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Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes

(cytokine/cellular immunity/inflammation/epidermis/keratinocyte)

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ABSTRACT **Psoriasis is a common papulosquamous skin disease. The histopathology is characterized by epidermal hyperplasia and inflammation. Recent studies suggest that keratinocyte proliferation and inflammation in psoriasis are manifestations of the same underlying pathological process. Interleukin 6 (IL-6), a cytokine that is a major mediator of the host response to tissue injury and infection, is produced by both keratinocytes and leukocytes in culture. IL-6 expression was studied in psoriatic plaques by immunoperoxidase staining with two different polyclonal anti-recombinant IL-6 antisera and by *in situ* nucleic acid hybridization with IL-6 cRNA probes. Epidermal and dermal cells in active psoriatic plaques from 35 psoriasis patients stained heavily for IL-6 as compared with nonlesional skin and with plaques after treatment with antimetabolic and antiinflammatory agents. Absorption of the anti-recombinant IL-6 antisera with purified fibroblast-derived IL-6 or with recombinant IL-6, but not bovine serum albumin, removed the immunostaining. Increased levels of IL-6 were detected in the plasma of patients with active psoriasis (mean 3 ng/ml) by using two different bioassays. IL-6 production by proliferating keratinocytes was suggested by IL-6-specific immunostaining in cultured normal and psoriatic keratinocytes and by the detection of mRNA specific for IL-6 in psoriatic epidermis by *in situ* hybridization. IL-6 stimulated the proliferation of cultured, normal human keratinocytes as assessed by two different assays. Thus, IL-6 could directly contribute to the epidermal hyperplasia seen in psoriatic epithelium as well as affect the function of dermal inflammatory cells.**

Psoriasis is a chronic papulosquamous skin disease affecting approximately four million Americans. Although there is a genetic predisposition to this disorder, environmental factors such as streptococcal infection, AIDS, injury to the skin (Koebner phenomenon), reaction to such medications as β-adrenergic blockers, anti-malarials, lithium salts, and stress play a role in precipitating active psoriasis (1, 2). The histopathologic features of psoriatic plaques are epidermal hyperplasia and the presence of inflammatory cells. Components of the infiltrate include polymorphonuclear leukocytes, activated T cells, Langerhans cells, and macrophages (3–5).

The possibility that psoriatic epidermal hyperplasia results from increased local cytokine or growth factor production by keratinocytes or by activated cellular elements in the inflammatory infiltrate has drawn increasing attention (1, 3, 4, 6, 7). The cytokine interleukin 6 (IL-6) is a major mediator of the host response to injury and infection (8, 9). IL-6 stimulates the production of "acute phase" plasma proteins by the liver; enhances B- and T-cell proliferation; and enhances B-cell, T-cell, and macrophage activation (for review, see ref. 8). IL-6 is produced by a number of different cell types such as fibroblasts, macrophages, endothelial cells, and keratinocytes in response to induction by a variety of stimuli, which include other cytokines such as interleukin 1 (IL-1), tumor necrosis factor, and platelet-derived growth factor (6, 8–10). In this paper we report that IL-6 mRNA and protein increase in psoriatic plaques and IL-6 levels increase in the plasma of psoriasis patients. We also report that IL-6 stimulates the proliferation of human keratinocytes in culture. These observations suggest that IL-6 may play an important role in the pathophysiology of psoriasis.

MATERIALS AND METHODS

Patients and Skin Biopsies. Biopsies were performed after informed consent was obtained in accordance with a protocol approved by the Rockefeller University Hospital Institutional Review Board. Skin biopsies were obtained from plaques from 32 patients with psoriasis vulgaris, 2 with erythrodermic psoriasis, and 1 with pustular psoriasis of the Von Zumbusch type. Nine of these patients received treatment with topical tar and UV B irradiation for 3–6 weeks. One of these patients received methotrexate simultaneously, and another received topical steroids. One patient was treated exclusively with methotrexate. Biopsies of normal skin were obtained from two volunteers.

