Studies of the Effect of Cyclosporine in Psoriasis In Vivo: Combined Effects on Activated T Lymphocytes and Epidermal Regenerative Maturation

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Cyclosporine (CSA) decreases lymphokine synthesis and keratinocyte proliferation in vitro, but its in vivo mechanism of action in treating recalcitrant psoriasis is incompletely understood. Ten psoriasis patients were treated with CSA (2–7.5 mg/kg/d) with clinical improvement in nine of 10 patients. Skin biopsies before and after 1–3 months of CSA treatment were studied for evidence of immune and keratinocyte activation using immunoperoxidase and Northern blotting analysis. The number of activated, IL-2 receptor$^+$ T cells in plaques after CSA treatment was reduced in all patients by a mean of 60%. Seven of 10 patients showed a decrease in keratinocyte HLA-DR expression; five of seven showed a decrease in gamma-IP-10 immunoreactivity, suggesting a decline in gamma interferon levels in plaques after CSA therapy.

We studied the effect of CSA treatment in vivo on TGF-α, IL-6, and keratin K16 expression, three markers of keratinocyte growth activation. Expression of keratinocyte TGF-α and IL-6, which are elevated in active psoriatic epidermis, did not change in these patients after CSA treatment. The majority of patients (five of eight) continued to express the hyperproliferative keratin K16 after CSA treatment. Our results suggest that the predominant direct mechanism of action of Cyclosporine in vivo is a diminution of T-cell activation in plaques, with attendant decreased lymphokine production. *J Invest Dermatol 98:302–309, 1992*

Psoriasis is a papulosquamous disease that affects approximately 5 million individuals in the United States. Hallmarks of psoriasis are epidermal hyperplasia and chronic inflammation in affected skin.

The increased frequency of certain mixed histocompatibility complex antigens (HLA), especially HLA-Cw6, in populations of psoriasis patients suggests that immune mechanisms may be important in the pathogenesis of psoriasis [1]. Active psoriatic plaques demonstrate a similar immunologic phenotype as do ongoing cellular immune responses: activated, IL-2 receptor$^+$, and HLA-DR$^+$ T cells are found in psoriatic plaques in significantly higher numbers than are found in uninvolved skin or in treated plaques [2–5]. Furthermore, epidermal keratinocytes in active plaques express HLA-DR, ICAM-1, and gamma-IP-10 proteins, which could directly affect leukocyte activation or trafficking in lesional skin [2–4,6–10]. Increased expression of these immune-related molecules by epidermal keratinocytes in active psoriasis is similar to their expression in cutaneous cellular immune reactions and may result from interferon gamma produced by activated lymphocytes [2–4, 6–10]. A centrol role for interferon gamma is further suggested by its ability to induce psoriatic plaques upon local injection in uninvolved skin [11].

Keratinocytes in psoriatic plaques also have an increased proliferation rate and display markers of regenerative growth identical to those in acute healing wounds [12–19]. One measure of this growth-related activation is the expression of keratin K16 by suprabasal keratinocytes in hyperplastic epidermis of lesional psoriatic skin [19]. Increased keratinocyte proliferation might be regulated by exaggerated expression of both transforming growth factor-alpha (TGF-α) and its receptor in lesional skin [12–16]. Alternatively, epidermal proliferation, as well as some features of immunologic activation, might be directly modulated by increased interleukin-6 (IL-6), which can serve as keratinocyte mitogen under some conditions [17]. Thus, determination of TGF-α, IL-6, and

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Abbreviations:
BSA: body surface area
CSA: cyclosporine
HLA: histocompatibility complex antigens
IL: interleukin
IL-2R: IL-2 receptor
NA: not applicable
ND: not determined
NL: normal
RM: regenerative maturation
SD: standard deviation
SI: severity index
TGF-α: transforming growth factor-α

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K16 keratin levels and distribution in psoriatic plaques gives a measure of growth-related keratinocyte activation in the epidermis.

Accumulated data suggest that both the inflammatory and epidermal components in psoriatic plaques are activated. However, it is not known if the epidermal hyperplasia is the result of the action of cytokines produced by activated T lymphocytes/accessory cells or if lymphocytes are non-specifically activated by cytokines produced by the abnormal keratinocytes [3]. The relative contributions of epidermal activation and immunologic activation to maintenance of active psoriatic lesions might be determined by examining the specific effects of cyclosporine (CSA) on these tissue types in lesional psoriatic skin. Acting through cyclophilin, a cellular CSA receptor expressed in lymphoid cells and keratinocytes, cyclosporine could potentially affect either epidermal or immunologic activation in psoriatic skin [20–34]. Cyclosporine inhibits proliferation of chronic inflammatory cells by diminishing transcription of cytokines such as IL-2 and interferon gamma [22,27,28,30,34]. Cyclosporine also inhibits proliferation of epidermal keratinocytes, arresting growth in the G_1 phase of the cell cycle, though the mechanism of this effect is not known [14,24–26].

In this paper we report that CSA treatment decreased expression of activated T cells and gamma interferon–induced proteins in psoriatic plaques, which showed clinical improvement after treatment. In contrast, markers associated with keratinocyte activation, e.g., elevated TGF-α, IL-6, and K16 keratin expression, were relatively resistant to CSA treatment. These data suggest that the predominant direct mechanism of action of CSA in psoriasis plaques in vivo is to decrease T-cell activation with resultant decreases in the production of lymphokines such as gamma interferon, rather than to decrease keratinocyte activation.

