

PUVA Bath Therapy Strongly Suppresses Immunological and Epidermal Activation in Psoriasis: A Possible Cellular Basis for Remittive Therapy

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Summary

Psoriasis is characterized by alterations in both the epidermis and dermis of the skin. Epidermal keratinocytes display marked proliferative activation and differentiate along an "alternate" or "regenerative" pathway, while the dermis becomes infiltrated with leukocytes, particularly interleukin 2 (IL-2) receptor-bearing "activated" T cells. Psoralens, administered by the oral route, have therapeutic effects in psoriasis when photochemically activated by ultraviolet A light (PUVA therapy). Recently psoralen bath therapy has been introduced to more effectively deliver this agent to the diseased skin. We have correlated the efficacy of PUVA bath therapy with its effects on specific molecular and cellular parameters of disease, in 10 consecutive patients with recalcitrant psoriasis. Rapid clearing of lesions occurred in 8 out of 10 patients. Biopsies were taken from lesional and nonlesional skin before and after a single round of therapy, and observation was continued in our Clinical Research Center at The Rockefeller University. Enumeration of cycling keratinocytes with the Ki-67 monoclonal antibody showed that PUVA reduced cell proliferation by 73%. The pathological increase in insulin-like growth factor 1 (IGF-1) receptors was reversed, whereas epidermal growth factor (EGF) receptors, which are also increased in psoriasis, remained unchanged. Keratinocyte proteins that are expressed in abnormal sites of the epidermis during psoriasis, i.e., keratin 16, filaggrin, and involucrin, were, after PUVA treatment, localized to their normal sites. Epidermal and dermal T-lymphocytes (CD3⁺), as well as CD4⁺, CD8⁺, and IL-2 receptor⁺ subsets, were strongly suppressed by PUVA, with virtual elimination of IL-2 receptor⁺ T cells in some patients. Consistent with diminished lymphocyte activation, HLA-DR expression by epidermal keratinocytes was markedly reduced in treated skin. In comparison to cyclosporine treatment of psoriasis, PUVA therapy leads to more complete reversal of pathological epidermal and lymphocytic activation, changes which we propose to be the cellular basis for a more sustained remission of disease after PUVA treatment.

Psoriasis is a common skin disease characterized by marked changes in tissue architecture and by simultaneous activation of a variety of distinct cell types, including epidermal keratinocytes, vascular elements, and leukocytes. Much of the clinical appearance of psoriasis (red, raised, scaly plaques) can be attributed to alterations in the growth and maturation of epidermal keratinocytes. The thickened psoriatic epidermis is characterized by keratinocyte hyperplasia and by a number of differentiation-related alterations, including maturation of keratinocytes with retained nuclei (parakeratosis), and altered expression of keratins and other differentiation-specific proteins along a "regenerative" or "alternate" pathway (1-3). It seems likely that important interaction occurs between psoriatic keratinocytes and T-lymphocytes, since psoriatic keratinocytes inappropriately synthesize a number of immune-related

molecules, including HLA-DR (4-6), intercellular adhesion molecule 1 (ICAM-1) (7, 8), and the IP-10 protein (9). Much of the cellular and molecular activation in psoriatic skin may be explained, in turn, by increased expression of cytokines or cytokine receptors within lesional skin (10-12). Psoriatic epidermis is characterized by increased expression of epidermal growth factor (EGF)¹ receptors (13), insulin-like growth factor 1 (IGF-1) receptors (14), and IL-1 receptors (15), all molecules which might directly regulate epidermal hyper-

¹ Abbreviations used in this paper: 8-MOP, 8-methoxypsoralen; EGF, epidermal growth factor; IGF-1, insulin-like growth factor 1; K16, keratin 16; PUVA, psoralens plus ultraviolet A light.

proliferation. A complex milieu of cytokines is also present in psoriatic lesions. There is an increased production of TGF- α , amphiregulin, IL-2, IL-6, IL-8, TNF- α , and IFN- γ , each of which may contribute to activation of epidermal or immunological cell types within active lesions (16).

Although one can point to specific alterations in cytokine circuits to explain many of the cellular features of psoriasis, it is not known whether psoriasis is intrinsically a disease of epidermal keratinocyte dysfunction or a disease triggered by inappropriate T-lymphocyte accumulation and activation within focal skin areas (17). Attempts to reproduce the histopathological features of psoriasis by overexpression of specific cytokines or HLA molecules in transgenic rodents has met with only limited success (18–20). As no animals develop psoriasis-like skin lesions, one of the few means to judge the relative contribution of different cellular elements to its pathogenesis is to study changes brought about by therapy-induced resolution of skin pathology. By studying the response of epidermal and immunological cells and regulating cytokines or receptors to different therapeutic agents, one can hope to identify critical pathogenic components.

Two emerging therapies for severe psoriasis are treatment with cyclosporine (21–23) and bath PUVA (psoralens, administered in bath water rather than systemically, plus UVA) (24–26), forms of treatment that can produce clinical resolution in a high percentage of individuals. In a recent study (27), we noted that cyclosporine induced marked reductions in T-lymphocytes, as well as in IL-2R⁺ lymphocytes in affected psoriatic skin; however, molecular markers of epidermal activation such as keratin 16 (K16) and TGF- α continued to be variably overexpressed. These data suggested that the observed clinical improvement correlated more closely with reduced lymphocyte activation rather than reduced keratinocyte activation. However, in another recent study that compared the action of cyclosporine with systemic PUVA treatment (28), it was noted that psoriasis was improved by cyclosporine treatment without marked reductions in tissue-infiltrating T-lymphocytes, whereas PUVA treatment produced marked reductions in T-lymphocytes. Although markers of epidermal activation were not examined, these authors suggested that the predominant action of cyclosporine might be on the epidermal compartment (28), a possibility supported by the direct effects of cyclosporine on epidermal keratinocytes (29).

To better dissect the relative mechanistic actions of PUVA and cyclosporine in psoriasis, we have now examined the ability of bath PUVA to modulate cellular and cytokine activation in a group of 10 consecutive patients with recalcitrant disease. Bath PUVA is a new form of PUVA administration in which psoralen is applied to the skin from bathing in a dilute aqueous solution, instead of administering psoralen to the skin from systemic dosing. Compared with systemic administration, bathing selectively concentrates the psoralen in the epidermis (for a review see reference 30), and thus, bath PUVA may have more selective effects on epidermal cells compared with PUVA treatment using systemic psoralens. We report that bath PUVA treatment leads to a more complete reversal

of the pathologic alterations in epidermal and immune cells, especially when compared with cyclosporine treatment. Specifically, we describe how bath PUVA leads to: (a) a profound reversal of several molecular markers of keratinocyte disease; (b) a virtual elimination of tissue-infiltrating T lymphocytes; and (c) a longer suppression of clinical disease activity relative to cyclosporine.

Materials and Methods

Patients. A protocol using topical/bath water delivery of psoralens plus UVA irradiation in patients with moderate to severe psoriasis was approved by The Rockefeller University Hospital Institutional Review Board. All patients entered into this protocol had either failed management with topical agents or had such extensive disease that management with topical agents was not feasible. Patients were at least 18 yrs old (ranging from 28 to 74 yr of age) and had no pre-existing photosensitizing diseases, history of melanoma/squamous cell carcinoma, or evidence of cataracts before therapy. If patients were skin types I or II, or if they were on any potentially photosensitizing medications, a small area on their backs was phototested before the initiation of therapy.

