Increased IL-6 Production by Monocytes and Keratinocytes in Patients with Psoriasis

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Interleukin-6 (IL-6) is a multifunctional inflammatory cytokine that is produced by monocytes and keratinocytes upon stimulation. Because psoriasis is a skin disease characterized by a hyperproliferative activity of keratinocytes and an inflammatory infiltrate, in the present study IL-6 production of monocytes and keratinocytes of patients with psoriasis was investigated. Peripheral blood mononuclear cells (PBMC) derived from psoriatics, atopics, and healthy controls were incubated for 24 h and, subsequently, supernatant IL-6 activity was measured using an IL-6–dependent hybridoma cell line (B9). Compared to controls and atopics, PBMC of psoriatics produced significantly increased amounts of biologically active IL-6. These findings were also confirmed by Western blot analysis using a specific antiserum directed against IL-6. Moreover, when the sera of the same patients were tested for IL-6 activity, sera of psoriatics contained significantly elevated amounts of circulating IL-6 in comparison to samples from atopics and healthy controls. In contrast to normal or uninvolved skin, keratinocytes in psoriatic lesions were remarkably positive for IL-6 as detected by immunohistochemistry and in situ hybridization. In addition, IL-6 also was found to induce its own synthesis and release by monocytes. These findings indicate that keratinocytes and monocytes in psoriasis are activated to produce increased amounts of IL-6, which may be one of the mediators involved in the regulation of both local and systemic inflammatory reactions occurring in skin diseases such as psoriasis. *J Invest Dermatol 97:27–33, 1991*

Psoriasis is a multifactorial skin disease whose pathogenesis has not been clarified so far. Although the principal clinical defect of a psoriatic lesion appears to be rapidly proliferating epidermis, interaction of keratinocyte proliferation with inflammatory and immunologic processes is obvious. The very early lesion of psoriasis is characterized by an inflammatory infiltrate of mononuclear cells in the upper dermis with only minimal changes in the epidermis [1]. Thus, mononuclear cells may be important in the induction of epidermal cell proliferation [2]. Macrophages have been demonstrated to exhibit the capacity to release various cytokines that function as important mediators of growth and differentiation of inflammatory and non-inflammatory cells [3]. Therefore, any alteration in the production of such secretory products could result in a disturbance of the immune and inflammatory response. Keratinocytes also have turned out to be a source of different cytokines; a partial listing includes interleukin (IL) -1, tumor necrosis factor, colony-stimulating factors, and IL-6 [4]. Interleukin-6 is a recently discovered cytokine whose principal functions currently include activation of lymphocytes and induction of an acute phase response. Through a multiplicity of its effects, IL-6 has been projected to play a role in the pathogenesis of local as well as systemic immunity and inflammation [5]. Because both macrophages and keratinocytes upon various stimuli synthesize and release remarkable amounts of IL-6 [4,6], it was investigated whether IL-6 production is altered in psoriasis.

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Abbreviations:
BSA: bovine serum albumin
EDTA: ethylendiamine tetracetic acid
IL-6: interleukin-6
LPS: lipopolysaccharide
PBMC: peripheral blood mononuclear cells
PHA: phytohemagglutinine
PUVA: psoralen plus UVA
rhIL-6: recombinant human interleukin-6
SDS-PAGE: sodium-dodecylsulfate polyacrylamid gelelectrophoresis
SSC: standard sodium citrate
UVB: ultraviolet B light

MATERIALS AND METHODS

Patients Twenty patients (12 male, 8 female; mean age, 49 years) with chronic psoriasis vulgaris were included in the study (Table I*A*). All had severe psoriasis with more than 25% of the skin surface affected. For comparison, 20 patients with atopic dermatitis (10 male, 10 female; mean age, 30 years; more than 25% of the body surface area involved) and 13 healthy volunteers (nine male, four female; mean age; 25 years) were studied. Patients had not undergone any systemic or topical antipsoriatic therapy for at least 4 weeks, except bland emollients, for at least 2 weeks before study began. In order to evaluate the influence of therapy on the production of IL-6, nine additional psoriatics (Table I*B*) were recruited, whose characteristics are shown in Table I*B*. All patients were in

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Table I. Psoriatics Evaluated Before and After Therapy

Patient Number	Age (years)	Type	Percent Body Surface Involved	Therapy (duration)
		A		
1	37	Plaques	40	
2	46	Plaques	35	
3	51	Plaques	35	
4	23	Guttate/plaques	35	
5	48	Plaques	30	
6	50	Guttate/plaques	30	
7	54	Guttate/plaques	35	
8	58	Plaques	45	
9	61	Plaques	30	
10	58	Guttate/plaques	40	
11	47	Guttate/plaques	35	
12	52	Plaques	40	
13	34	Plaques	35	
14	52	Plaques	30	
15	59	Guttate/plaques	35	
16	50	Guttate/plaques	35	
17	49	Plaques	40	
18	53	Plaques	30	
19	45	Plaques	30	
20	53	Plaques	35	
		B		
1	23	Plaques	30	PUVA (20 d)
2	52	Plaques	30	PUVA (27 d)
3	33	Plaques	25	UVB (14 d)
4	55	Guttate/plaques	25	UVB (23 d)
5	58	Guttate/plaques	25	UVB (26 d)
6	45	Guttate	25	UVB (18 d)
7	63	Plaques	40	Systemic corticosteroids (14 d)
8	28	Guttate	20	Local corticosteroids (14 d)
9	67	Plaques + psoriatic arthropathy	35	Methotrexate (30 d)

good physical condition and were free of current infections. After informed consent was obtained blood was collected into heparinized syringes. After each blood withdrawal a differential blood cell count was performed. In three psoriatics 6-mm punch biopsy specimens from marginal areas from developing lesions and uninvolved areas, respectively, were obtained for immunoperoxidase staining and in situ hybridization. As a control, normal skin was obtained from two healthy volunteers.