MATERIALS AND METHODS

Patients A protocol using CSA in doses ranging from 2.0–7.5 mg/kg/d in patients with recalcitrant psoriasis was approved by The Rockefeller University Hospital Institutional Review Board. Patients were initially screened with a panel of pre-treatment blood, urine, and skin tests to rule out pre-existing immunodeficiency, renal, hepatic, and cardiac disorders. Patients were at least 18 years of age and had biopsy-proven psoriasis. Patients did not receive treatment with methotrexate, retinoids, or ultraviolet irradiation for at least two weeks prior to or during CSA treatment. Patients with hypertension, positive HIV antibody test, active microbial infections, or history of malignancy, and women of child-bearing potential were excluded.

Patients were examined and photographed, and laboratory tests were performed on a weekly basis. Disease activity was assessed by quantitating percent body coverage by degrees of erythema, scaling, and skin thickness in the psoriatic plaques. Erythema, scaling, and skin thickness were quantitated 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, 7 = severe. The scores for erythema, scaling, and skin thickness were summed for a maximum obtainable score of 21. The sum was called the Severity Index. On each visit, blood pressure was assessed; weight measured; and blood tests for complete blood count, biochemistry profile, measurement of fasting cholesterol and triglyceride levels, CSA trough levels, urinalysis, and 24-h urine collection for protein and creatinine were performed. A total of 10 patients received CSA for a period ranging from 1–3 months depending upon clinical response. Skin biopsies of psoriatic plaques and uninvolved skin were obtained before and after CSA treatment. In some individuals, there was not enough material to assess every element of the immunoperoxidase and Northern blotting study panel. All patients treated were male, between the ages of 25 and 55.

Immunoperoxidase Studies Immunoperoxidase studies of fresh-frozen skin biopsies were done by using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) as described [2,3,6,12,17,35–37]. IL-2 receptor$^+$, CD3$^+$, CD4$^+$, and CD8$^+$ T cells were quantitated by averaging the number of positively staining cells in three ×40 fields. Statistics were performed using the Wilcoxon Rank Sum Test (two tailed). The antibodies directed against HLA-DR [2], the IL-2 receptor [2], the gamma interferon–induced protein, IP-10 [6], TGF-α [12], IL-6 [17], and K16 keratin (monoclonal antibody AE-1$^+$) [18] have been previously reported. Monoclonal antibody AE-1 was a generous gift from Dr. T.-T. Sun. Negative controls for these immunoperoxidase studies included matched isotype monoclonal antibodies with irrelevant antigenic specificities, the omission of the mouse monoclonal antibody or the rabbit antibody preparation, and, in the case of the rabbit antibody preparations, the pre-immune sera.

RNA Isolation and Analysis The RNA was extracted from 6-mm skin biopsies of psoriatic plaques and uninvolved skin or from cultured normal human foreskin keratinocytes grown in serum-free medium [17] with acid guanidinium thiocyanate–phenol-chloroform by the method of Chomczynski and Sacchi [38,39]. RNA concentration was determined by absorption at 260 nm. Equal quantities of RNA were size fractionated by electrophoresis in 1% agarose and then transferred to nitrocellulose [14]. The TGF-α probe was made by random priming with ^{32}P dCTP using the random primed DNA-labeling kit obtained from Amersham (Arlington Heights, IL). A full-length cDNA clone for TGF-α [40] was obtained from Dr. R. Derynck. A 40-base synthetic oligonucleotide of human beta-actin (Oncogene Science, Manhasset, NY) was 5′-end labeled with gamma[^{32}P]-ATP using T4 polynucleotide kinase (New England Biolaboratories, Beverly, MA). After hybridization with the probes, the membranes were washed and then autoradiographed on Kodak XAR film at −70°C using Cronex intensifying screens. The autoradiographic intensity was quantitated by densitometric scanning using an LKB Ultrascan XL Laser Densitometer (Bromma, Sweden).

RESULTS

Cyclosporine Treatment Decreases the Numbers of IL-2 Receptor$^+$ T Cells and Immunostaining Levels of Gamma-Interferon–Induced Proteins in Psoriatic Plaques CSA inhibits both lymphocyte and keratinocyte growth in vitro; however, the effects of CSA on human keratinocytes and lymphocytes in vivo are incompletely characterized. The goal of this study was to determine if the inflammatory (lymphocyte) and the hyperproliferative (keratinocyte) component of psoriatic plaques showed differential sensitivity to CSA administered in vivo in patients at comparatively low doses.

Ten severe psoriasis patients were treated with CSA at a relatively low dose range of 2–7.5 mg/kg/d for 1 to 3 months. Skin biopsies, obtained before and after treatment, were studied for evidence of immune activation and keratinocyte regenerative maturation (activation) using immunoperoxidase and Northern blotting techniques. Nine of ten patients had psoriasis vulgaris and patient 7 had erythrodermic psoriasis (Table I). The mean percent body surface area (%BSA) covered by psoriatic plaques in all patients before treatment was 60%. After CSA therapy, the mean %BSA was only 30%. Nine of ten patients showed clinical improvement in erythema, thickness, and scale as a result of treatment with CSA. The severity of psoriatic plaques was expressed as a Severity Index, which is the sum of the severity scores for erythema, skin thickness, and scale as outlined in the *Materials and Methods* section. The mean pre-treatment Severity Index for erythema, scaling, and thickness was 17.4 (maximal attainable score of 21) as compared with a mean index of only 5.4 in plaques from patients treated with CSA. Skin biopsies, obtained before and after CSA treatment, were studied for evidence of immune activation and features of epidermal growth activation or regenerative maturation using immunoperoxidase and Northern blotting techniques.