Procedures. If patients met entry criteria, they underwent pretreatment biopsies and were begun on therapy three times per week. Patients bathed in 200 liters of water at body temperature, to which the dissolved 8-methoxypsoralen (8-MOP) had been added (final concentration, 0.5 mg/liter). UVA was delivered with a phototherapy unit (model 57000; Psoralite, Columbia, SC) using an initial UVA dose of 0.5 J/cm² as recently described (30). Clinical evaluations and photographs were performed weekly to assess efficacy. Disease activity was assessed by quantitating percent body coverage by degrees of erythema, scaling, and skin thickness in the psoriatic plaques. Each was rated by assigning a score from 1 to 7 based upon severity as follows: 1, absent; 2, trace; 3, mild; 4, mild to moderate; 5, moderate; 6, moderate to severe; and 7, severe. The scores for erythema, scaling, and skin thickness were summed for a maximum obtainable score of 21 and a minimum of 3. The sum was called the severity index (27). The patients were treated until complete resolution of their disease took place, or until it became clear that treatment was ineffective.

Immunoperoxidase Studies. Immunoperoxidase studies of fresh-frozen skin biopsies were done using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) as described (4, 31). IL-2R⁺, CD3⁺, CD4⁺, and CD8⁺ T cells were quantitated by averaging the number of positively staining cells in three $\times 40$ fields in each of three regions—epidermis, dermo-epidermal junction, and dermis. Statistics were performed using either the Parametric *t* test, or the Wilcoxon Sign Rank Test if the data were not normally distributed. The antibodies directed against HLA-DR (4), IL-2R (4), Langerhans cells (4), K16 (2, 32), TGF- α (33), involucrin (34), filaggrin (34), IL-6 (31), IGF-I receptors (35), and EGF receptors (35) have been reported previously.

Epidermal Thickness. Measurements were made directly on cryostat-processed biopsy specimens using a calibrated microscope micrometer. The thickness of the stratum corneum, and stratum Malpighi were calculated in four separate areas of each biopsy and averaged. Pre- and posttreatment measurements were performed on lesional and nonlesional skin.

In Vitro Cell Culture and UVA Irradiation. Human keratinocytes were cultured as previously described in keratinocyte growth medium (KGM; Clonetics, Corp., San Diego, CA) (35) and were used after the first or second subculture. Mononuclear leukocytes from human blood were prepared by centrifugation on Ficoll den-

sity gradients and were activated with PHA for 3 d in RPMI 1640 medium containing 10% fetal bovine serum as previously described (29). 8-MOP dissolved in ethanol was added directly to PBS so that ethanol did not exceed 0.01% final concentration and diluted 8-MOP solutions were incubated with cultured cells for 15 min before UVA irradiation. Standard UVA fluorescent bulbs with a peak emission at 360 nm and a power output of 10 mW were used to irradiate cultures after the light was passed through a 335-nm bandpass glass filter (WG335, Shott Glass, Germany), which removed all detectable ultraviolet below 320 nm. The light intensity at the culture surface was measured with a calibrated UVA detector (IL 1700; International Light, Inc., Newburyport, MA). After incubation of cells for 24 h after UVA irradiation, cell proliferation was measured by incorporation of [³H](methyl) thymidine, 1 μ Ci/ml (3-ml/assay) in the appropriate medium for 4 h, or by direct cell counting of trypsinized keratinocytes as previously described (35).

Results

10 patients with recalcitrant psoriasis were enrolled into our PUVA bath therapy protocol. The majority (80%) of patients showed remarkably good clearing of their psoriasis after an average of 17 treatments. In these patients, the severity index was reduced from an average of 13.6 before treatment to 3.7 after treatment, with 6 of 8 showing complete clearing of their disease. Before treatment, an average of 28% of the skin surface area was affected by psoriasis, whereas after treatment only 4% was affected. Patients 9 and 10 (Table 1) had only 16–20% improvement in their psoriasis severity indices and were considered treatment failures. However, one of these patients was poorly compliant with therapy and received only intermittent PUVA treatment. The average time-to-clearing was \sim 6 wk (17 treatments) in patients responding to this form of PUVA therapy.

Effects of PUVA Treatment on Immunological Parameters. Since psoriasis is defined in pathological terms by inappropriate activation of immunological and epidermal cells, we sought to characterize the effect of PUVA bath therapy on expression of aberrant immunological and keratinocytic components in affected skin. Immunological alterations in active psoriatic skin include heavy infiltration of epidermal and dermal tissue by T-lymphocytes, many expressing IL-2Rs, and induced expression of HLA-DR and IP-10 proteins in epidermal keratinocytes. Fig. 1 shows a representative histopathological analysis of CD3⁺ lymphocytes and IL-2R⁺ lymphocytes in psoriatic tissue before and after PUVA treatment (A–D). There are marked reductions in total T-lymphocytes and in those expressing IL-2Rs after PUVA therapy. Table 1 presents a quantitative analysis of CD3⁺, CD4⁺, CD8⁺, and IL-2R⁺ lymphocytes in nonaffected skin and in psoriatic lesional skin for each patient before and after PUVA therapy. Lymphocyte numbers were counted separately in the epidermis, the dermis, and in the region of the epidermal-dermal interface (Table 1). The distribution of T cells infiltrating psoriatic lesions was about one-third in the epidermis and two thirds in the dermis. Independent quantification of CD4⁺ and CD8⁺ cells indicates that CD8⁺ cells preferentially infiltrate the epidermis, e.g., in patients 1–8, the CD4/CD8 ratio was 0.95

in the epidermis, whereas it was 1.86 in the dermis. All T cell subsets were reduced in psoriatic tissue by PUVA bath therapy. In the interface region, those patients with good clinical responses (nos. 1–8) averaged a mean reduction in total T cells by 10.5-fold and a reduction in T cells expressing IL-2Rs by 14.2-fold. Concomitant reductions in CD4⁺ and CD8⁺ lymphocyte subsets were observed (Table 1). It is interesting that PUVA bath therapy induced larger relative reductions in epidermal T-lymphocytes compared with effects on dermal T-lymphocytes (Table 1). In patients responding to therapy, epidermal CD3⁺ T cells were reduced by an average of 14.9-fold, whereas dermal cells were reduced by an average of 4.7-fold. Marked reductions in HLA-DR expression by epidermal keratinocytes were also present in PUVA-treated psoriatic skin (Fig. 1, E and F, and Table 1), but constitutive expression of HLA-DR by epidermal Langerhans cells was not apparently affected. As HLA-DR is induced in keratinocytes by IFN- γ , which is synthesized by activated T-lymphocytes, its reduction might be caused by either immunosuppressive effects of PUVA on T-lymphocytes or direct effects of PUVA on HLA-DR production by epidermal cells.

A number of previous studies (36–41), most using oral dosing or higher topical psoralen concentrations, have suggested that PUVA treatment largely destroys epidermal Langerhans cells. However the focal expression of HLA-DR in PUVA bath-treated skin suggested the continued presence of Langerhans cells. Thus, we investigated the distribution of CD1⁺ (Langerhans) cells within nonlesional and lesional psoriatic epidermis before and after PUVA bath treatment (Fig. 2). In lesional psoriatic skin (Fig. 2 A), CD1⁺ cells were primarily located in the mid-to-upper spinous layer of the epidermis. No obvious decrease in density of epidermal CD1⁺ cells occurred in PUVA-treated lesional skin (Fig. 2 B). In two patients, comparison of pretreatment normal skin (Fig. 2 C) with posttreatment normal skin (Fig. 2 D) showed a marked reduction in epidermal Langerhans cells. However, in normal skin of all other patients studied, there was little difference in the distribution of CD1⁺ epidermal cells in pre- vs. posttreatment normal skin (Fig. 2, E and F). This analysis, while not rigorously quantitative, certainly suggests that low psoralen concentrations employed in PUVA bath therapy do not uniformly reduce the abundance of epidermal Langerhans cells. Thus, there was no consistent relationship between the abundance of epidermal Langerhans cells and either psoriatic disease activity or induction of disease remission.