Isolation of Monocytes Peripheral blood mononuclear cells (PBMC) were obtained by density gradient sedimentation via Lymphoprep (Nycomed AS, Oslo, Norway) as described [7]. PBMC (1×10^6/ml) were plated in 24-well culture plates (Costar, Cambridge, MA) and cultured for 24 h at 37°C in a humidified 5% CO_2 atmosphere. Immediately after plating PBMC were stimulated either with lipopolysaccharide (LPS, 50 μg/ml, Difco, Detroit, MI) or phytohemagglutinine (PHA, 5 μg/ml, Gibco, Paisley, Scotland), or left untreated. After 24-h incubation supernatants were harvested, filter sterilized, and stored at −70°C.

IL-6 Bioassay For the detection of IL-6 the murine hybridoma cell line B9 was used [8]. Briefly, B9 cells (5×10^4/ml) were cultured in supplemented RPMI 1640. Cells were labeled at 68–74 h with 1 μCi (^{3}H)-thymidine and radioactivity was measured. Samples to be tested were titrated at various dilutions in triplicate, and compared to a standard IL-6 preparation containing 10 U/ml IL-6 activity. Results are expressed as counts per minute ± SEM of triplicate cultures or in U/ml. Plasma samples were thawed immediately before being tested and heat inactivated at 56°C for 30 min.

Statistical analysis was performed by using the Student t test.

Western Blot Analysis Supernatants derived from unstimulated PBMC cultures obtained as described above were supplemented with 50 μl/ml aprotinin (Trasylol 100.000 KIE Bayer, Leverkusen, FRG) and with ethylendiamine tetracetic acid (EDTA, 50 mM final concentration). After adding dithiothreitol (50 mM final concentration) samples were incubated at 37°C for 30 min, dialyzed excessively against distilled water at 4°C overnight (cutoff 1000), and finally lyophilized.

SDS-PAGE (6–16% total acrylamide concentration, 2.6% of this as methylenbisacrylamid) was performed under reducing conditions as described [9]. Following SDS-PAGE proteins were electrotransferred to nitrocellulose and stained with a rabbit antiserum directed against recombinant human IL-6 (rhIL-6), kindly provided by Dr. L. T. May and Dr. P. B. Sehgal (Rockefeller University, New York, NY) according to standard procedures [10]. For detection of rabbit IgG a Vecta-stain ABC Kit (PK 4001, Vector Laboratories, Burlingame, CA) was used. Biotinylated molecular weight markers were obtained from Sigma (St. Louis, MO).

Immunoperoxidase Staining Snap-frozen 5-μm tissue sections were stained with a rabbit antiserum directed against recombinant human IL-6. Briefly, slides were mounted with 100 μl of diluted antiserum. After washing, sections were incubated with peroxidase-labeled swine–anti-rabbit immunoglobulin (Dakopatt, Klostrup, Denmark) and, subsequently, peroxidase-labeled rabbit–anti-swine immunoglobulin was added. As substrate 3-amino-9-ethylcarbazole (Sigma) was used [11]. Irrelevant antibodies served as negative controls.

In Situ Hybridization In situ hybridization was performed using Oncor in situ hybridization kit (Oncor Inc., Gaithersburg, MD). Frozen sections were dried for 10 min, fixed in 4% paraformaldehyde for 3 min, and stored at 4°C in 70% ethanol. Before hybridization slides were treated with 0.25% acetic anhydride in a 0.1-M triethanolamine (pH 8.0) solution followed by a 0.1-M tris (pH 7.0)–0.1-M glycine solution. Single-stranded ^{35}S-labeled RNA probes were transcribed for hybridization by using a PGEM1 vector containing a 0.9-kb cDNA probe encoding for IL-6, kindly provided by L. May, Rockefeller University, New York. Plasmids were linearized with the appropriate restriction endonuclease before transcription with either SP6 or T7 RNA polymerase. Hybridization was performed at 52°C in a humid chamber. Slides were washed in 50% formamide/2× SSC, followed by digestion with RNase A (20 μg/ml) and three further washes in 50% formamide/2× SSC. Dried sections were prepared for autoradiography using the NTB2 emulsion (Eastman Kodak, Rochester, New York), were developed after 6 d, and counterstained with hematoxylin [13].

RESULTS

PBMC Derived from Psoriatics Exhibit Enhanced IL-6 Production Conditioned medium derived from unstimulated PBMC cultured for 24 h was tested in the B9 bioassay; significantly increased amounts of IL-6 activity were detected in the supernatants derived from PBMC of psoriatics in comparison to identically treated samples from atopics or healthy controls (Table II). However, there was no significant difference between the IL-6 levels released by PBMC derived from the atopics and those from healthy controls. In order to study the inducibility of IL-6 production, PBMC were stimulated either with LPS or PHA. Addition of both LPS and PHA significantly enhanced IL-6 production, but resulted in an equally increased IL-6 activity in all three groups tested (psoriatics, atopics, healthy controls), indicating that the capacity to release IL-6 upon stimulation is not affected in any of these groups (Table II).