Active psoriatic plaques demonstrate a similar immunologic phenotype as do ongoing cellular immune responses: activated, IL-2 receptor$^+$, and HLA-DR$^+$ T cells are found in psoriatic plaques in significantly higher numbers than are found in uninvolved skin or in treated plaques [2,3]. In this study, the average number of activated IL-2 receptor$^+$ T cells per 40× microscopic field before treatment was 28 ± 27. The number of activated, IL-2 receptor$^+$ T cells

Table I. Cyclosporine Treatment Decreases Immune Activation More Than Keratinocyte Growth Activation

					Altered Expression After CSA Treatment[b,f]																
	%BSA[a]		SI[a]		IL-2R[c]				CD3			CD4			CD8						
					Cells/hpf		Percent		Cells/hpf		Percent	Cells/hpf		Percent	Cells/hpf		Percent				
Patient	Pre	Post	Pre	Post	Pre	Post	Change	HLA-DR	IP-10	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change	IL-6	TGF-α	AE-1
1	50	<10	19	6	47	10	−80	→	ND	179	62	−70	100	40	−60	100	20	−80	↑	U	RM
2	60	<25	18	3	15	10	−30	U	U	217	50	−80	91	30	−70	132	10	−90	↑	↑	RM
3	90	40	19	6	10	5	−50	U	→	100	44	−60	52	22	−60	75	33	−60	U	↑	RM
4	60	15	18	12	20	15	−30	→	→	119	43	−60	78	48	−40	25	8	−70	U	U	RM
5	50	30	16	3	15	8	−50	→	→	30	20	−30	ND	ND	ND	15	5	−70	U	↑	NL
6	70	30	18	6	20	10	−50	U	U	97	49	−50	ND	ND	ND	ND	ND	ND	ND	U	ND
7	95	50	19	9	5	0	−100	→	→	ND	ND	ND	ND	ND	ND	ND	ND	ND	→	U	ND
8[d]	50	75	18	21	57	28	−50	→	→	50	50	0	37	33	−10	30	30	0	U	U	RM
9	15	2	12	3	11	1	−90	→	ND	300	18	−90	178	50	−70	90	13	−90	U	U	NL
10	30	10	17	6	75	0	−100	→	ND	324	50	−80	137	42	−70	79	26	−70	↑	↑	NL
Mean ± SD[b]					28 ± 27	9 ± 8	−60 ± 30	NA	NA	157 ± 105	43 ± 15	−60 ± 15	96 ± 49	38 ± 10	−50 ± 22	68 ± 41	18 ± 11	−70 ± 30	NA	NA	NA
						$p = 0.01$					$p = 0.004$			$p = 0.007$			$p = 0.01$				
Number of patients showing improvement					10/10			7/10	5/7	7/9			5/7			7/8			0/7	0/8	3/8

[a] %BSA, percent body surface area; SI, severity index.
[b] U = unchanged; ↓ = decreased; ↑ = increased; ND = not determined; RM = regenerative maturation; NL = normal pattern of expression; NA = not applicable; SD = standard deviation.
[c] *IL-2R, IL-2 receptor.*
[d] Patient 8 refused doses of CSA above 4 mg/kg/d and did not clinically improve on lower doses.
[e] The patients were male between the ages of 28 and 55 years old with a mean age of 37 years.
[f] Statistics were performed using the Wilcoxon-Rank sum test (two tailed).

in plaques after CSA treatment was reduced by an average of 60% in all 10 patients (Table I). IL-2-receptor expression by lymphocytes in pre- and post-treatment plaques is illustrated for one patient in Fig 1. The number of IL-2 receptor$^+$ T lymphocytes was markedly decreased in the CSA-treated plaque as compared with the pre-treatment plaque. The average numbers of CD3$^+$, CD4$^+$, and CD8$^+$ cells in the inflammatory infiltrate prior to CSA treatment were 157 ± 105, 96 ± 49, and 68 ± 41, respectively. Decreases in the number of CD3$^+$, CD4$^+$, and CD8$^+$ T cells in excess of 50% after CSA treatment were observed in seven of nine, five of seven, and seven of eight patients, respectively. However, it should be noted that in all cases there were T cells still present in post-treatment plaques. The observed decrease in the number of IL-2 receptor$^+$ T cells after cyclosporine treatment was not solely the result of a decrease in the total number of T cells. Prior to CSA treatment, the average percentage of IL-2 receptor$^+$ T cells in the mononuclear inflammatory infiltrate was 16 ± 10. Compared to the total mononuclear cell infiltrate, the proportion of IL-2-receptor$^+$ T cells were diminished by 70%, whereas the total percentage of CD3$^+$ T cells were reduced by only 35%. These data suggest that there is a selective loss of IL-2 receptor$^+$ T cells in addition to a loss in total T-cell numbers in plaques as a result of CSA treatment.