Effects of PUVA Therapy on Epidermal Parameters. PUVA therapy also strongly modulates the psoriatic epidermal phenotype. Pathological features of psoriatic epidermis include: (a) increased keratinocyte proliferation (hyperplasia); (b) a tremendously thickened (acanthotic) epidermis with elongated rete; (c) an altered sequence of keratinocyte differentiation called “the alternate differentiation pathway” or “regenerative maturation,” which is associated with altered transcription of numerous keratinocyte genes; and (d) an altered epidermal structure which includes an absent granular layer, retention of keratinocyte nuclei in the stratum corneum (parakeratosis), and focal accumulation of leukocytes within the stratum

Table 1. Bath PUVA Decreases Immune and Epidermal Keratinocyte Activation in Psoriatic Plaques

Patient	Altered Expression after Bath PUVA Treatment (cells/high-power field [hpf])													
	IL-2R			CD-3			CD-4			CD-8			HLA-DR	
	E	DEJ	D	E	DEJ	D	E	DEJ	D	E	DEJ	D	E	E
1	Normal	0.0	1.7	1.0	1.0	16.3	16.6	0.7	11.3	9.3	4.3	7.0		
	Pre	6.3	22.7	17.7	16.0	64.0	61.0	3.0	27.3	29.0	38.3	34.3		
	Post	0.3	1.8	1.3	1.3	14.3	11.3	0.3	11.3	3.3	5.3	7.3	↕	↕
2	Normal	0.0	0.0	0.0	0.7	3.0	24.3	0.7	3.3	18.3	0.3	6.0		
	Pre	9.3	20.3	9.0	26.7	71.7	75.0	12.0	51.0	56.7	15.7	22.3		
	Post	0.0	0.0	0.7	0.0	2.7	19.3	0.3	1.7	16.6	0.0	4.7	↕	↕
3	Normal	0.7	0.7	1.3	0.0	3.6	12.7	0.0	1.7	3.0	2.0	10.6		
	Pre	8.3	20.7	19.0	11.3	101.3	101.3	3.6	72.0	62.7	8.3	40.0		
	Post	0.0	0.0	0.6	0.0	0.7	0.7	0.0	0.3	0.3	0.0	0.3	↕	↕
4	Normal	0.3	1.3	0.3	N/A	N/A	N/A	2.0	3.3	N/A	3.3	3.6		
	Pre	13.0	29.7	23.7	39.3	42.0	91.3	21.7	78.7	67.0	18.3	25.3		
	Post	5.3	11.7	7.3	14.3	21.6	39.0	6.3	21.7	27.0	9.3	13.7	↕	↕
5	Normal	0.0	0.0	0.3	0.0	8.0	13.7	0.3	4.3	5.0	4.3	8.0		
	Pre	26.7	37.3	N/A	74.0	155.0	63.6	20.7	126.0	46.0	55.7	24.3		
	Post	0.3	0.7	N/A	1.0	15.6	23.3	0.3	12.7	18.3	0.7	6.3	↕	↕
6	Normal	0.3	1.0	0.0	25.0	31.0	46.7	24.0	26.3	38.7	5.7	7.0		
	Pre	12.3	34.3	21.3	46.7	140.0	74.7	57.0	102.0	64.3	16.7	26.3		
	Post	0.3	0.3	0.3	1.0	3.0	1.0	1.7	1.3	40.3	0.3	0.7	↕	↕
7	Normal	0.0	0.3	0.0	1.0	4.0	1.7	1.0	2.0	0.7	2.0	0.7		
	Pre	9.0	40.3	9.3	36.3	52.3	7.7	20.0	25.7	2.0	26.7	6.7		
	Post	1.7	0.7	0.3	0.7	4.3	1.7	0.3	1.3	1.0	3.3	1.0	↕	↕
8	Normal	0.3	0.3	1.3	1.0	6.3	24.0	0.7	2.7	18.3	4.0	6.0		
	Pre	2.3	15.7	36.7	23.3	34.3	59.0	10.7	20.3	40.0	12.3	18.7		
	Post	0.3	0.3	0.3	0.0	0.7	16.3	0.0	0.7	15.3	0.0	1.0	↕	↕

continued

Table 1. (continued)

Patient	Altered Expression after Bath PUVA Treatment (cells/high-power field [hpf])													
	IL-2R			CD-3			CD-4			CD-8			HLA-DR	
	E	DEJ	D	E	DEJ	D	E	DEJ	D	E	DEJ	D	E	E
9*	Normal	0.0	0.3	0.7	0.0	4.0	1.3	0.3	3.3	0.7	0.3	0.3	0.3	0.7
	Pre	3.0	5.0	5.0	9.0	49.7	7.0	6.7	21.0	1.7	3.3	30.0	30.0	5.7
	Post	0.0	2.0	9.7	1.3	30.7	9.3	1.3	26.7	5.3	0.3	4.0	4.0	4.7
10†	Normal	0.3	0.3	0.3	0.7	27.0	22.3	0.3	19.0	8.3	0.3	9.7	9.7	14.0
	Pre	4.0	22.3	14.7	16.0	104.7	78.3	7.0	78.7	52.7	10.0	28.0	28.0	25.7
	Post	8.0	21.3	14.0	63.3	127.7	109.0	35.3	68.0	50.7	30.0	57.3	57.3	58.7
	Mean	0.2 ± 0.23	0.9 ± 0.9	0.6 ± 0.7	3.0 ± 7.8	11 ± 10	17 ± 13	3.0 ± 7.4	7.7 ± 8.5	11 ± 11	0.5 ± 0.7	3.6 ± 2.8	6.4 ± 4.1	6.4 ± 4.1
	± SD	9.3 ± 6.7	25 ± 10.2	17 ± 9	29 ± 19	83 ± 44	71 ± 42	16 ± 16	60 ± 37	42 ± 24	13 ± 4.7	28 ± 7.5	23 ± 11	23 ± 11
	Post	1.5 ± 2.6	4.9 ± 7.5	5.0 ± 6.0	7.8 ± 19	25 ± 37	27 ± 33	4.6 ± 11	15 ± 21	18 ± 17	4.2 ± 9.5	10 ± 18	10 ± 18	10 ± 18
	<i>p</i> -Values† (pre vs. post lesional)	0.0125	0.0007	0.0098	0.0542	0.0140	0.0108	0.0645	0.0055	0.0044	0.0645	0.0371	0.0645	0.0645
	% Reduction	~80%	~80%	~70%	~70%	~70%	~60%	~70%	~80%	~60%	~70%	~70%	~70%	~60%
	No. of patients who improved	9/10	8/10	8/10	8/10	8/10	8/10	8/10	8/10	8/10	9/10	9/10	9/10	9/10

* Patients 9 and 10 showed a poor clinical response to therapy.

† Statistics were performed using the Parametric *t* Test and Wilcoxon Sign Rank Test.

D, dermis; DEJ, demo-epidermal junction; E, epidermis; N/A, not available; ↓↓, markedly decreased.