Antibodies. A polyclonal rabbit antiserum (C-11) to human recombinant IL-6 (rIL-6) was prepared as described (11). The immunogen was an immunoaffinity-purified rIL-6 fusion protein produced in *Escherichia coli* (11). The IgG fraction of an anti-rIL-6 antiserum was purchased from Genzyme. This antibody was prepared by immunization of a rabbit with human IL-6 expressed in yeast.

Immunoperoxidase Studies. Immunoperoxidase studies of fresh-frozen skin biopsies or keratinocytes grown on coverslips in serum-free medium (12) were done by using the Vectastain ABC kit (Vector Laboratories) as described (3).

Immunofluorescence Studies. Normal human keratinocytes grown on coverslips in serum-free medium (12) were fixed in neutral phosphate-buffered formalin and permeabilized with 1% Triton X-100. Bound rabbit antibodies were visualized with fluorescein isothiocyanate-conjugated $F(ab)'_2$ fragments of a goat anti-rabbit IgG antibody (Tago).

Absorptions. The anti-IL-6 antibody preparations were absorbed with equal volumes of purified human IL-6 (2 μg/ml) derived from IL-1-stimulated human fibroblasts (10). Absorptions were carried out for 1 hr at 37°C followed by overnight incubation at 4°C. Sham absorptions were carried

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Abbreviations: IL-1, -4, and -6, interleukins 1, 4, and 6, respectively; rIL-6, recombinant IL-6.

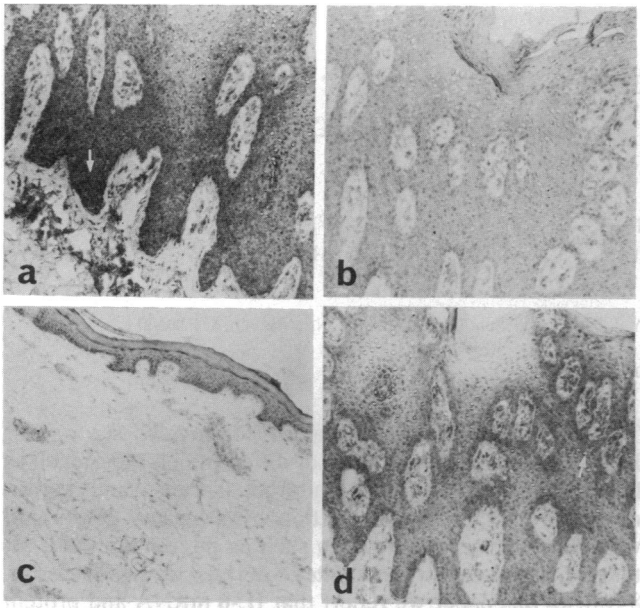

FIG. 1. Increased immunoreactive IL-6 in psoriatic plaques. (*a*) Active plaque shows cytoplasmic immunoperoxidase staining by the C-11 anti-rIL-6 antibody of most keratinocytes (white arrow), the dermal infiltrate, and endothelial cells. (*b*) Staining of the same plaque with preimmune serum shows only trace reactivity. (*c*) Uninvolved skin from the same patient demonstrates little reactivity. (*d*) Staining of the same active plaque with a different commercial anti-rIL-6 shows cytoplasmic staining of most keratinocytes (white arrow), the dermal infiltrate, and endothelial cells. (×85.)

out under the same conditions with bovine serum albumin at 2 μg/ml.

Proliferation Assays. Fifty thousand normal human keratinocytes were seeded into each chamber of a 24-well tissue culture plate and were allowed to attach and spread for 16–24 hr in modified MCDB 153 medium supplemented with defined growth factors (keratinocyte growth medium, Clonetics, San Diego, CA). To assess growth inhibition, fresh growth medium containing human rIL-6 at 10 ng/ml (Genzyme) or growth medium containing human recombinant γ interferon at 20 ng/ml (Collaborative Research) was added. To assess growth stimulation, growth medium was replaced with basal medium (keratinocyte basal medium, Clonetics) or basal medium containing growth factors. After 24-hr cell proliferation was assessed by incorporation of [6-methyl-^{3}H]thymidine (4 μCi/ml, 4-hr pulse; 1 Ci = 37 GBq). Proliferation was also assessed at 48 hr after factor addition by trypsinization and counting of cells in a hemocytometer.

