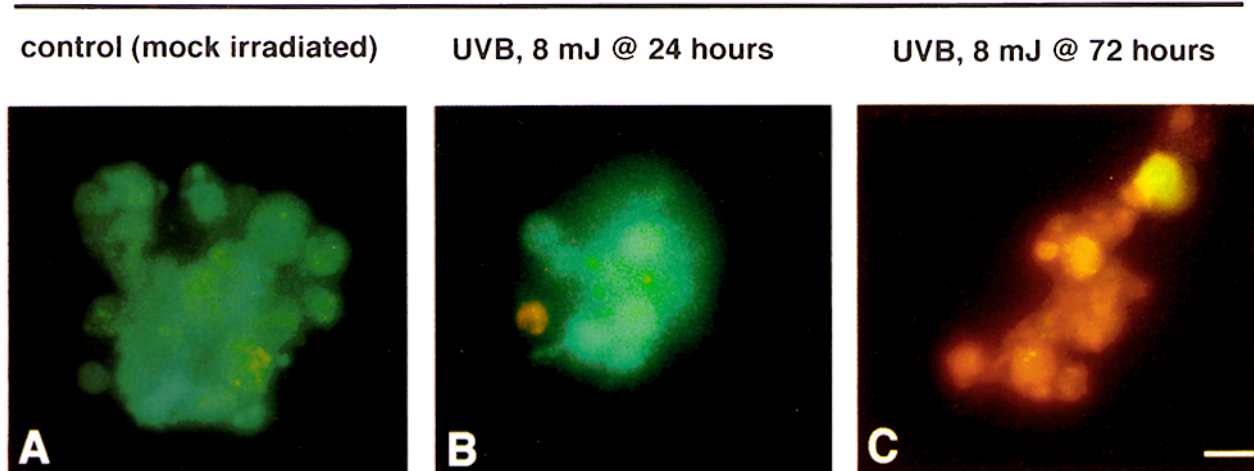

Figure 5. Comparative study of antiproliferative effects of UVB in human keratinocytes or PBMC in vitro. (*A*) Keratinocytes grown in serum-free medium were irradiated with 0–20 mJ/cm² UVB, and cell growth was assessed after 1, 2, 3, or 4 d by counting of cell numbers. Mitogen-activated PBMC (*B*) or HUT 102 cells (*C*) were exposed to 0–20 mJ/cm² UVB, and cell proliferation was assessed after 1, 2, or 3 d by [³H]thymidine incorporation. Values are graphed for exposure to 1–20 mJ/cm² in relationship to mock irradiated cultures. Legends to the right of each figure define symbols for different levels of UVB exposure.

by uptake of calceinAM (nonfluorescent substrate), which is converted by cellular esterases to calcein which emits green fluorescence. Conversely, esterase-negative cells with compromised plasma membranes (nonviable) do not cleave calceinAM but will allow ethidium homodimer to enter, and this is detected as red nuclear fluorescence. Hence, every