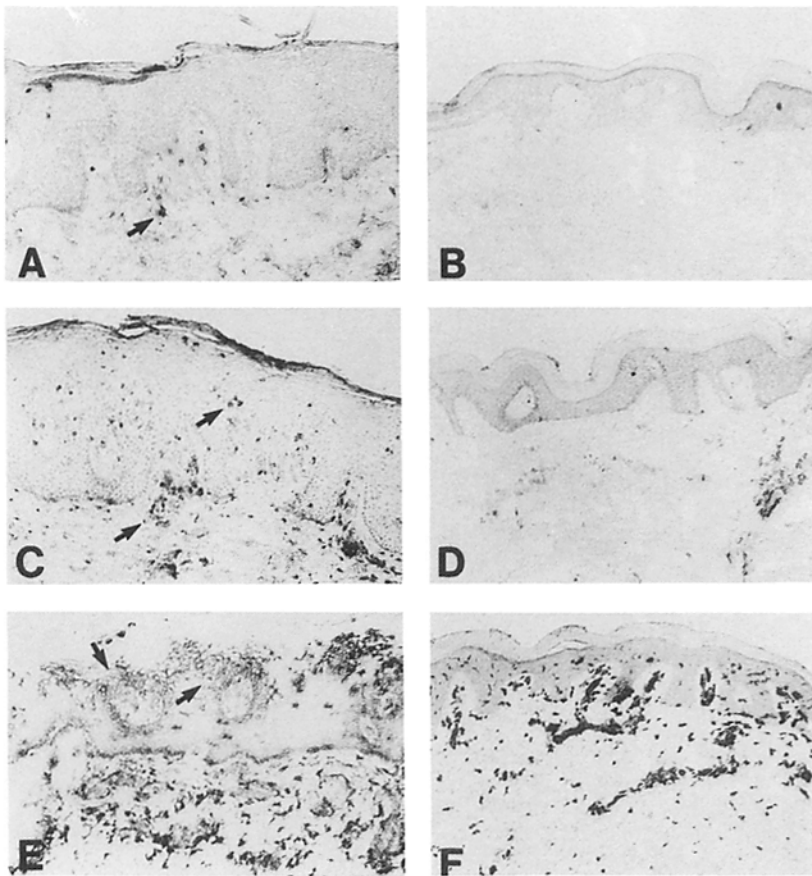


Figure 1. PUVA therapy suppresses immunological activation in psoriatic lesional skin. The expression of IL-2R⁺ T cells (A and B), CD3⁺ T cell (C and D), and HLA-DR molecules (E and F) is shown in pretreatment psoriatic lesional skin (A, C, and E) vs. posttreatment skin (B, D, and F). Note thinner epidermis after PUVA treatment and reduced numbers of CD3⁺ and IL-2R⁺ lymphocytes in PUVA-treated psoriatic lesions, as well as absence of keratinocyte HLA-DR staining. $\times 200$.

corneum (microabscesses). We thus examined the effect of PUVA therapy on these epidermal parameters, which combined with T-lymphocyte infiltration, define psoriasis as a unique disease entity.

Psoriatic epidermal acanthosis is strongly reversed by PUVA therapy. Overall epidermal thickness and the thickness of living epidermal layers (stratum Malpighi) and the stratum corneum were measured in histological sections of normal and lesional psoriatic skin before and after PUVA therapy (Fig. 3). There was an overall reduction of epidermal acanthosis in lesional psoriatic skin by 58%. Thinning of both the stratum Malpighi and stratum corneum occurred in psoriatic lesional skin (Fig. 3). PUVA treatment of lesional psoriatic skin also led to return of a granular layer, restoration of keratinocyte maturation with nuclear elimination (orthokeratotic maturation), and elimination of neutrophils from the stratum corneum. In contrast, PUVA treatment of nonlesional skin led to a slight overall increase in epidermal thickness (Fig. 3). It is interesting to note that this slight acanthosis could all be attributed to a statistically significant increase in the thickness of the stratum corneum ($p < 0.025$). Histological sections showing this effect of PUVA therapy on nonlesional skin can be seen in Fig. 2, C and D.

In active psoriatic skin, keratinocyte differentiation is shifted into an alternate or regenerative pathway. As epidermis can display a regenerative phenotype without showing gross

histopathological alterations (2, 34), we sought to determine whether PUVA therapy reversed regenerative epidermal activation in psoriasis. Three markers of regenerative maturation were studied: K16, expressed only in hyperplastic epidermis, and the differentiation-related proteins filaggrin and involucrin, expressed by granular layer keratinocytes in normal epidermis and lower spinous-to-granular layer keratinocytes in regenerative epidermis (2). Micrographs depicting expression of the regenerative markers in lesional psoriatic skin before and after PUVA therapy are displayed in Fig. 4. In active, lesional psoriatic skin, K16 is strongly expressed throughout the stratum Malpighi (Fig. 4 A); involucrin and filaggrin are expressed by keratinocytes from lower spinous-to-granular keratinocytes (Fig. 4, C and E). In lesional psoriatic skin exposed to PUVA therapy, the expression of each of these proteins is altered. K16 expression by suprabasal keratinocytes is eliminated (Fig. 4 B). The staining of basal and acrosyringeal keratinocytes with K16 antibodies in posttreatment skin (Fig. 4 B) also occurs routinely in normal epidermis and is due to an apparent cross-reaction of these antibodies with basal-specific keratins (2). The expression of involucrin (Fig. 4 D) and filaggrin (Fig. 4 F) in spinous keratinocytes is eliminated and these proteins are expressed by granular layer keratinocytes, as in normal skin. Table 2 summarizes the effects of PUVA therapy on regenerative maturation markers in lesional psoriatic skin for each patient studied. Although PUVA

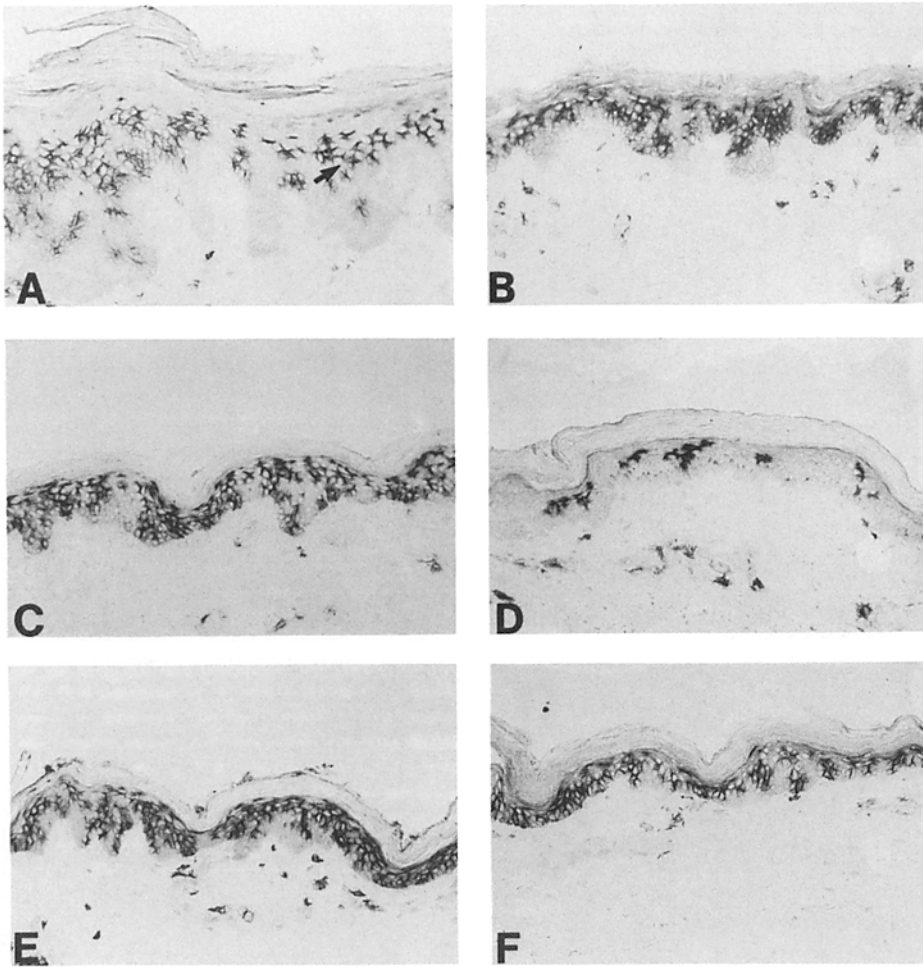


Figure 2. Effect of PUVA therapy on Langerhans cell distribution in non-lesional and lesional psoriatic epidermis. Pretreatment skin tissue (A, C, and E) is paired with post-PUVA treated tissue (B, D, and F) from the same patient. All have been reacted with CD1 antibodies. Biopsies from lesional psoriatic skin (A and B); biopsies from uninvolved areas of skin (C-F). $\times 200$.

altered keratinocyte differentiation in nonlesional skin, it did not activate alternative or regenerative epidermal maturation (data not shown).