Keratinocytes in active psoriatic plaques are HLA-DR$^+$ and IP-10$^+$, similar to the expression of these gamma interferon-induced proteins in ongoing cellular immune responses [2-4,6-10]. Seven of ten patients treated with CSA showed a decrease in keratinocyte HLA-DR expression and five of seven showed a decrease in IP-10 reactivity, suggesting a decline in gamma interferon levels in plaques after CSA therapy. This is illustrated for both HLA-DR and IP-10 in Fig 1. Most keratinocytes are HLA-DR$^+$ in the pre-treatment plaque. After CSA treatment, there is a decrease in the proportion of keratinocytes that are HLA-DR$^+$. HLA-DR staining of the mononuclear infiltrate was decreased to a lesser extent by CSA treatment than was keratinocyte HLA-DR expression. There was decreased IP-10 positivity in CSA-treated plaques that was most pronounced in the spinous keratinocytes (Fig 1). In six of seven patients concordance was observed between changes in HLA-DR and IP-10 expression after CSA treatment (Table I). In eight of ten patients decreases in the number of IL-2 receptor$^+$ T cells were accompanied by a concurrent decrease in HLA-DR and/or IP-10 expression. In patients 2 and 6, decreases in the number of IL-2-receptor$^+$ lymphocytes were observed without accompanying decreases in expression of HLA-DR and IP-10.

Cyclosporine Treatment Does Not Decrease the Expression of TGF-α and IL-6 in Psoriatic Plaques and Causes Only Patchy Decreases in K16 Keratin Expression In contrast, CSA treatment did not decrease the expression of most keratinocyte growth activation-associated proteins in psoriatic plaques. Increased TGF-α expression in psoriatic plaques has previously been demonstrated by immunohistochemistry [12] with monoclonal antibody A1.5, which is specific for TGF-α. This correlated with increased TGF-α mRNA detected in psoriatic plaques by Northern analysis [13,14].

TGF-α expression was previously studied in 18 psoriasis patients and in normal individuals using immunoperoxidase techniques [12]. In psoriatic plaques, intense membrane staining was apparent in basal and spinous keratinocytes. In contrast, in uninvolved skin from psoriasis patients and from unaffected individuals, membrane staining was most marked in only the basal layer. In all patients in the present study, TGF-α immunoreactivity remained elevated in psoriatic plaques after CSA treatment. Intense membrane staining was observed throughout the entire epidermis in both pre-treatment and CSA-treated plaques, as illustrated in Fig 2 (patient 2). In fact, TGF-α staining in this patient appeared to be more intense in the post-treatment plaque compared with the pre-treatment plaque. In contrast, intense membrane TGF-α staining was seen in only the basal layer of uninvolved skin from the same patient. These observations were extended in psoriatic plaques of patient 2 before and after CSA treatment using Northern blotting with a TGF-α-