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Viability - PBMC

control (mock irradiated)   UVB, 8 mJ @ 24 hours   UVB, 8 mJ @ 72 hours

Viability - Keratinocytes

UVB, 16 mJ @ 72 hours   UVB, 64 mJ @ 72 hours   UVB, 64 mJ @ 72 hours

TUNEL reactions

lymphocyte -TdT   lymphocyte +TdT   keratinocyte +TdT

UVB, 8 mJ @ 48 hours   UVB, 8 mJ @ 48 hours   UVB, 16 mJ
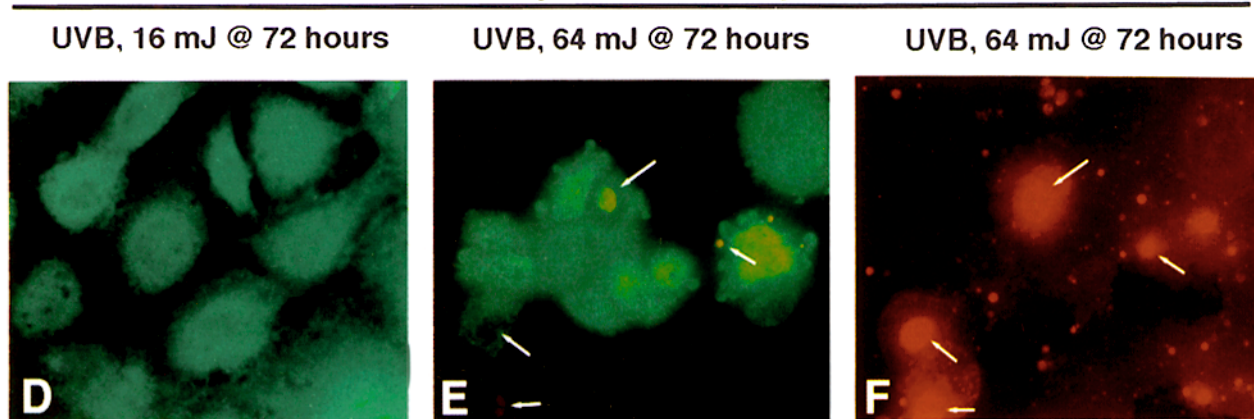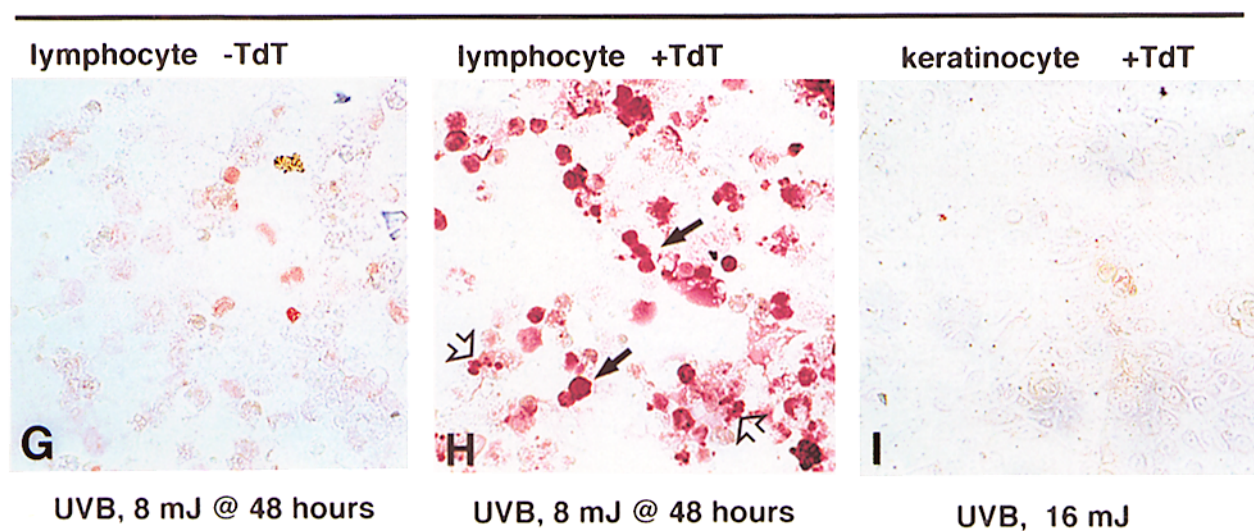
Figure 6. Comparative study of cytotoxic effects of UVB in human keratinocytes or leukocytes in vitro. Micrographs are shown for PBMC (*A–C*) or keratinocytes (*D–F*) after incubation of cells in calceinAM (substrate for green fluorescence, live cells) and ethidium dimer (red fluorescence, dead cells). Lymphocytes were mock irradiated and examined after 72 h in culture (*A*), or they were irradiated with 8 mJ/cm^2 and examined after 24 (*B*) or 72 h (*C*). Keratinocytes were examined 72 h after irradiation with 16 (*D*) or 64 mJ/cm^2 (*E* and *F*). Micrographs for *E* and *F* show the same field of cells photo-

cell visualized in an assay can be scored for viability or non-viability. Exposure of lymphocytes to 8 mJ/cm^2 of UVB led to massive cell death (>90% nonviable cells) 3 d after UVB exposure (Fig. 6, A–C). Lethality was not immediately evident, since lymphocytes were fully viable 1 d after exposure to 8 mJ/cm^2 UVB. Exposure of lymphocytes to 4 mJ/cm^2 produced ∼50% cell death at 3 d using this assay, whereas exposure to 2 mJ/cm^2 produced <20% cell death. In contrast, keratinocytes were more resistant to the cytotoxic effects of UVB. 3 d after exposure to 8 or 16 mJ/cm^2, keratinocytes appeared fully viable (Fig. 6 D). Exposure to 64 mJ/cm^2 produced death in ∼50% of keratinocytes (Fig. 6, E–F), a level of cytotoxicity produced in lymphocytes after exposure to only 4 mJ/cm^2 of UVB. Keratinocytes treated with 64 mJ/cm2 showed cytoplasmic blebbing characteristic of apoptosis (22).