Active psoriasis is also characterized by epidermal hyper-

plasia. In lesional psoriatic epidermis, a higher fraction of germinative keratinocytes is proliferating and the cell cycle length is dramatically shortened compared with nonlesional or normal skin (1). We assessed the ability of PUVA bath

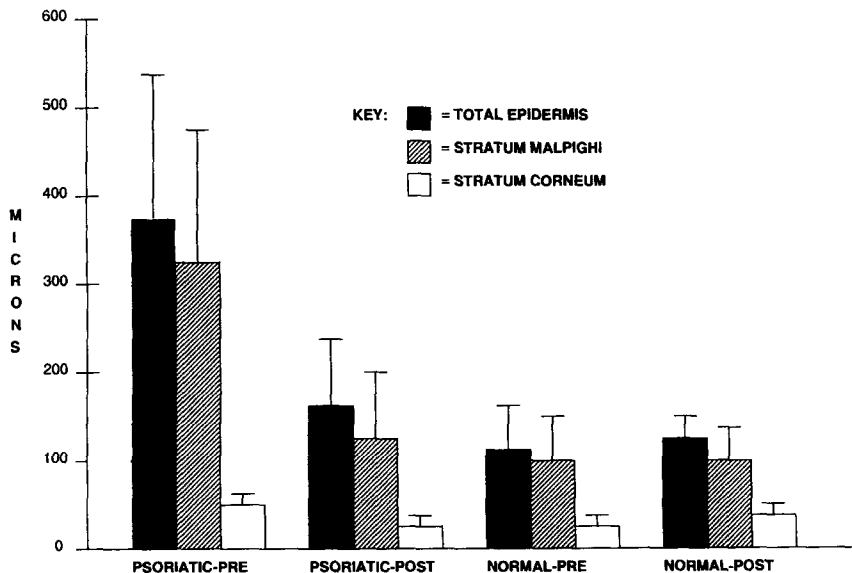


Figure 3. PUVA therapy reverses epidermal acanthosis in lesional psoriatic skin. Thickness of entire epidermis (solid bars), thickness of stratum Malpighi or living epidermal layer (hatched bars), and thickness of stratum corneum (open bars) was measured in histological sections of lesional and uninvolved psoriatic skin before and after PUVA treatment. Error bars show standard deviation of measurements.

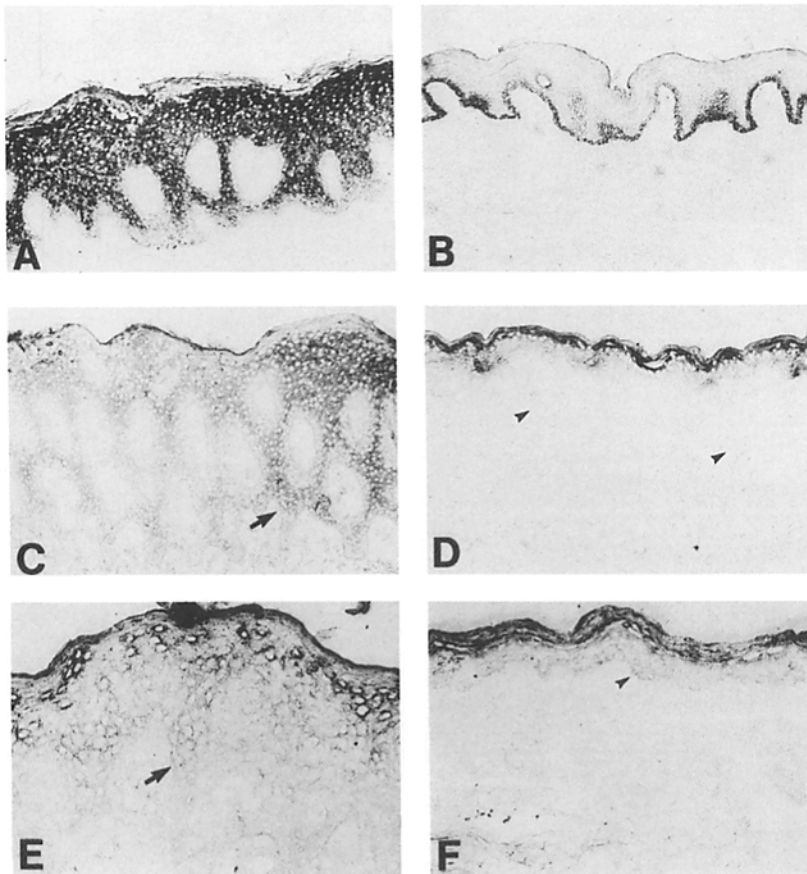


Figure 4. PUVA therapy reverses regenerative phenotype of lesional psoriatic skin. Pretreatment skin biopsies (A, C, and E) are compared with posttreatment skin biopsies (B, D, and F) from the same patient. Reaction with antibodies to K16 (A and B), involucrin (C and D), and filaggrin (E and F) are shown. (D and F) Arrowheads mark the epidermal-dermal junction (note that involucrin and filaggrin are expressed in the granular layer of the epidermis in PUVA-treated skin, whereas they are expressed by lower spinous keratinocytes in psoriatic lesions before treatment). As in normal skin, K16 is not expressed by suprabasal keratinocytes in PUVA-treated psoriatic lesions. Thus keratin expression and epidermal differentiation in PUVA-treated psoriatic epidermis are like those found in normal human skin. $\times 200$.

Table 2. Bath PUVA Decreases Keratinocyte Growth Activation

Patient	Altered Expression after Bath PUVA*						
	K16	Involucrin	Filaggrin	IGF-1R	TGF- α	EGF-R	IL-6
1	NL	\downarrow RM	NL	NL	U	U	U
2	NL	NL	NL	NL	U	U	U
3	NL	NL	NL	NL	U	U	U
4	\downarrow RM	NL	NL	\downarrow RM	U	U	U
5	NL	NL	NL	\downarrow RM	U	U	U
6	NL	\downarrow RM	NL	NL	U	U	U
7	NL	\downarrow RM	NL	NL	\uparrow	U	U
8	NL	NL	NL	NL	\downarrow	U	U
9	NL	NL	NL	NL	\uparrow	U	U
10	U	U	U	U	U	U	U
No. patients							
Improved	9/10	9/10	9/10	9/10	1/10	0/10	0/10

* Improved is defined as a reversion to, or a significant trend towards the phenotype seen in NL or nonlesional skin. NL, normal expression; \downarrow RM, a trend towards NL expression with persistent evidence of RM; U, no change; \uparrow , increase; \downarrow , decrease.

therapy to reverse epidermal hyperplasia using nuclear staining with Ki67 antibody. The Ki67 protein is a proliferating cell nuclear antigen that is synthesized by cells in mid-S phase, continues to be expressed at high levels throughout G2 and M phases, and is degraded rapidly upon cell entry into G1 (42). Fig. 5, *A* and *B* illustrates representative micrographs of pre- and posttreatment psoriatic lesional skin reacted with Ki67 antibodies. Ki67 antibodies react with numerous basal and immediately suprabasal keratinocytes in active psoriatic skin (Fig. 5 *A*) in a pattern that is virtually identical to *in situ* labeling with [³H]thymidine (1). With PUVA treatment, there is a reduction in the density of Ki67⁺ keratinocyte nuclei and positive cells reside largely in the basal epidermal layer (Fig. 5 *B*), similar to nonlesional skin or skin from normal controls (data not shown). Fig. 6 presents a quantitative analysis of the Ki67⁺ nuclei in pre- and posttreatment lesional psoriatic skin for all patients responding to PUVA bath therapy. The number of Ki67⁺ cells was reduced in each patient after PUVA treatment, with a mean reduction of 73% ($p = 0.004$).