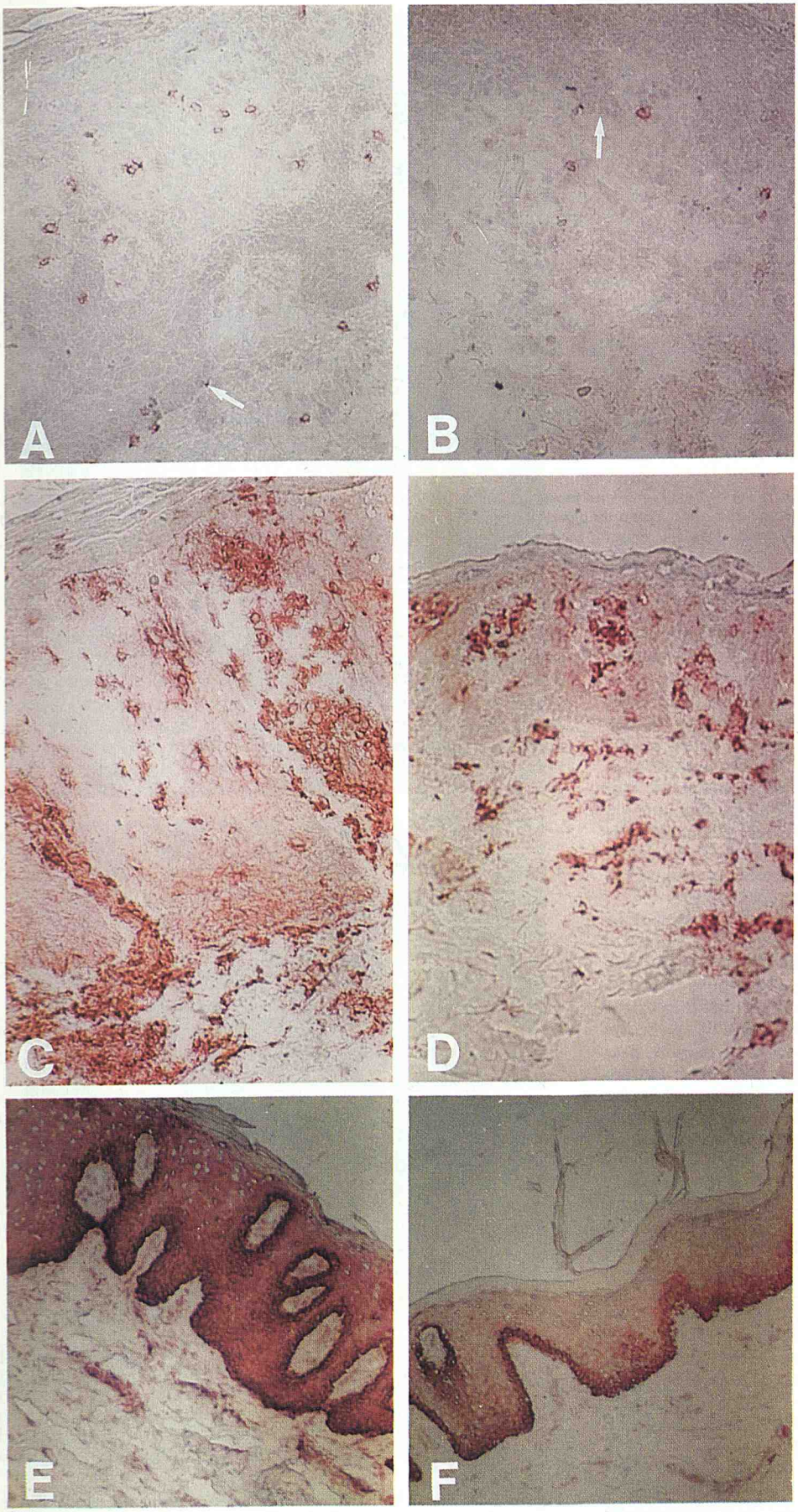

Figure 1. Immunoperoxidase reactivity of psoriatic plaques before and after cyclosporine treatment. The number of IL-2 receptor$^+$ T lymphocytes was markedly decreased in the CSA-treated plaque *(B)* as compared with the pre-treatment plaque *(A)*; magnification ×195. Most keratinocytes were HLA-DR$^+$ in the pre-treatment plaque *(C)*. After CSA treatment *(D)*, there was a decrease in the proportion of keratinocytes that were HLA-DR$^+$; magnification ×195. There was decreased IP-10 positivity in the CSA-treated plaque *(F)* as compared with the pre-treatment plaque *(E)*, which was most pronounced in the spinous keratinocytes; magnification ×97.5. *White arrow*, dermal-epidermal junction.

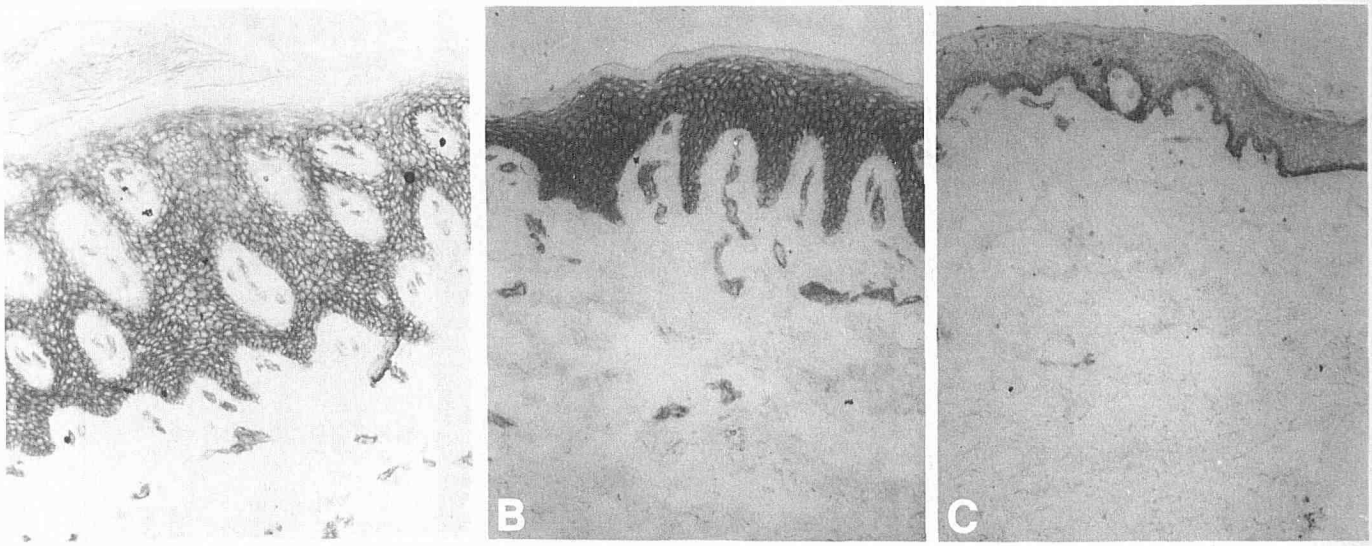

Figure 2. Immunoperoxidase staining of lesional and uninvolved skin from a psoriasis patient before and after cyclosporine treatment with an anti-TGF-α-specific monoclonal antibody; magnification ×94. Intense membrane staining was observed throughout the entire epidermis in both the pre-treatment *(A)* and CSA-treated plaque *(B)*. In contrast, intense membrane TGF-α staining was seen in only the basal layer of uninvolved skin from the same patient *(C)*.

specific probe (Fig 3). A single 4-kb mRNA species hybridizing with the TGF-α probe was detected in RNA isolated from cultured keratinocytes *(lane 1)* or psoriatic tissue (*lanes 2* and *3*). Densitometric analysis of hybridization signals from pre- and post-treatment RNA samples indicated no significant decrease in TGF-α mRNA after CSA treatment. Control hybridization with actin-specific probes (*lanes 5* and *6*) showed no significant difference in actin-specific mRNA between pre- and post-treatment plaques. Note that the immunohistochemical data in Fig 2 and mRNA data in Fig 3 were performed on biopsy material from the same plaque. Similar analysis of TGF-α mRNA expression in pre- and post-treatment psoriatic plaques in a second patient also showed no decrease in TGF-α expression after CSA treatment (data not shown).