To explore further the ability of UVB to induce apoptosis in lymphocytes or keratinocytes, cells that had been UVB treated were examined for DNA fragmentation by the TUNEL procedure. Transformed lymphocytes or PBMC exposed to 8 mJ/cm^2 UVB showed clear TUNEL positivity 24–48 h after UVB treatment. Fig. 6 H illustrates TUNEL positivity in both intact nuclei (arrows) and in nuclear fragments (open arrows), which are morphological indicators of advanced apoptosis. As a control for the TUNEL reaction, Fig. 6 G shows absence of nuclear staining in UVB-treated lymphocytes when only TdT enzyme was omitted from the reaction. In contrast to the lymphocyte results, no significant TUNEL reactivity was detected in cultured keratinocytes 1–3 d after exposure to 1–16/cm^2 mJ UVB (Fig. 6 I), but some TUNEL-positive cells could be detected after exposure to 64 mJ/cm^2 (not shown). Thus, TUNEL reactivity paralleled viability measurements made in keratinocytes with calcein/ethidium uptake. Both viability and TUNEL reactions indicate greater lethality of UVB in lymphocytes than in epidermal keratinocytes, and the TUNEL reactions suggest that apoptosis is induced in lymphocytes after exposure to relatively low levels of UVB.

Discussion

One of the most common treatments for moderately severe psoriasis is skin irradiation with UVB, often given after skin lubrication with tar-containing emollients. A number of earlier studies have demonstrated effects of UVB on normal skin that are paradoxical in relationship to therapeutic improvements. In normal skin, UVB increases TGF-α synthesis by keratinocytes, accelerates keratinocyte proliferation, and increases overall epidermal thickness (23–25). Several cytokines thought to initiate or enhance psoriatic pathology are also increased after UVB irradiation of skin or cultured keratinocytes: IFN-γ (25), TNF-α (20, 26),

IL-1 (27), IL-6 (28) and IL-8 (29). Furthermore, UVB irradiation increases leukocytic infiltration (30) and activates neutrophil effector functions (31). Therefore, it might be anticipated that UVB treatment of psoriatic skin would worsen hyperplasia and inflammation in lesions, but the entirely opposite effect was observed. We observed reduced epidermal thickness, diminished keratinocyte proliferation, decreased abundance of immunoreactive IL-6 in lesional tissue (32), and virtual elimination of interferon-γ–induced proteins (HLA-DR and ICAM-1) in psoriatic keratinocytes. In effect, UVB treatment reversed the hyperplasia and regenerative growth to levels associated with normal skin, even though existing evidence predicted the opposite.

One explanation for this paradox is that UVB may operate primarily on T lymphocytes in psoriatic tissue. In fact, UVB treatment produced consistent and profound depletion of T lymphocytes from psoriatic epidermis. T cell activation appeared to be decreased even further, as judged by expression of the IL-2 receptor α chain (CD25). Dermal lymphocytes were much less affected by UVB treatment. Our observations clearly oppose the view that therapy-related improvements are mediated by increased numbers of epidermal lymphocytes (33).

As used therapeutically, 5–10% of incident radiation in the UVB spectrum should penetrate the epidermis to the basal layer (the remaining energy is scattered at the epidermal surface or is absorbed by keratinocyte proteins or other molecules) (34). Our psoriatics were treated on a daily basis with UVB averaging 174 mJ/cm^2. Thus, at the midpoint of therapy, one would expect an average penetration of 8–17 mJ/cm^2 to the basal layer. UVB penetration beyond the basal layer would be rapidly attenuated by the dermal–epidermal interface and upper dermis (34). Irradiation of transformed lymphocytes or mitogen-activated PBMC with 4–8 mJ/cm^2 of UVB in vitro immediately inhibited proliferation and then induced apoptotic cell death over a somewhat longer time frame. Thus, the expected delivery of UVB to psoriatic epidermis near the midpoint of therapy approximates the energy levels sufficient to kill activated lymphocytes. The minimal penetration of UVB into dermal tissue is then the most logical explanation for the minimal effects of UVB observed on dermal lymphocytes. In contrast, keratinocytes appear to be relatively resistant to the cytotoxic effects of UVB, since UVB was cytotoxic for keratinocytes at levels that were at least 10-fold higher than those for lymphocytes. The deletion of activated lymphocyte clones from psoriatic tissue might then explain the "remittive" nature of UVB treatment, i.e., its ability to produce sustained therapeutic improvement, whereas noncytotoxic immunosuppressive agents, e.g., corticosteroids or cyclosporine, do not produce this sustained therapeutic benefit (5).