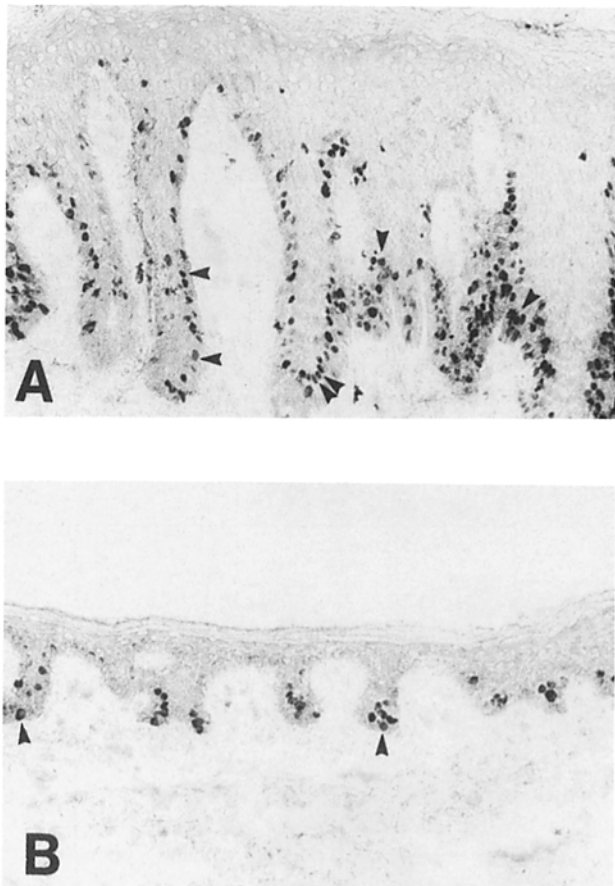


Figure 5. PUVA therapy reduces expression of the Ki67 proliferating cell nuclear antigen in lesional psoriatic skin. Pretreatment lesional skin (*A*) is contrasted with posttreatment skin from a resolved lesional area (*B*). Note the appearance of numerous cycling keratinocytes in basal and suprabasal psoriatic epidermis before treatment and the restoration of cycling keratinocytes largely to the basal layer after PUVA treatment. $\times 200$.

Even so, the number of Ki67⁺ keratinocytes in some post-treatment specimens was higher than in uninvolved psoriatic skin controls (Fig. 6). The effects of PUVA therapy on cell cycle length could not be calculated from these data, but if it also normalized this parameter, then the overall rate of keratinocyte production in posttreatment skin might be reduced by 15-fold or more compared with pretreatment lesional skin.

Several growth factor or cytokine pathways are altered in lesional psoriatic skin and have been proposed as molecular regulators of psoriatic epidermal hyperplasia (10, 14, 31, 33, 43). Accordingly, we sought to determine the relationship between expression of these putative growth-regulating pathways and reversal of epidermal hyperplasia using PUVA therapy. IGF-1 receptors are expressed by basal keratinocytes in normal skin, but both basal and suprabasal keratinocytes in lesional psoriatic epidermis express membranous IGF-1 receptors (Fig. 7 *A*), coincident with the increased keratinocyte proliferative pool in psoriasis (1, and Fig. 5 *A*). Membranous IGF-1 receptor expression was restored to the basal epidermal compartment in lesional skin treated with PUVA (Fig. 7 *B*). In contrast, EGF receptors were expressed in a membranous pattern by most basal and spinous keratinocytes in psoriatic skin pre- and post-PUVA treatment (Fig. 7, *C* and *D*). Lesional psoriatic skin also typically expresses high levels of immunoreactive TGF- α and IL-6, two cytokines that can serve as keratinocyte mitogens. However, little reduction in expression of these proteins was seen in lesional psoriatic epidermis after PUVA treatment (Table 2).

Antiproliferative Effects of PUVA on Cultured Human Lymphocytes and Keratinocytes. As PUVA therapy produced pro-

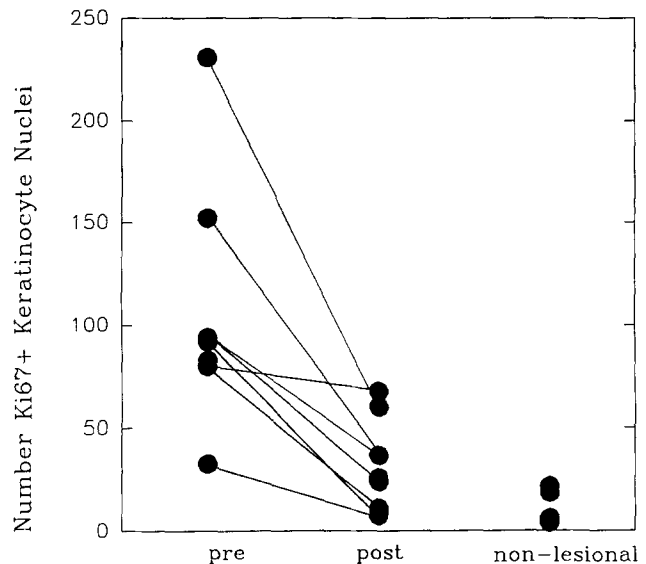


Figure 6. Quantitative analysis of Ki67 nuclear staining in psoriatic lesional skin before and after PUVA treatment. Lines connect measurements made in individual patients in pre- vs. posttreatment lesional skin for all patients responding to therapy. The measurement of Ki67 in uninvolved skin from six patients is also shown. Each measurement is the number of Ki67⁺ keratinocyte nuclei in 600 μm of epidermis (a linear measurement made across the width of the spinous epidermal layer).

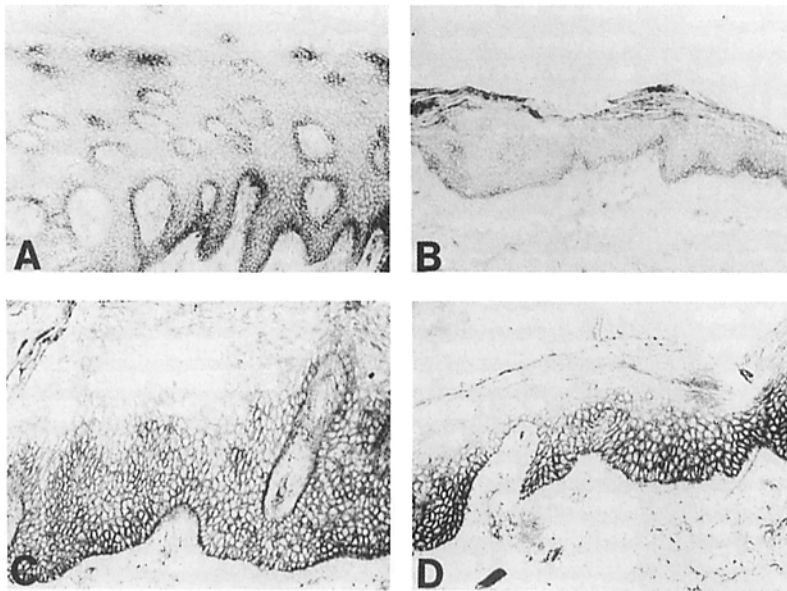


Figure 7. Effects of PUVA therapy on expression of IGF-1 and EGF receptors by keratinocytes in lesional psoriatic epidermis. Skin tissue before therapy (*A* and *C*) and after treatment (*B* and *D*) was reacted with anti-IGF-1 receptor (*A* and *B*) or anti-EGF receptor (*C* and *D*) mAbs. IGF-1 receptors are expressed at the cell surface by basal and numerous suprabasal keratinocytes in psoriatic epidermis, whereas only basal keratinocytes express these receptors at the cell surface after PUVA treatment. In contrast, numerous basal and suprabasal keratinocytes express EGF receptors at the cell surface before and after PUVA-treatment. $\times 400$.