In order to examine whether the B9-promoting activity found in PBMC supernatants was specific for IL-6, an antiserum directed against recombinant human IL-6 was added to some of the supernatants derived from PBMC of psoriatics. The antiserum significantly suppressed IL-6 activity in a dose-dependent manner (Fig 1). In addition, Western blot analysis was performed (Fig 2). The anti-

Table II. IL-6 Activity in Supernatants of PBMC Derived from Psoriatics, Atopics, and Healthy Controls

Stimulant[a]	IL-6 Activity (U/ml, mean ± SD)[b] Healthy Controls (n = 13)	Atopics (n = 20)	Psoriatics (n = 20)
	141 ± 18	82 ± 14	780 ± 42[c]
PHA (5 μg/ml)	2050 ± 225	2451 ± 382	2122 ± 227
LPS (50 μg/ml)	1292 ± 338	1264 ± 329	1479 ± 34

[a] Peripheral blood mononuclear cells (1×10^6/ml) were incubated 24 h either in RPMI medium alone or in the presence of PHA or LPS.
[b] IL-6 was measured using the B9 bioassay. Results are expressed in U/ml (mean ± SD).
[c] Significantly different from unstimulated PBMC of healthy controls and atopics ($p < 0.01$).

serum reacted with recombinant human IL-6, which was used as a positive control, and revealed specific bands in supernatants of unstimulated PBMC of both psoriatics and healthy controls. The amount of IL-6 detected in the psoriatic supernatants, however, was much larger compared to that in PBMC supernatant derived from the healthy controls.

Plasma IL-6 Levels in Psoriatics In order to investigate whether the increased release of IL-6 by PBMC of psoriatics is not only an in vitro phenomenon, we tested whether psoriatics exhibit elevated circulating IL-6 levels. Plasma samples obtained from the psoriatics (Table I*A*) contained higher amounts of IL-6 in comparison to atopics or healthy controls (Fig 3). The B9 cell-promoting activity detected in the circulation could be blocked by the IL-6–antiserum in a dose-dependent manner, indicating that the activity is really due to IL-6 (Table III).

Effect of Therapy on IL-6 Production in Psoriatics In order to test whether antipsoriatic therapy (PUVA, UVB, corticosteroids, methotrexate) affects the enhanced activity of PBMC to release IL-6, PBMC were collected from patients (Table I*B*) before initiation and after completion of therapy (Table I). After application of PUVA, UVB, systemic corticosteroids, or methotrexate the amounts of IL-6 released by unstimulated PBMC were significantly reduced, whereas topical application of corticosteroids did not affect IL-6 production (Table IV). Stimulation of PBMC with LPS (50 μg/ml) resulted in an equally increased IL-6 activity irrespective of whether the PBMC were collected before or after therapy. Elevated IL-6 serum levels, however, did not change significantly during treatment (data not shown).

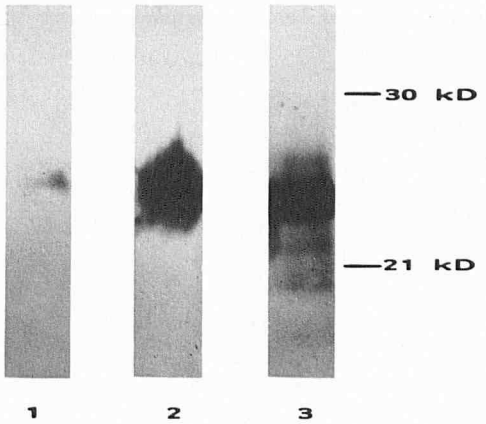

Figure 2. Western blot analysis of IL-6 using a rabbit antiserum to rhIL-6. PBMC (1×10^6/ml) obtained from a healthy control individual *(lane 1)* and from a patient with psoriasis *(lane 2)* were incubated 24 h in RPMI. Subsequently, 5 ml supernatant was dialysed against distilled water, lyophilized, and used for Western blot analysis. Human rhIL-6 was included as a control *(lane 3)*.

IL-6 Induces IL-6 in PBMC As the release of IL-6 by macrophages can be induced by various cytokines such as IL-1, tumor necrosis factor α, IL-4, and interferons [14,15], it was tested whether IL-6 by itself may stimulate its own release. Therefore, PBMC of healthy controls were isolated as described above and incubated in the presence of 1 U/ml rhIL-6. After incubation for 6 h supernatants of PBMC treated with rhIL-6 were tested in the B9 assay (Fig 4). Supernatants of IL-6–stimulated PBMC contained larger amounts of IL-6 when compared to supernatants of untreated PBMC or to 1 U/ml recombinant IL-6 alone. This was further confirmed by Western blot analysis. Briefly, 1×10^6/ml PBMC were pulsed with 1 U/ml rhIL-6 incubated for 24 h and washed extensively to get rid of exogenously added IL-6. Cells were subsequently cultured for another 6 h. Western blot analysis demonstrated increased amounts of IL-6 protein in IL-6–pulsed PBMC (Fig 5, *lane 2*) in comparison to untreated cells (Fig 5, *lane 1*). In order to exclude the possibility that IL-6 induction is due to LPS contamination of the rhIL-6 added to PBMC, boiled IL-6 (100°C,

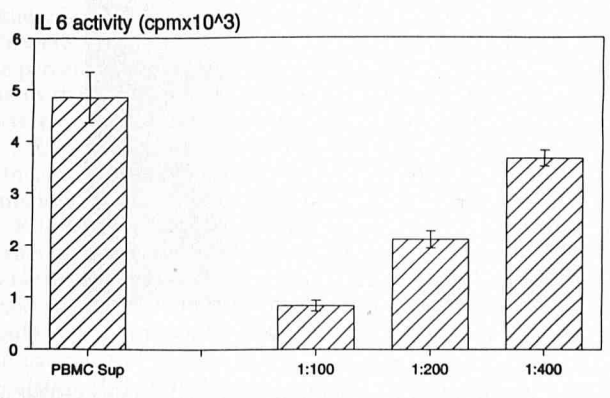

Figure 1. Effect of IL-6 antibody on IL-6 activity released by PBMC from psoriatics. Supernatants derived from PBMC of psoriatics were incubated with an IL-6 antibody in various concentrations and subsequently tested in the B9 bioassay. Results are expressed as cpm ± SEM. The IL-6 antibody alone (1:100) had no effect in the B9 assay.