Increased levels of Il-6 protein and mRNA have been demonstrated by us previously in psoriatic plaques. Because IL-6 promotes keratinocyte proliferation in vitro [17,41], it could potentially promote epidermal hyperplasia in psoriasis as an autocrine growth factor. The expression of IL-6 protein in psoriatic plaques (Fig 4) was not decreased by CSA treatment. IL-6 was expressed by essentially all keratinocytes, endothelial cells, fibroblasts, and mononuclear dermal infiltrate cells in active plaques before treatment. No decrease in IL-6 immunoreactivity in plaques was observed in the seven patients who were studied after CSA treatment. However, marked decreases in IL-6 immunoreactivity were observed in patients treated with conventional therapy [17].

Epithelial hyperplasia psoriasis is marked by expression of keratins (K6, K16) found only in hyperproliferative epidermis (undergoing "regenerative maturation") [19]. The hyperplastic growth activation in psoriatic epidermis is readily detected with the AE-1 monoclonal antibody to acidic keratins, which detects expression of K16 in suprabasal keratinocytes of growth-activated epidermis. K16 (monoclonal antibody AE-1$^+$) keratin is expressed throughout the suprabasal epidermis of active psoriatic plaques, whereas AE-1 reactivity is confined to the basal layer of normal epidermis or non-lesional skin of psoriatics. Normalization of K16 keratin expression was seen in only three of eight patients after CSA treatment. In the remaining five patients, suprabasal keratinocyte expression of K16 keratin was still evident after treatment (Fig 5).

DISCUSSION

Both the inflammatory and epidermal components in psoriatic plaques are activated and produce cytokines that could contribute to ongoing cellular activation in affected tissues. However, it is not known if the epidermal keratinocyte hyperplasia is the result of the action of cytokines produced by activated T lymphocytes and other white blood cells, or if lymphocytes are nonspecifically activated by cytokines produced by abnormal keratinocytes in psoriatic plaques. Because CSA affects both lymphocyte and keratinocyte growth in vitro and the effects of CSA on human keratinocytes in vivo are

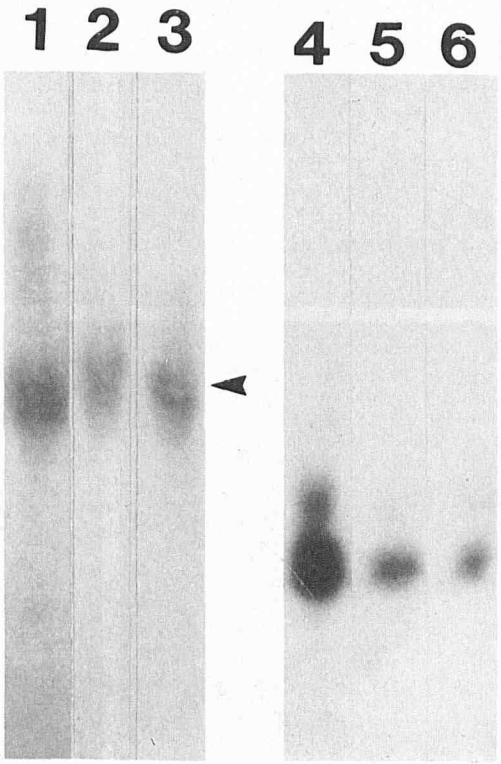

Figure 3. Northern blotting analysis of psoriatic plaques before and after cyclosporine treatment using TGF-α *(lanes 1–3)* and actin-specific cDNA probes *(lanes 4–6)*. *Lanes 1* and *4*: cultured normal human keratinocytes. *Lanes 2* and *5*: pre-treatment plaque. *Lanes 3* and *6:* post-treatment plaque.