found effects on both epidermal keratinocytes and T-lymphocytes in psoriatic tissue, we compared the ability of PUVA to suppress growth of these cell types in vitro. As shown in Fig. 8, *A* and *B*, a 15-min incubation of cultured cells with 8-MOP concentrations between 1 and 50 ng/ml followed by exposure to 2 J/cm² of UVA light (from which UVB had been removed by a band-pass filter), produced a dose-dependent reduction in proliferation of both cell types. Maximal growth inhibition was achieved at 24 h after treatment with 10 ng/ml 8-MOP in these cell types, with a similar dose-response to varying psoralen concentrations. For our patient study, the bath solution contained 500 ng/ml of 8-MOP and UVA doses of 5–15 J/cm² were typical. In another study (44) which used a slightly lower 8-MOP concentration (400 ng/ml), typical 8-MOP levels in the epidermis ranged between 25 and 66 ng/g of tissue after bath application. It can thus be seen that proliferating epidermal keratinocytes and T-lymphocytes are strongly inhibited by psoralen and light concentrations that are easily achieved in vivo in human epidermis. Accordingly, it seems likely that both cell types would be direct targets of PUVA therapy in psoriatic skin. Our measurements indicate somewhat greater sensitivity of mitogen activated human lymphocytes to the effects of 8-MOP and UVA light than a previous study (45) in which complete inhibition of [³H]thymidine in lymphocytes was produced by 100 ng/ml of psoralen followed by treatment with 3 J/cm² UVA.

Relapse of Psoriasis after Bath PUVA Therapy. Fig. 9 illustrates the rate at which psoriatic plaques return to baseline intensity of thickness, scaling, and erythema (and also cover a significant percentage of the pretreatment body surface area). For most patients treated with bath PUVA, treated psoriasis lesions generally remain flat and clear for weeks to months

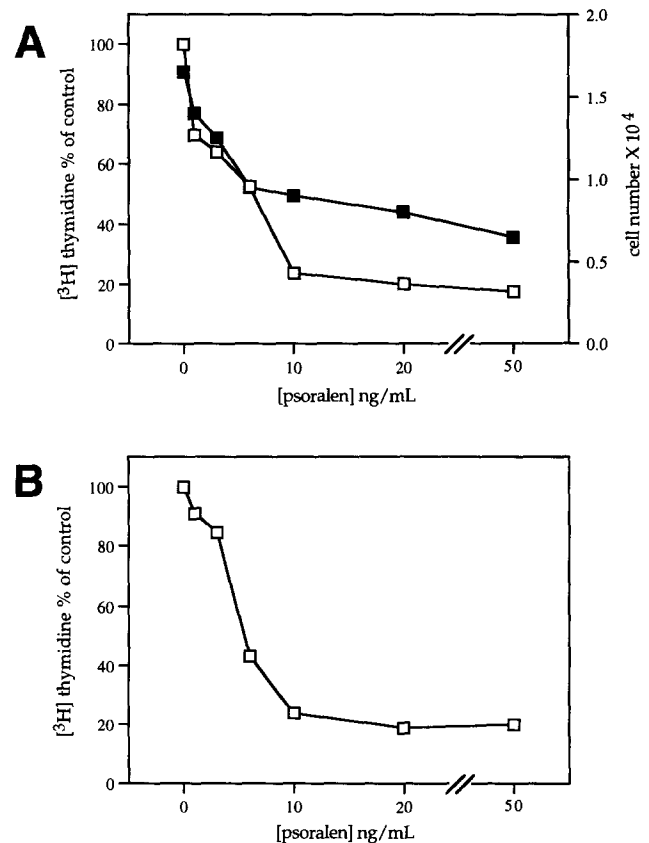


Figure 8. Antiproliferative effects of PUVA on human keratinocytes and lymphocytes in culture. (*A*) The response of keratinocytes to varying concentrations of 8-MOP and irradiation with 2 J/cm² of UVA was assessed by [³H]thymidine incorporation (□) or by direct cell counting (■). (*B*) A parallel experiment using mitogen-activated human lymphocytes.

following discontinuation of therapy, with usual recurrence as small papules that enlarge gradually over weeks or months to become clinically significant large plaques of psoriasis. 4 mo after discontinuation of therapy, about half of the treated patients were free from clinically significant psoriasis and some patients had resolution lasting for a year or more (Fig. 9). In sharp contrast, when cyclosporine was discontinued after attaining clinical clearing to the same extent as bath PUVA treatment, most patients had return of active, large plaques of psoriasis (at sites of prior disease) within 2 to 3 wk (Fig. 9). In many of these patients, psoriasis has returned fully to its baseline extent by 4 wk after discontinuation from cyclosporine. For patients whose psoriasis was treated to clinical clearing with the Goeckerman protocol (UVB and tar), we observed a relapse rate between that seen with cyclosporine and bath PUVA (Fig. 9). The ability of cyclosporine vs. bath PUVA treatment to produce significantly different therapeutic outcomes may be strongly based in the ability of each of these therapies to variably suppress cellular activation in psoriatic lesions and is discussed more fully below.

Discussion

In this study we found that 8 of 10 psoriatic patients treated with topical PUVA, administered as a diluted bathwater solution, responded with virtual clearing of their psoriasis. Although we used a lower psoralen concentration than a prior study (0.5 vs. 3.7 mg/liter), our clinical response rate was similar to both that study (24) and to the general population of patients treated with PUVA via systemic dosing (46–48).

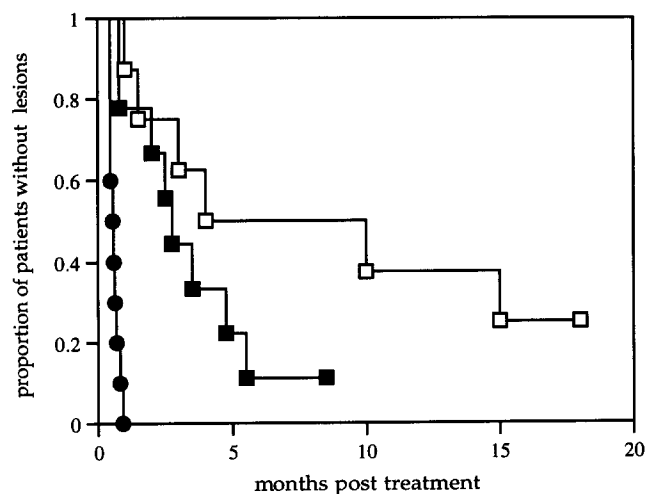


Figure 9. Recurrence of psoriasis after PUVA treatment in comparison with other therapies. Psoriasis was cleared in patients with comparable disease activity by cyclosporine treatment for at least 8 wk (●), by treatment with bath PUVA (□), or by an inpatient Goeckerman treatment with UVB light and topical tar treatment (■). All patients began follow-up with visually resolved psoriatic lesions. For each treatment, the time interval to relapse of clinically significant psoriatic plaques is shown. Cyclosporine provides a very transient benefit upon discontinuation, whereas weeks-to-months of clear skin is produced by bath PUVA treatment.