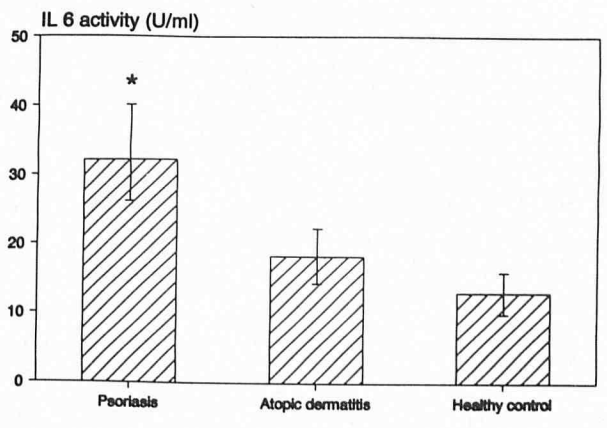

Figure 3. IL-6 activity (U/ml) in plasma of patients with psoriasis (n = 20), atopic dermatitis (n = 20), and healthy controls (n = 13). Plasma samples were tested for IL-6 activity using the B9 bioassay. Results are expressed as U/ml (mean ± SD). *Significantly different from atopics and healthy controls ($p < 0.01$).

Table III. Effect of Anti-IL-6 on Plasma IL-6 Activity

	IL-6 Activity[a] (cpm)				
	Rabbit Serum[b]	Anti-IL-6[b]			
	1:100	1:100	1:200	1:400	1:800
rhIL-6 (10 U)	33,422	861	2,793	13,331	32,565
Plasma 1[c]	5,305	625	3,828	4,340	5,034
Plasma 2[c]	10,164	571	4,054	5,436	7,854
Plasma 3[c]	5,547	422	3,591	4,527	4,624

[a] IL-6 activity was measured using the B9 hybridoma bioassay. Results are expressed as cpm of triplicates; SD were within 10%. ^{3}H-thymidine uptake of B9 cells alone was 336 cpm.

[b] rhIL-6 or plasma samples were incubated with various dilutions of rabbit anti-IL-6 antiserum or normal rabbit serum.

[c] Plasma samples obtained from three different patients with psoriasis (final dilution 1:40).

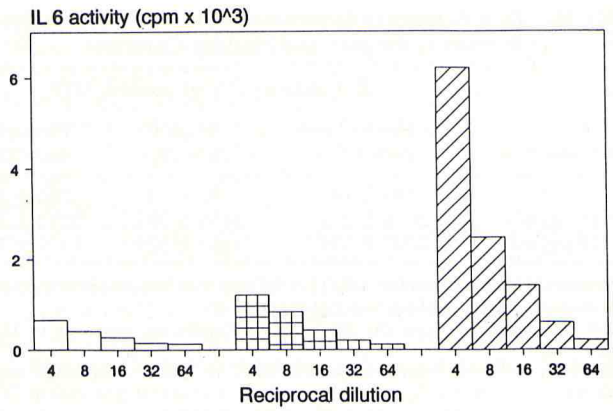

Figure 4. IL-6 production by human PBMC stimulated with rhIL-6. PBMC (1×10^6/ml) stimulated with rhIL-6 (1 U/ml) (▨) or unstimulated PBMC (■) were incubated 24 h. Subsequently, supernatants as well as 1 U rhIL-6 alone (□) were tested for IL-6 activity using the B9 bioassay. Results are expressed as cpm of two experiments.

30 min) was used in an identical manner, which completely failed to induce IL-6 (Fig 5, *lane 3*).

Keratinocytes Produce Enhanced Amounts of IL-6 in Psoriatic Plaques Because keratinocytes previously have been shown to synthesize IL-6 it was studied whether epidermal cells in psoriatic lesions express increased amounts of IL-6. Immunoperoxidase staining with an IL-6 antiserum revealed significant amounts of IL-6 within lesional psoriatic epidermis, whereas no or only minimal IL-6 was found in non-lesional psoriatic epidermis and in skin of healthy controls (Fig 6). As IL-6 visualized by immunohistochemistry in psoriatic epidermis could be macrophage derived and just accumulated intraepidermally, in situ hybridization using an antisense probe encoding for IL-6 was performed. Keratinocytes in psoriatic lesions expressed IL-6 mRNA (Fig 7*a*), whereas epidermal cells both in uninvolved and normal healthy skin turned out to be negative (Fig 7*c*). As a negative control hybridization with the respective sense probe was performed and showed no specific pattern (Fig 7*b,d*).

Table IV. IL-6 Activity Released by PBMC of Patients with Psoriasis Before and After Therapy

		IL-6 Activity (mean cpm)[a]			
		Spontaneous Release[b]		LPS-Induced Release[b]	
Patient Number	Treatment	Before Therapy	After Therapy	Before Therapy	After Therapy
1	PUVA	13,586	2,463	18,230	16,793
2	PUVA	9,266	2,050	18,626	18,923
3	UVB	2,896	806	18,329	18,970
4	UVB	3,370	1,093	18,626	16,653
5	UVB	8,356	913	21,100	20,873
6	UVB	3,436	620	16,220	15,880
7	Systemic corticosteroids	16,186	736	16,600	18,536
8	Local corticosteroids	9,090	13,270	18,220	18,740
9	Metothrexate	3,270	920	18,230	18,216

[a] IL-6 activity was measured in the B9 assay. Results are expressed as mean cpm of three experiments. SD were within 10%. The spontaneous thymidine uptake of B9 cells was 578 cpm.

[b] PBMC were cultured without treatment or after stimulation with LPS (50 μg/ml) for 24 h and supernatants tested for IL-6 activity.

DISCUSSION

The present study demonstrates that PBMC obtained from psoriatics and cultured for 24 h release higher amounts of IL-6 compared to identically treated mononuclear cells obtained from atopic patients or healthy controls. Moreover, Western blot analysis using an antiserum directed against recombinant human IL-6 revealed specific bands that appeared more pronounced in the psoriatic group.