Bioassays for IL-6 Detection in Plasma. IL-6 in plasma samples from patients with active psoriasis was assayed by using the B9 hybridoma proliferation assay as described (6) or the hepatocyte-stimulating factor assay with the Hep3B2 cell line (10).

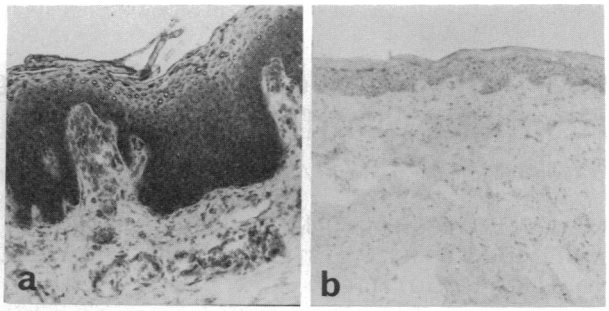

FIG. 2. Medical therapy decreases immunoreactive IL-6 in plaques. (*a*) Active plaque shows diffuse cytoplasmic staining of keratinocytes, the dermal infiltrate, and endothelial cells. (*b*) After 4 weeks of topical tar and UV B therapy, acanthosis and IL-6 reactivity are no longer present. (×85.)

***In Situ* Hybridization.** Cryostat sections were air dried and fixed for 20 min in 4% (wt/vol) paraformaldehyde. Prehybridization and hybridization were performed as described (13). The pBSF2.38 cDNA probe (14) was subcloned in pGEM4 (Promega). Antisense and sense RNA were transcribed with T7 or SP6 RNA polymerases.

RESULTS

Increase in Immunoreactive IL-6 in Psoriatic Plaques. Keratinocytes, endothelial cells, and most cells of the dermal infiltrate in psoriatic plaques from 35 patients were reactive with the C-11 polyclonal anti-rIL-6 antibody. In all cases the staining pattern was cytoplasmic. In some instances nuclear and plasma membrane staining were also seen. Basal keratinocytes stained more intensely than spinous keratinocytes (Fig. 1*a*). Staining with the preimmune serum showed only trace reactivity (Fig. 1*b*). In 14 of 17 patients, IL-6 staining in plaques was greater in intensity and/or density than in normal-appearing skin from the same patients or from normal volunteers (Fig. 1*c*). Of the three plaques in which IL-6 staining was not increased, one was obtained from a patient treated with systemic antiinflammatory drugs, which may have played a role in decreasing IL-6 reactivity. Why the IL-6 staining in the remaining two plaques was not increased is unclear. There was no unifying clinical characteristic to account for the findings in these three patients. Staining of psoriatic plaques with the commercially available polyclonal anti-IL-6 antibody confirmed the IL-6 immunoreactivity detected with the C-11 anti-rIL-6 antibody (Fig. 1*d*).

In 10 patients, biopsies of psoriatic plaques were obtained before and after medical therapy. In seven of these patients,