UVB might also exert immunosuppressive actions inde-

pendent of direct cell cytotoxicity in the psoriatic lesions. In human skin, direct UVB irradiation ablates or reduces contact hypersensitivity to a number of antigens (14, 15, 35). In various systems, UVB depletes Langerhans cells from the epidermis, decreases the activation capability of residual Langerhans cells, and decreases the ability of Langerhans cells to present viral antigens to lymphocytes (15, 35–39). UVB also alters tyrosine phosphorylation of T lymphocyte proteins, inhibiting T cell activation but not directly inducing cytotoxicity (40). UVB also increases synthesis of cis-uroconic acid, IL-10, the IL-1 receptor antagonist, and TGF-β, which are all potentially immunosuppressive mediators (14, 41, 42). Thus CD25 expression in UVB-treated psoriatic epidermis, which is reduced overall more than are T cells, might be modulated via decreased antigen–APC interactions or by chemical mediators. The number of Langerhans cells in UVB-treated skin, however, did not appear to be reduced by repetitive UVB exposures (Fig. 4 F), an observation that suggests that UVB selectively affects the T lymphocytes that infiltrate psoriatic epidermis. The apparently minimal effects of UVB on Langerhans cells might reflect their relative resistance to suberythrogenic UVB levels. It is possible, however, that continued trafficking of Langerhans cells into the epidermis might replace those cells damaged by repetitive UVB exposure.

Intensive UVB treatment of psoriasis leads to relatively durable clinical remission (5, 43). Our data establish that a major cellular correlate of this outcome is the elimination of intraepidermal lymphocytes and restoration of a normal growth/differentiation sequence in the epidermis. Psoralen + UVA (PUVA) treatment has similar effects on the elimination of epidermal T lymphocytes, the reversal of regenerative epidermal growth, and clinical remissions (5). These data, when considered with effects produced by DAB$_{389}$IL-2 and other more selective antilymphocyte agents (11), suggest that disease-mediating lymphocytes reside primarily in the epidermis or at the dermal–epidermal interface, where T cells could exert direct effects on epidermal keratinocytes. The predominant epidermal lymphocytes are CD8$^+$, and a recent report indicates that these CD8 cells contain only a limited number of clones (9). The association of MHC class I alleles with disease susceptibility, the selective accumulation of CD8$^+$ lymphocytes in the epidermis, and clonality in CD8$^+$, but not CD4$^+$ cells (9), imply that spe-

cific immune activation is MHC class I restricted in psoriasis. Epidermal cells could be important targets of effector CD8$^+$ cells. Keratinocyte injury produced by CTLs in the epidermis might trigger these cells into a repair or regenerative program, or, alternatively, cytokines elaborated by activated CD8$^+$ lymphocytes might directly stimulate keratinocyte proliferation (44). Some cytokines that are elevated in psoriatic lesions, e.g., IL-2, IL-6, and IFN-γ, may promote efficient maturation of immature CD8$^+$ lymphocytes into effector CTLs (45, 46), and IFN-γ can enhance CD8$^+$-mediated injury to keratinocytes by modulating MHC class I expression (47).

Although either CD4$^+$ or CD8$^+$ lymphocytes could elaborate disease-associated cytokines, the majority of CD4$^+$ clones isolated from psoriatic lesions elaborate cytokines that suppress keratinocyte proliferation (44). The pathogenic significance of CD4$^+$ lymphocytes in psoriatic tissue is thus somewhat uncertain. CD4$^+$ lymphocytes are frequently abundant in MHC class I–restricted inflammatory tissue reactions mediated by effector CD8$^+$ lymphocytes (48). For example, in cutaneous GVH reactions, many CD8$^+$ effector lymphocytes are localized in the epidermis (49), but the majority of dermal lymphocytes are CD4$^+$ (50), as in psoriatic tissue. Since the CD4$^+$ lymphocytes that have been analyzed from psoriatic lesions display no preferential TCR usage (9), infiltration by this cell type, though quantitatively dominant in the dermis, may reflect nonspecific recruitment by inflammatory cytokines and altered vascular adhesion molecules (51). Furthermore, since most dermal T lymphocytes persist in psoriatic dermis after UVB treatment, it appears that these cells are not immediately pathogenic. In fact, the most direct explanation for the prolonged clinical remissions that are obtained with UVB or PUVA therapies is that probably through cytotoxic actions, these modalities produce profound depletion of effector lymphocytes residing at the dermal–epidermal interface or in the epidermis (5). Longer periods of clinical remission attained by PUVA treatment might be best explained by the treatment's ability to deplete more dermal lymphocytes from psoriatic skin than UVB treatment does (5). In turn, these findings suggest a rational basis for use of specific immune-modulating agents to treat psoriasis and to potentially decrease inadvertent toxicity-associated effects on epidermal keratinocytes.

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