PBMC were separated by Lymphoprep centrifugation, yielding a cell population containing mainly monocytes and lymphocytes. In spite of this heterogeneity, monocytes may be regarded as the main source for IL-6 [6], because the recently reported induction of IL-6 production in normal T cells required an incubation period of 2-3 d [16], whereas in the present study supernatants were collected after 24 h. Nevertheless, we cannot exclude from the present study design the possibility that lymphocytes activated during psoriatic flare may secrete factors that stimulate monocytes to release IL-6. En-

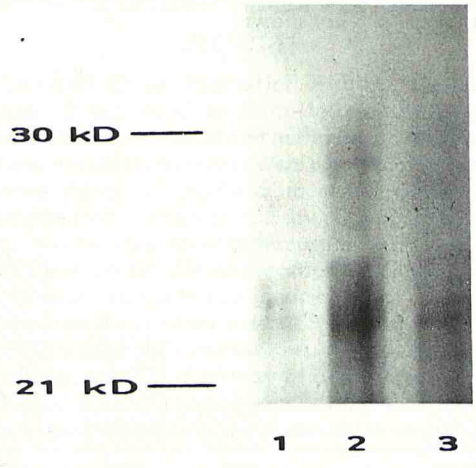

Figure 5. Western blot analysis of PBMC-derived IL-6 using a rabbit antiserum to rhIL-6. PBMC (1×10^6) were untreated *(lane 1)*, pulsed with 10 U/ml rhIL-6 *(lane 2)*, or treated with boiled rhIL-6 *(lane 3)*. After 2 h cells were washed and incubated for a further 6 h. Subsequently, 5 ml supernatant was dialysed against distilled water, lyophilized, and used for Western blot analysis.

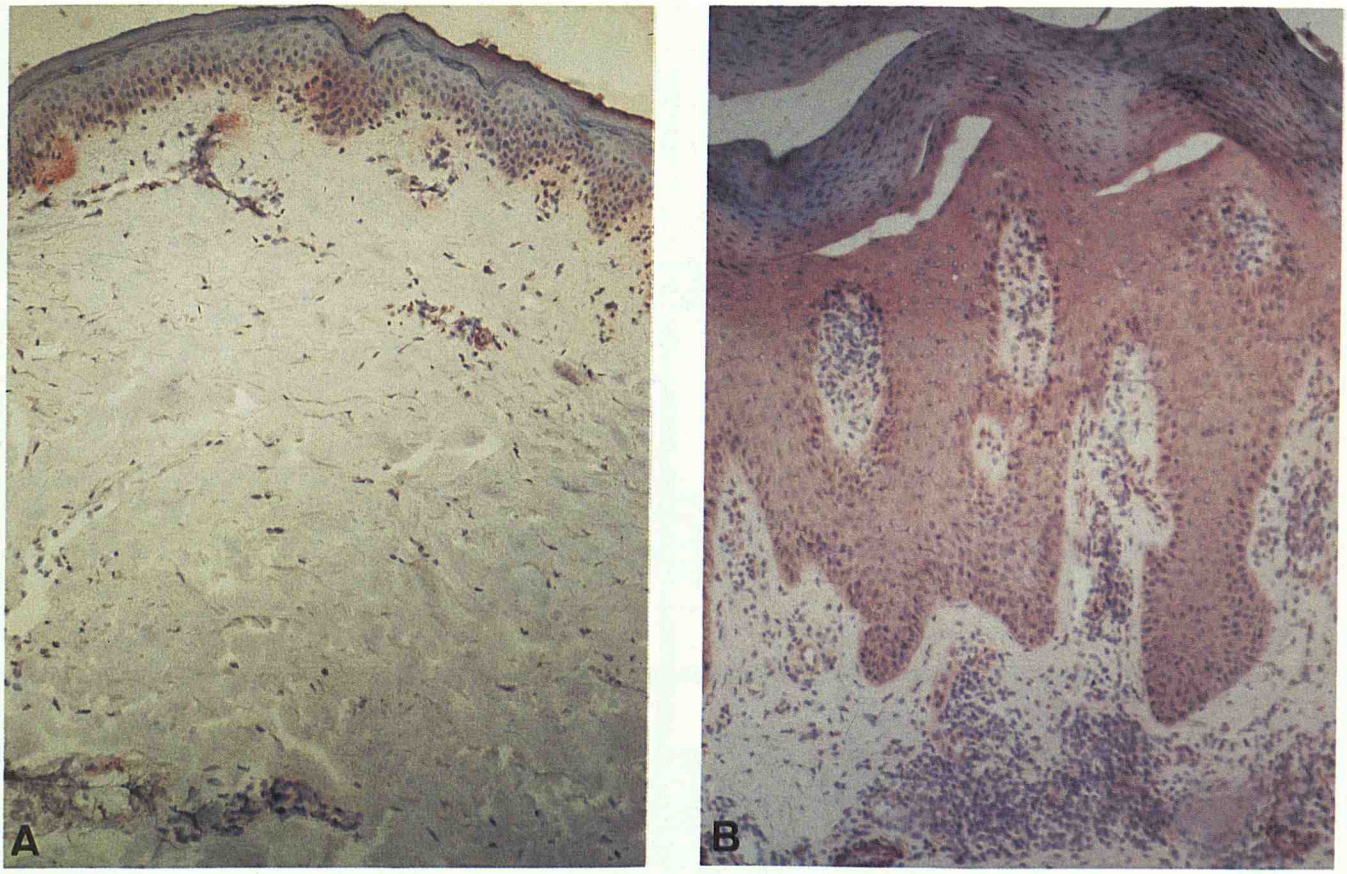

Figure 6. Immunoperoxidase staining using a rabbit antiserum to rhIL-6 of normal *(A)* and psoriatic lesional *(B)* skin.