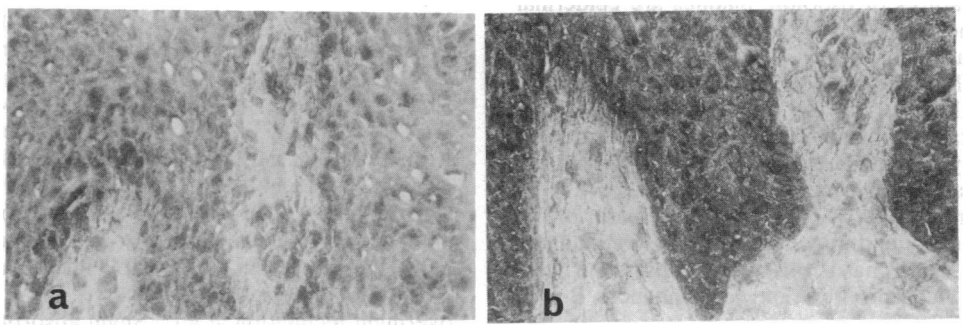

FIG. 3. Preincubation of the anti-rIL-6 antibody with IL-6 reduces staining activity. (*a*) Plaque treated with the anti-rIL-6 antibody absorbed with purified human IL-6 shows diminished immunoperoxidase staining of both the epidermis and dermis. (*b*) Plaque treated with the anti-rIL-6 antibody absorbed with bovine serum albumin shows cytoplasmic staining of both the epidermis and dermis. (×360.)

there was a decrease in IL-6 staining in plaques after therapy (Fig. 2). Posttreatment plaques showed a decrease in scale, erythema, and skin thickness. Histologically these plaques showed a decrease in hyperkeratosis, acanthosis, and dermal infiltration. Analysis of skin biopsies of the three remaining patients taken before and after treatment showed little change in IL-6 staining. Comparison of pre- and posttreatment plaques in two of these patients revealed only a minimal decrease in hyperkeratosis and acanthosis, possibly accounting for the persistent increased IL-6 reactivity.

To confirm the specificity of both anti-rIL-6 antibody preparations, absorption experiments were performed with purified IL-6 derived from IL-1-stimulated human fibroblasts (10). Absorption of the commercial anti-rIL-6 antibody with purified human IL-6, but not with bovine serum albumin removed most staining activity (Fig. 3). Similar results were obtained with the C-11 anti-rIL-6 antibody and with *E. coli*-derived rIL-6 (data not shown).

Sixty-one ± 23% of keratinocytes cultured in serum-free medium from psoriatic plaques from three patients were stained by the C-11 anti-IL-6 antiserum in immunoperoxidase studies. In contrast, only 35 ± 2% of keratinocytes cultured from uninvolved skin from two of these patients, and only 40 ± 2% of keratinocytes cultured from the skin of two normal individuals were stained by the anti-IL-6 antiserum. Therefore, the increased IL-6 staining seen in sections of psoriatic plaques still remained after culture of these keratinocytes *in vitro*. These data suggest a higher frequency of IL-6 expression by keratinocytes derived from psoriatic plaques.

Expression of IL-6 Transcripts in Psoriatic Plaques. The presence of IL-6 mRNA in cells of psoriatic skin lesions was evaluated using the *in situ* nucleic acid hybridization technique. Hybridization with an antisense RNA probe (Fig. 4*a*), but not with a sense RNA probe, revealed that most epidermal cells and cells in the dermal infiltrate expressed IL-6 mRNA. More autoradiographic grains were found in basal keratinocytes than in mature spinous keratinocytes. The level of IL-6 mRNA in active psoriatic plaques was greater than that in plaques after therapy and in normal-appearing skin (data not shown). These results show that the IL-6 gene is actively expressed in psoriatic skin lesions.

Subcellular Localization of Anti-IL-6 Staining in Cultured Human Keratinocytes. Reaction of the C-11 anti-IL-6 antise-