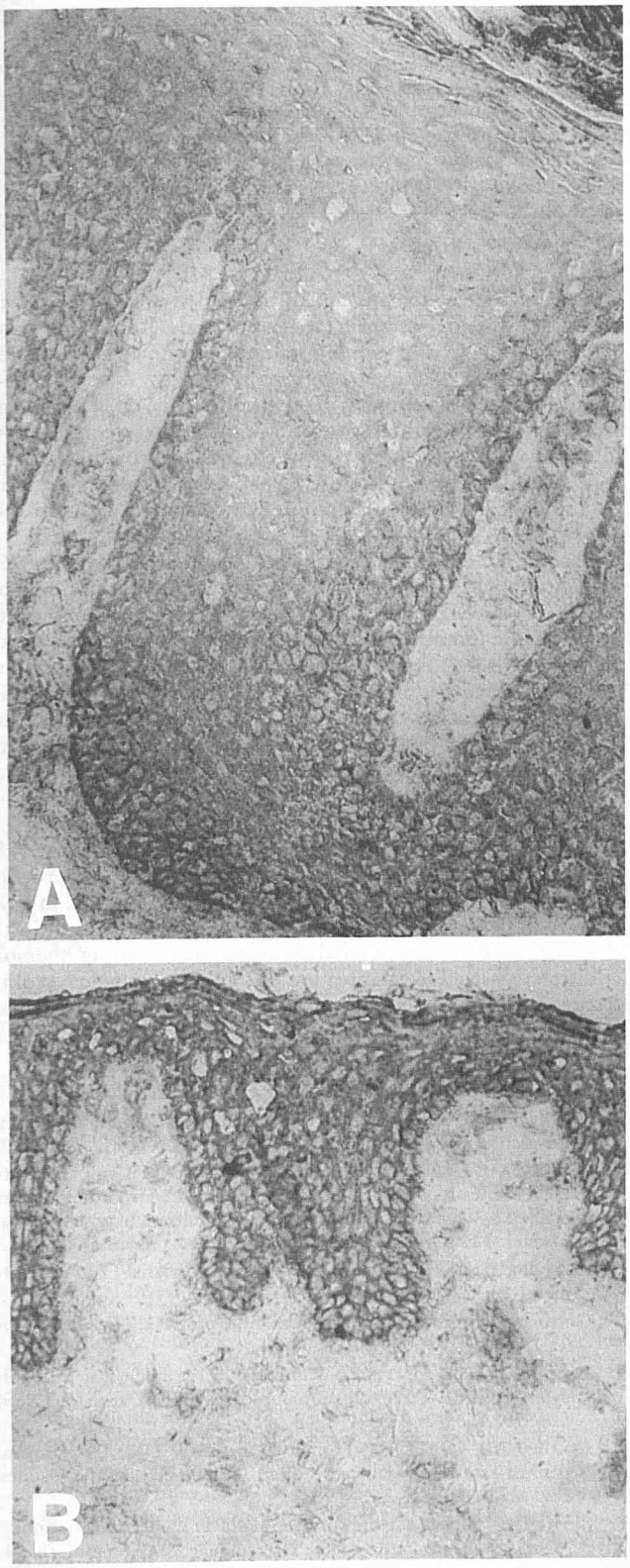

Figure 4. Immunoperoxidase reactivity of a psoriatic plaque with an anti-IL-6 antibody before *(A)* and after *(B)* cyclosporine treatment: magnification ×260. IL-6 was expressed by essentially all keratinocytes, endothelial cells, fibroblasts, and mononuclear dermal infiltrate cells in the active plaque before treatment. No decrease in IL-6 immunoreactivity in the plaque was observed after cyclosporine treatment.

incompletely characterized, the goal of this study was to determine if the inflammatory (lymphocyte) or the hyperproliferative (keratinocyte) component of psoriatic plaques showed differential sensitivity to CSA administered in vivo at comparatively low doses. Using a panel of markers that detect evidence of immune activation, and a second panel that recognizes keratinocyte growth activation–associated proteins, we were able to demonstrate that the predominant in vivo effect of CSA in psoriasis patients was to decrease immune activation. Keratinocyte growth activation was less sensitive to the inhibitory effects of CSA in vivo.

CSA has been successfully used to treat recalcitrant psoriasis in doses ranging from 1 to 15 mg/kg/d [20,21]. However, active psoriatic plaques return soon after cessation of CSA therapy. In vitro studies have demonstrated effects of CSA on both lymphocytes and keratinocytes. In lymphocytes, CSA inhibits mRNA transcription for a number of lymphokines including IL-2, gamma-interferon, and IL-4 [27–30,34]. However, IL-2 receptor mRNA transcription is not directly affected by CSA [22,28]. Similar results have been observed in experimental animals where CSA administered in vivo blocks mRNA transcription for IL-2 and gamma-interferon [42]. However, there are T-cell functions that appear to be relatively resistant to inhibition by CSA. These include the maturation of gamma/delta$^+$ T cells in the thymus [43] and the activation of the T cells in vitro by a combination of a monoclonal antibody directed against the CD28 membrane protein and a phorbol ester [44]. In vitro, CSA inhibits keratinocyte proliferation and specifically inhibits its keratinocyte cell cycle progression in the G1 phase, but the required concentrations are much higher than those needed to decrease mitogen-induced lymphocyte proliferation [14]. The concentrations required to significantly inhibit keratinocyte growth in vitro are difficult to achieve in skin even in patients treated with high doses of CSA (14 mg/kg/d) in vivo and are not attained in patients treated with CSA at doses between 2.0 and 7.5 mg/kg/d [20,26,45].

Previous studies of psoriasis plaques from patients treated with high doses of CSA showed a decrease in the number of T cells in plaques, although CD4$^+$ and CD8$^+$ T cells appeared to be equally affected. IL-2 receptor expression was not studied [21]. After treatment with high doses of CSA (14 mg/kg/d), keratinocyte HLA-DR expression decreased in plaques [46]. However, these observations were not confirmed in subsequent studies using lower doses of cyclosporine [47].

The data in this report demonstrate a significant decrease in the number of activated T cells in psoriatic plaques treated with low doses of CSA in vivo. The observed decreases in the number of IL-2 receptor$^+$ T cells in CSA-treated plaques could not be accounted for simply by a decrease in the total number of T cells because the proportion of IL-2 receptor$^+$ cells in the inflammatory infiltrate decreased twofold compared with the observed decreases in the proportion of CD3$^+$ cells. These data extend those of Gorrocks et al [48] in which decreased numbers of IL-2 receptor$^+$ T cells were demonstrated in a few patients treated with CSA. In the present study, the decrease in IL-2 receptor$^+$ T-cell number was accompanied by marked decreases in keratinocyte expression of the gamma interferon–induced proteins, HLA-DR, and IP-10, in treated plaques. The observed decrease in these gamma interferon–induced proteins is unlikely to be mediated via CSA-induced suppression of the keratinocyte response to gamma interferon because we have previously demonstrated that CSA does not directly inhibit IP-10 mRNA synthesis in gamma-interferon treated cultured human keratinocytes [14]. Therefore it is likely that CSA treatment in vivo decreases the levels of gamma interferon–induced proteins by inhibiting T-cell activation with resultant declines in gamma interferon production.