hanced IL-6 production detected in the PBMC of psoriatics was not due to an elevated number of monocytes in the periphery, as differential blood cell counts did not reveal significant differences within the three patient groups studied. The increased spontaneous release of IL-6 by PBMC of psoriatics obviously was not due to an elevated capacity to produce IL-6, because there were no differences in the amounts of IL-6 secreted after stimulation with PHA or LPS within the different groups. This demonstrates that there is no essential alteration in the capacity of mononuclear cells to produce IL-6 in psoriasis. PBMC from psoriatics, however, appear to be more activated during the acute phase of the disease. The enhanced activity of 24-h cultured PBMC does not appear to be an in vitro phenomenon because IL-6 plasma levels were found elevated in psoriatics as well. We were not able to observe a correlation between IL-6 release and the percentage of body surface involved. This, however, could be due to the fact that the extent of lesions did not vary remarkably between the individual patients because only individuals with more than 25% of body surface affected were included.

In order to study whether the enhanced IL-6 production may somehow be related to the pathogenesis or disease activity of psoriasis, PBMC were isolated from patients before and after completion of therapy. The increased spontaneous IL-6 release by PBMC was found reduced to normal levels after photo- (PUVA, UVB) or systemic treatment (corticosteroids, methotrexate), whereas, as one would expect, topical application of steroids, though studied only in one patient, had no effect. In contrast, no significant reduction in circulating IL-6 could be observed after completion of therapy. The discrepancy between the data obtained from the circulation and from the PBMC is not quite clear. Although it is hard to correlate in vivo and in vitro data, one explanation could include the possibility that the monocyte is not the only cell releasing IL-6 in vivo; fibroblasts, endothelial cells, and keratinocytes have to be considered as a source for IL-6 as well. Moreover, the present data show that IL-6 in an autocrine manner can induce its own release by mononuclear cells, suggesting a self-perpetuating process. The particular signal responsible for elevated IL-6 release in psoriasis, however, has not yet been detected.

LPS, a potent inducer of IL-6 in vivo and in vitro [6,17], does not appear to be involved in enhanced IL-6 release in psoriasis, as, in spite of the presence of an increased number of staphylococcus areus and other aerobic organisms on psoriatic skin, there is no evidence for higher incidence of septicemia or local infections [18]. In vitro LPS contamination during PBMC preparation or during incubation appears to be unlikely because blood samples from all patients, including controls, were treated in an identical manner.

Currently, it has been demonstrated that in vitro cultured human keratinocytes synthesize and release IL-6 [19]. In addition, by immunohistochemistry and in situ hybridization, IL-6 was found more widely distributed and present at a higher concentration in lesional psoriatic skin [20,21]. These observations clearly are confirmed by our data. Upregulation of IL-6 production in psoriatic keratinocytes may at least partly account for their hyperproliferative activity, because it recently has been shown that IL-6 in vitro may function as a growth factor for keratinocytes [21].

Interleukin-6, through its multiple biologic activities, appears to be a mediator involved in acute-phase and immunologic responses to injurious agents [5]. Accordingly, elevated IL-6 serum levels have been demonstrated in patients with burns [22], septicemia [23], those undergoing surgical procedures [24], or after administration of endotoxin [17]. In view of the present data, which are in accordance with a recent study [21], severe psoriasis can be added to the list of diseases in which circulating IL-6 is increased. Although this observation may not be claimed to be specific for psoriasis, no elevation of serum IL-6 was found in patients with atopic dermatitis,