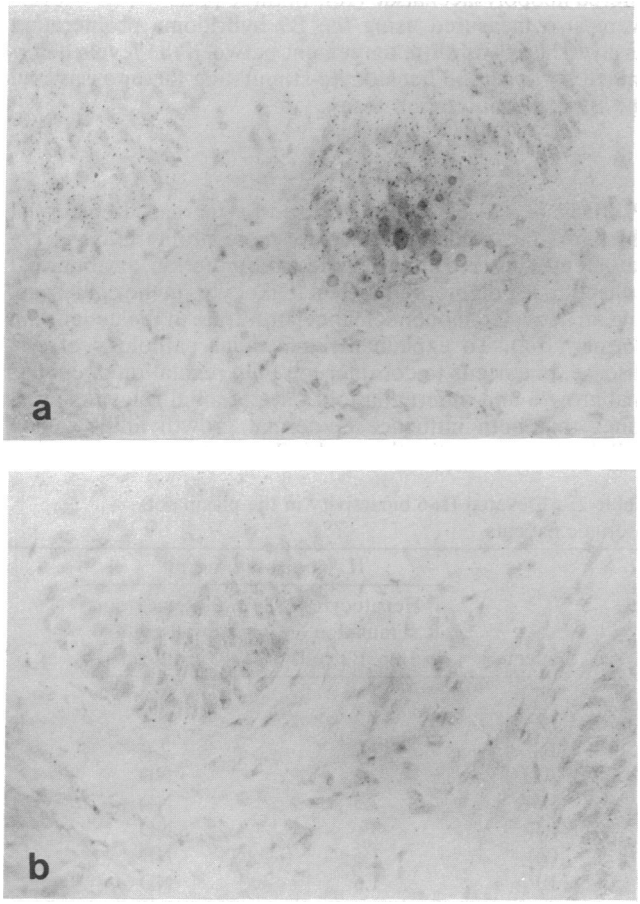

FIG. 4. Detection by *in situ* hybridization of IL-6 mRNA in psoriasis plaques. Fixed sections were hybridized with the anti-sense cRNA probe (*a*) and sense cRNA probe (*b*). (×190.)

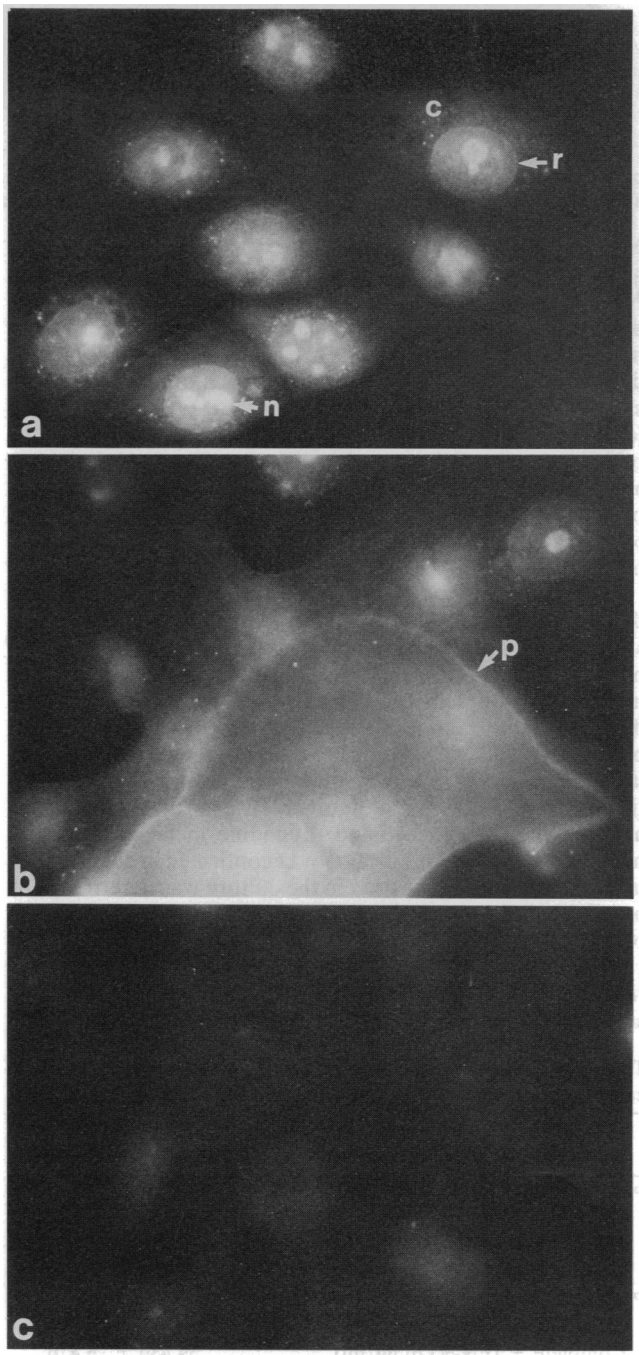

FIG. 5. Immunofluorescence staining of cultured human keratinocytes with the anti-rIL-6 antibody. (*a*) Basaloid human keratinocytes show nuclear rim (r), nucleolar (n), nucleoplasmic, and cytoplasmic (c) staining. (*b*) Keratinocytes beginning to stratify show additional plasma membrane (p) staining. (*c*) Preimmune serum shows no reactivity. (×410.)