In contrast, CSA treatment in vivo did not decrease expression of growth factors associated with keratinocyte activation and whose expression is increased in active psoriatic plaques. Although keratin K16 was diminished by CSA treatment in some individuals, it remained positive in others, suggesting CSA-treated epidermis maintains features of hyperplastic growth activation or regenerative growth. It would be interesting to directly measure keratinocyte

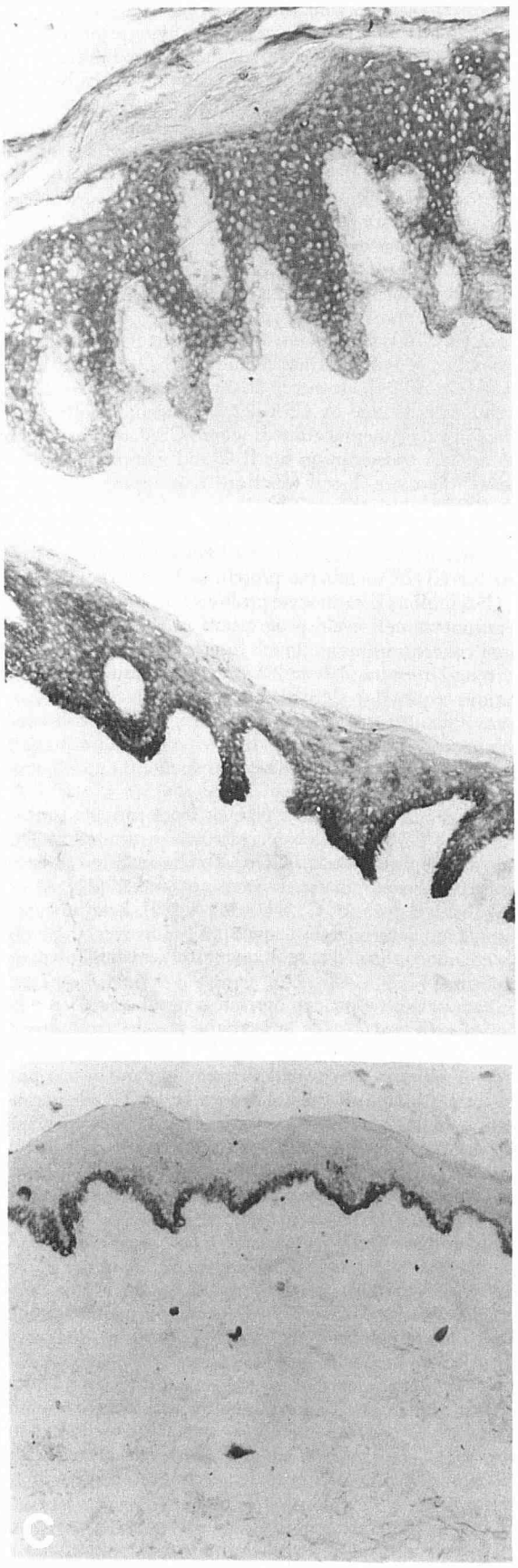

Figure 5. Immunoperoxidase reactivity of lesional and non-lesional skin from a psoriasis patient with monoclonal antibody AE-1 (anti-K16 keratin) before and after cyclosporine treatment; magnification ×110.5. K16 keratin is expressed throughout the suprabasal epidermis of this active psoriatic plaque *(A)*. In normal epidermis, AE-1 reactivity is confined to the basal layer *(C)*. The cyclosporine-treated plaque showed patchy decreases in the immunostaining levels of K16 keratin in the spinous layer of the epidermis *(B)*.

proliferation in treated tissue, e.g., by tritiated thymidine incorporation, but this was not feasible in the present study. It should be noted that TGF-α mRNA and immunostaining protein expression were increased in some patients after treatment with low doses of CSA in vivo. These results confirm our observations on the effects of adding CSA to cultured human keratinocytes on TGF-α expression [14]. Addition of CSA (1 – 5 μg/ml) in vitro to actively growing keratinocytes did not decrease TGF-α mRNA expression. In some keratinocyte strains, CSA caused a significant increase in TGF-α mRNA expression [14].

Cutaneous IL-6 expression was not decreased by low-dose CSA treatment. This is consistent with the observation that CSA only partially decreases IL-6 mRNA expression is cultured lymphocytes [49]. The same immunocytochemical technique successfully demonstrated decreases in cutaneous IL-6 staining after topical tar and ultraviolet B irradiation therapy [17]. Therefore, the observed inability of CSA treatment to decrease IL-6 staining in plaques could not be accounted for by the insensitivity of the immunocytochemical assay used to detect such decreases. The inability of CSA to decrease TGF-α and IL-6 levels in plaques may also contribute to the recurrence of active disease after cessation of CSA therapy.

Diminution of T-cell activation in plaques, with resulting decreases in the production of cytokines such as gamma interferon, is a likely mechanism of action of CSA in psoriasis plaques in vivo. These studies suggest that immune activation is important in the pathogenesis of psoriasis and that immunomodulating therapies that interfere with T-cell activation or inhibit subsequent cytokine secretion will play a major role in the future treatment of psoriasis.

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