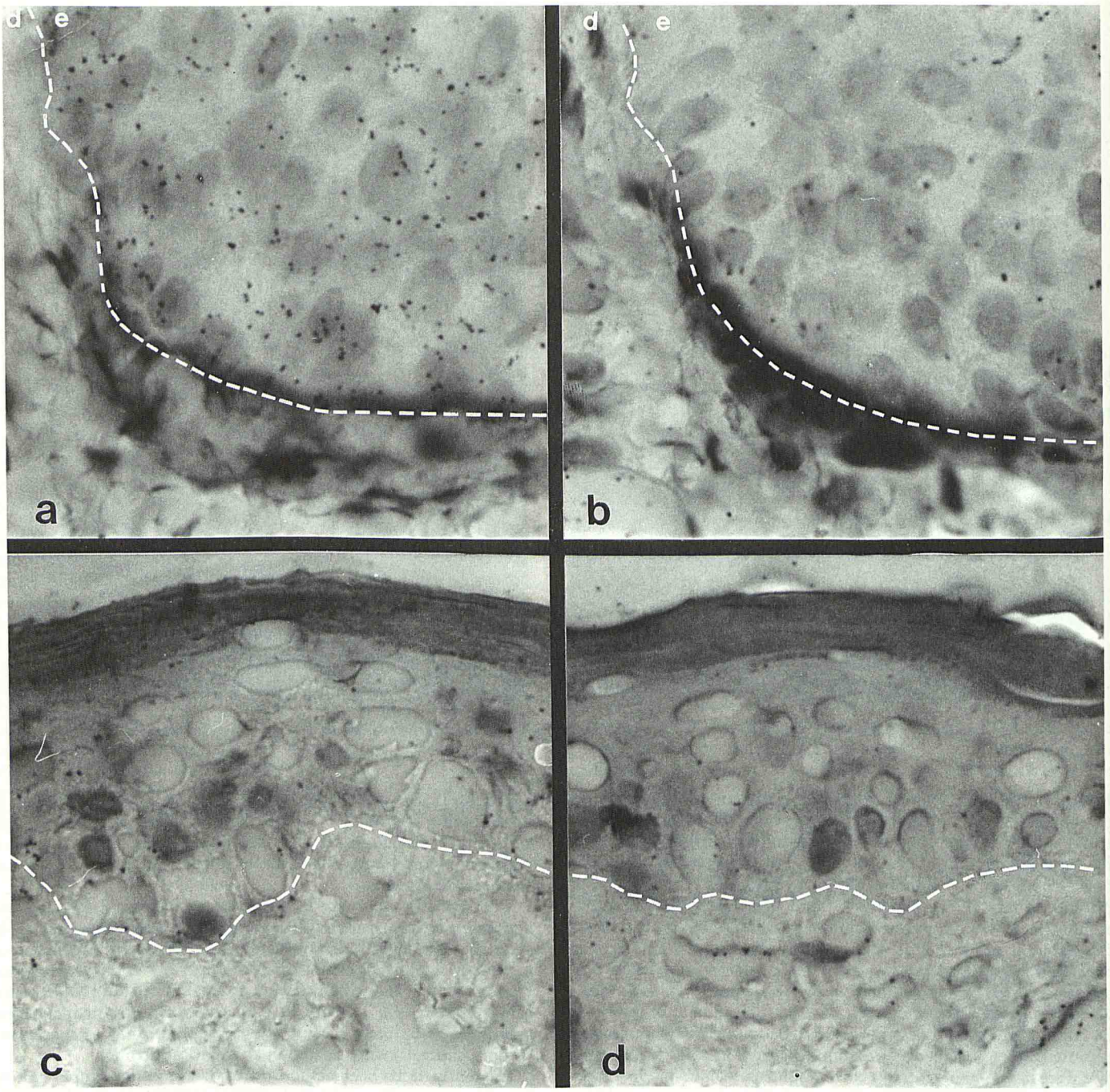

Figure 7. Analysis of IL-6 expression in normal *(c,d)* and psoriatic lesional *(a,b)* skin by in situ hybridization. Sections were hybridized either with an antisense IL-6 probe *(a,c)* or a sense IL-6 probe *(b,d)*. Hybridization with the sense probe *(b,d)* shows only unspecific grains both in the epidermis *(e)* and dermis *(d)*. The dermoepidermal junction has been outlined.

indicating that upregulation of IL-6 is not a common phenomenon in any skin disease affecting extensive surface areas. However, it does not seem to be justified to regard elevated IL-6 levels as marker for a psoriatic diathesis. Accordingly, psoriatics appear to be more prone to an acute-phase response, although the serum levels did not reach concentrations of biologic relevance. Therefore it remains to be determined whether there is a correlation between IL-6 levels and systemic changes in erythrodermic psoriasis, which is frequently associated with fever and acute-phase protein release [25]. IL-6 recently has been suggested to be involved in inflammatory joint diseases, like rheumatoid arthritis [26]; as in the present study, where only one patient was suffering from arthritis, it is now under investigation whether IL-6 plays a role in the pathogenesis of psoriatic arthropathy. Moreover, because activated T cells are found increased in fully developed psoriatic lesions [27], it is also possible that IL-6, through its T-lymphocyte co-stimulating capacities [5], may mediate these events.

One of the most significant histopathologic findings in psoriasis is the accumulation of polymorphonuclear leukocytes in the stratum corneum of psoriatic epidermis. At present there is no evidence that IL-6 is directly involved in the attraction of neutrophils. However, it is possible that IL-6 induces the release of other chemotactic mediators, because it appears to be integrated into the network of interacting cytokines. Therefore it remains to be studied whether

IL-6 can influence the release of recently described monocyte-derived mediators that attract and activate neutrophils [28,29]. These factors may play an important role in the pathogenesis of psoriasis [5,30].

The release of a variety of soluble mediators, including prostaglandins, leukotrienes, polyamines, and other cytokines, has been demonstrated to be dysregulated in psoriasis [31,32]. None of these findings has clarified the cause and pathogenesis of psoriasis. Thus, it appears likely that these changes are not the cause, but the consequence of the disease [32]. Although this may also be the case with IL-6, this mediator can be involved as an inflammatory mediator and in T-cell activation during the acute phase. Whether blocking of IL-6 activity, either by antibodies or specific inhibitors, may open new therapeutic aspects has yet to be demonstrated.