However, the major purpose of this investigation was to systematically study the response of activated epidermal and immunological cells to PUVA in a large group of patients.

Epidermal changes in psoriatic skin are critical to the altered appearance of the skin and to its pathological basis. The thickened psoriatic epidermis is caused in large part by hyperproliferative keratinocytes that transit from the basal epidermal layer to the stratum corneum in reduced time compared to normal skin (17). The differentiation sequence of psoriatic keratinocytes is along the alternate or regenerative pathway and is thus highly related, if not identical, to epidermal changes occurring in repair of acute wounds (2). Several cytokine pathways that control keratinocyte proliferation *in vitro*, e.g., those related to IGF-1, EGF, and IL-6, show increased expression in psoriatic epidermis (10, 16) and may thus control keratinocyte proliferation in activated epidermis. In particular, there appears to be a close spatial relationship between increased IGF-1 receptors in suprabasal spinous keratinocytes and increased proliferation of keratinocytes in these cells in psoriatic epidermis (cf. Figs. 5 A and 7 A). PUVA treatment dramatically reduces the number of cycling keratinocytes in psoriatic epidermis, and restores keratinocyte proliferation to the basal epidermal layer, changes which parallel reduced IGF-1 receptor expression by suprabasal keratinocytes. These observations suggest that the IGF-1 receptor may be particularly important to controlling keratinocyte proliferation in psoriasis, since comparatively few changes were seen in EGF receptor expression or IL-6 in PUVA-treated skin.

It is also noteworthy that molecular and cellular markers of regenerative epidermal differentiation in psoriasis were reversed by PUVA treatment. Thus the altered expression of filaggrin, involucrin, and K16, as well as the relative absence of a granular epidermal layer and the presence of parakeratosis (keratinocyte maturation with retained nuclei), features that largely define the histopathological psoriatic phenotype, must all be considered as conditional and reversible. As PUVA treatment strongly suppresses keratinocyte proliferation in culture (Fig. 8), an important effect of PUVA on psoriatic keratinocytes might be to diminish proliferation and, thereby, allow for slower and more orderly epidermal differentiation. PUVA treatment may also directly modulate keratinocyte differentiation in psoriatic epidermis, since it induces synthesis of a number of spinous layer differentiation proteins in cultured keratinocytes (49). The thicker stratum corneum in PUVA-treated skin presumably reflects conversion of large numbers of regenerating keratinocytes into a slower and more ordered differentiation pathway. The net effect of PUVA on keratinocyte proliferation and differentiation in psoriatic epidermis may then be to convert a relatively large pool of rapidly cycling, largely undifferentiated keratinocytes (3, 50) into a smaller pool of slower cycling cells with appropriate differentiation, a conversion that would be most important in cycling suprabasal keratinocytes in order to restore cell proliferation to the basal epidermal layer.

The role of activated T-lymphocytes must also be considered in the pathogenesis of psoriasis and in the effects of therapy upon this disease. It is likely that T-lymphocyte-derived cytokines, particularly IFN- γ , induce expression of HLA-

DR, IP-10, and ICAM-1 in psoriatic keratinocytes (10, 16). Activated lymphocytes, monocytes, and dendritic cells produce a number of other cytokines such as IL-1, IL-6, and GM-CSF which are mitogens for epidermal keratinocytes under some circumstances (10, 16, 17, 51). It should also be noted that psoriasis is often associated with expression of certain HLA class I alleles, an association that implies a role for CD8⁺ lymphocytes in its pathogenesis (17). As noted in Table 1, 8 of 10 psoriatics displayed a predominance of CD8⁺ lymphocytes in the epidermis, whereas CD4⁺ lymphocytes predominated in the dermis. The ability of intraepidermal CD8⁺ lymphocytes to contribute injury-producing cytokines or to directly injure epidermal keratinocytes might link their presence in the epidermis with epidermal activation that is appropriate to an injury-response (wound healing) program. Our *in vitro* studies establish that PUVA treatment strongly suppresses the proliferation of activated lymphocytes (Fig. 8), a response that might account for elimination of lymphocytes from psoriatic epidermis and dermis by PUVA treatment. It is likely that the diminished production of HLA-DR by PUVA-treated psoriatic keratinocytes reflects diminished production of IFN- γ from lymphocytes infiltrating psoriatic tissue. Potentially more important, however, is the possibility that removal of intraepidermal CD8⁺ lymphocytes by PUVA treatment leads to removal of an activating stimulus for epidermal keratinocytes, an effect that might indirectly convert regenerative epidermal growth to normal growth.

Given the potentially critical, but somewhat uncertain, relationship between epidermal and immunological activation in psoriasis, it is important to compare the relative effects of cyclosporine and PUVA treatment on cellular activation and disease activity in this condition. In a prior study (27) we treated 10 recalcitrant psoriatics with oral cyclosporine and 8 of 10 displayed clinical clearing of their psoriasis comparable to that seen with bath PUVA in this study. Regenerative epidermal activation as assessed by K16 expression was incompletely suppressed in most cyclosporine-treated patients, whereas K16 expression was fully reversed in 8 of 10 PUVA-treated patients in this study. Bath PUVA treatment also decreased total T-lymphocytes and T cell subsets (CD4⁺, CD8⁺, and CD25⁺) in lesional skin to a greater extent than did cyclosporine (27). For example, in cyclosporine-treated psoriasis lesions which were biopsied at clinical resolution, an average of 42 CD3⁺ lymphocytes and 7 CD25⁺ lympho-

cytes were counted in the region of the epidermal-dermal interface. In bath PUVA-treated psoriasis, an average of eight CD3⁺ lymphocytes and two CD25⁺ lymphocytes were seen in this region. The difference in residual T-cell infiltration of psoriatic tissue treated with these two modalities is highly significant ($p = 0.00007$). The ability of PUVA treatment to more completely suppress T-lymphocyte infiltration of psoriatic tissue compared with cyclosporine was also seen in a recent study using oral 5-MOP (28). In fact, these authors suggested that a predominant action of cyclosporine in psoriasis might be directly on the epidermis, since only small reductions in tissue-infiltrating T-lymphocytes were produced by cyclosporine (28). However, no markers of epidermal activation were studied (28), and based on results of the present study it can be ascertained that cyclosporine less completely suppresses epidermal activation than PUVA in psoriatic lesions. Although cyclosporine does not decrease tissue-infiltrating T-lymphocytes to the same extent as PUVA, cyclosporine strongly suppresses production of IL-2 and other TH1-type cytokines in psoriatic lesions (52), which is the probable basis of its immunomodulatory activity in this disease. However, as numerous T-lymphocytes continue to be present in clinically suppressed psoriatic lesions during cyclosporine treatment, these cells could reactivate quickly upon discontinuation of cyclosporine. This is the probable basis for rapid return of psoriasis upon discontinuation of cyclosporine (Fig. 9), although epidermal activation above that seen in normal skin could also contribute to the rapid disease recurrence. In contrast, PUVA treatment produced nearly complete ablation of tissue-infiltrating lymphocytes in psoriatic skin, a result that could reflect cytotoxic action of PUVA on activated lymphocytes. Thus, return of lymphocytes to PUVA-treated psoriatic lesions would require proliferation and/or increased trafficking from central compartments, in addition to local activation in skin. This process would be considerably more complex, and presumably slower, than reactivation of tissue-resident lymphocytes in cyclosporine-treated lesions. Thus the differential effects of PUVA vs. cyclosporine on T-lymphocytes and immunological activation may underlie the observed temporal differences in recurrence of psoriasis after discontinuation of each therapy (Fig. 9). The ability to correlate overall therapeutic benefit in psoriasis with suppression of specific cell types and related molecular pathways may provide a rational scientific means to elucidate its pathogenesis more precisely and to design targeted therapy.

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