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REFERENCES

1. Lever WF, Schaumburg-Lever G: Histopathology of the Skin. JB Lippincott Company, Philadelphia, 1975, pp 135–146
2. Hancock GE, Kaplan G, Cohn Z: Keratinocyte growth regulation by the products of immune cells. J Exp Med 168:1395–1402, 1988
3. Johnston RB: Current concepts in immunology. Monocytes and macrophages. N Engl J Med 318:747–752, 1988
4. Luger TA, Schwarz T: Epidermal cell derived secretory regulins. In: Schuler G (ed.). Epidermal Langerhans Cells. CRC Press, Boca Raton, 1989, pp 217–251
5. Wong GG, Clark SC: Multiple actions of interleukin-6 within a cytokine network. Immunol Today 9:137–139, 1988
6. Santhanam U, Tatter SB, Helfgott DC, Ray A, Ghrayeb J, May LT, Sehgal PB: Structure, genetics and function of human "β2-interferon/B-cell stimulatory factor-2/hepatocyte stimulating factor" (interleukin-6). In: Powanda MC, Oppenheim JJ, Kluger MJ, Dinarello CA (eds.). Monokines and Other Non-Lymphocytic Cytokines. Alan R. Liss Inc, New York, 1987, pp 29–34
7. Luger TA, Smolen JS, Chused TM, Steinberg AD, Oppenheim JJ: Human lymphocytes with either OKT4 or OKT8 phenotype produce interleukin 2 in culture. J Clin Invest 70:470–473, 1982
8. Aarden LA, DeGroot ER, Schaap OL, Lansdorp PM: Production of hybridoma growth factor by human monocytes. Eur J Immunol 17:1411–1416, 1987
9. Barret AJ, Brown MA, Sayers CA: The electrophoretically "slow" and "fast" terms of the α2-macroglobulin molecule. Biochem J 181:401–409, 1979
10. May LT, Ghrayeb J, Santhanam U, Tatter SB, Stoehger Z, Helfgott DC, Chiorazzi N, Grieninger G, Sehgal PB: Synthesis and secretion of multiple forms of "β2-interferon/B-cell differentiation factor BSF-2/hepatocyte stimulating factor" by human fibroblasts and monocytes. J Biol Chem 263:7760–7766, 1988
11. Köller U, Stockinger H, Majdic O, Bettelheim P, Knapp W: A rapid and single immunoperoxidase staining procedure for blood and bone marrow samples. J Immunol Methods 86:76–86, 1986
12. Hirano T, Taga T, Nakano N, Yasukawa K, Kaskiwamura S, Shimizu K, Nakajima K, Pyrun KM, Kishimoto T: Purification to homogeneity and characterization of human B cell differentiation factor (BCDF or BSF 2). Proc Natl Acad Sci USA 82:5490–5495, 1985
13. Takacs L, Kovacs EJ, Smith MR, Young HA, Durum SK: Detection of IL 1α and IL 1β gene expression by in situ hybridization. Tissue localization of IL 1 mRNA in the normal C57BL/6 mouse. J Immunol 141:3081–3087, 1988
14. VanDamme J, Opdenakker G, Simpson RJ, Rubira MR, Cayphas S, Vink A, Billiau A, VanSnick J: Identification of the human 26-kD protein, interferon β2 (IFN-β2) as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor. J Exp Med 165:914–919, 1987
15. Zhang Y, Lin JX, Yip YK, Vilcek J: Enhancement of cAMP levels and of protein kinase activity by tumor necrosis factor and interleukin 1 in human fibroblasts: role in the induction of interleukin-6. Proc Natl Acad Sci USA 85:6802–6805, 1988
16. Horii Y, Muraguchi A, Suematsu S, Matsuda T, Yoshizaki T, Hirano T, Kishimoto T: Regulation of BSF-2/IL-6 production by human mononuclear cells. J Immunol 141:1529–1535, 1988
17. Fong Y, Moldawer LL, Marano M, Wei H, Tatter SB, Clarick RH, Santhanam U, May LT, Sehgal PB, Lowry SF: Endotoxemia elicits increased circulating β2-interferon/interleukin-6 in man. J Immunol 142:2321–2323, 1989
18. Krueger CG, Hill HR, Jederberg WW: Inflammatory and immune cell function in psoriasis—a subtile disorder. I. In vivo and in vitro survey. J Invest Dermatol 71:189–194, 1978
19. Kirnbauer R, Köck A, Schwarz T, Urbanski A, Krutmann J, Borth W, Damm D, Shipley G, Ansel JC, Luger TA: IFN-β2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. J Immunol 142:1922–1928, 1989
20. Oxholm A, Oxholm P, Staberg B, Bendtzen K: Interleukin-6 in the epidermis of patients with psoriasis before and during PUVA treatment. Acta Dermatol Venereol (Stockh) 69:195–199, 1989
21. Grossman RM, Krueger J, Yourish F, Granelli-Piperno A, Murphy PD, May LT, Kupper TS, Sehgal P, Gottlieb AB: Interleukin-6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367–6371, 1989
22. Nijsten NWN, DeGroot ER, TenDuis HJ, Klasen HJ, Hack CE, Aarden LA: Serum levels of interleukin-6 and acute phase responses (letter). Lancet II:921, 1987
23. Helfgott DC, Tatter SD, Santhanam U, Clarick RH, Bhardway N, May LT, Sehgal PB: Interferon β2/interleukin-6 in plasma and body fluids during acute bacterial infection. J Immunol 142:948–953, 1989
24. Shenkin A, Fraser WD, Series J, Winstanley FP, McCartney AC, Burns HJG, Van Damme J: The serum interleukin-6 response to elective surgery. Lymphokine Res 8:123–127, 1989
25. Christophers E, Krueger GG: Psoriasis. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen K (eds.). Dermatology in General Medicine. McGraw Hill, New York, 1987, pp 461–501
26. Bhardway N, Santhanam U, Lau LL, Tatter SB, Ghrayeb J, Rivelis M, Steinman RM, Sehgal PB, May LT: IL-6/IFNβ2 in synovial effusions of patients with rheumatoid arthritis and other arthritides. J Immunol 143:2153–2159, 1989
27. Ramirez-Bosca A, Martinez-Ojeda L, Valcuende-Carero F, Castells-Rodelas A: A study of local immunity in psoriasis. Br J Dermatol 119:587–595, 1988
28. Schröder JM, Mrowietz U, Morita E, Christophers E: Purification and partial biochemical characterization of a human monocyte-derived, neutrophil-activating peptide that lacks interleukin 1 activity. J Immunol 139:3474–3483, 1987
29. Matsushima K, Morishita K, Yoshimura T, Lavu S, Kobayashi Y, Lew W, Appella E, Kung HF, Leonard EJ, Oppenheim JJ: Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J Exp Med 167:1883–1992, 1988
30. Van Damme J, Van Beeumen J, Opdenakker G, Billiau A: A novel, NH2-terminal sequence-characterized human monokine possessing neutrophil chemotactic, skin-reactive and granulocytosis promoting activity. J Exp Med 167:1364–1373, 1988
31. Grabbe J, Czarnetzki B, Risenbach T, Marin T: Identification of lipoxygenase products of arachidonate metabolism in psoriatic skin. J Invest Dermatol 82:477–479, 1985
32. Christophers E: Regulative Faktoren der psoriatischen Hautreaktion. Hautarzt 40:456–460, 1989