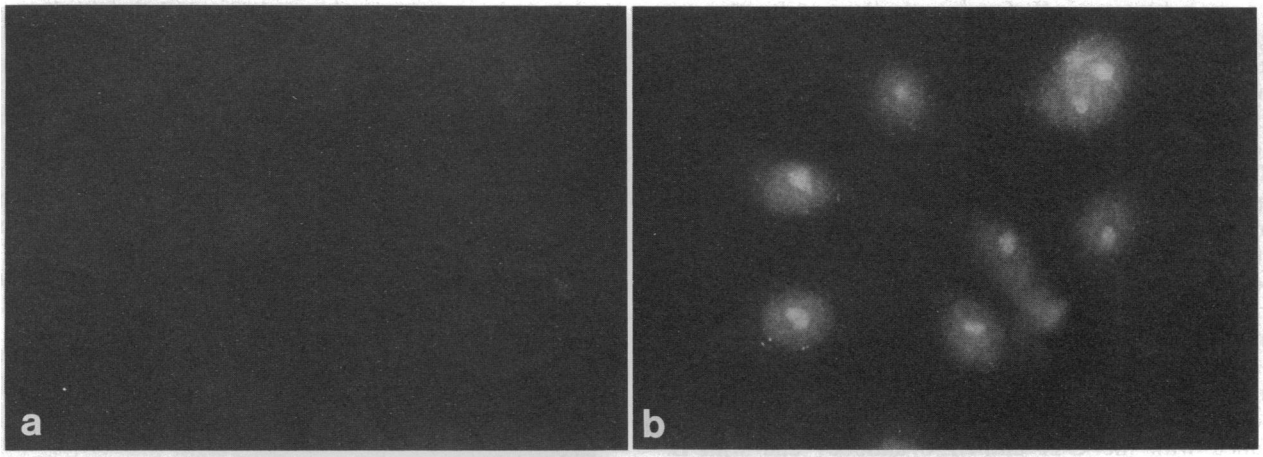

FIG. 6. Absorption with IL-6 removes immunofluorescence staining by anti-rIL-6 antibody. (*a*) The C-11 anti-rIL-6 antibody absorbed with purified human IL-6 shows decreased nuclear and cytoplasmic staining. (*b*) Immunofluorescence of keratinocytes treated with anti-rIL-6 absorbed with bovine serum albumin shows undiminished nuclear and cytoplasmic staining. (×410.)

rum with cultured human keratinocytes showed both nuclear and cytoplasmic immunofluorescence reactivity (Fig. 5*a*). Nuclear rim, nucleolar, and nucleoplasmic staining was evident; diffuse fluorescence was seen in the cytoplasm. Little or no membrane staining of cells with a basaloid morphology was detected. However, keratinocytes beginning to stratify showed a clear plasma membrane-staining pattern in addition to cytoplasmic and nucleolar fluorescence (Fig. 5*b*). The preimmune serum did not stain (Fig. 5*c*). The C-11 antiserum absorbed with IL-6 showed a marked decrease in nuclear rim, nucleolar, and cytoplasmic fluorescence in basaloid cells (Fig. 6*a*). In contrast, antiserum absorbed with bovine serum albumin showed no change in these staining patterns (Fig. 6*b*).

rIL-6 Stimulates Keratinocyte Proliferation *in Vitro*. To determine whether IL-6 or an IL-6-inducible cytokine could stimulate keratinocyte growth, rIL-6 was added to cultures of normal human keratinocytes grown either in basal medium or in medium supplemented with other growth factors (epidermal growth factor, insulin, hydrocortisone, and bovine pituitary extract) (Table 1). When keratinocytes were grown under conditions in which their proliferation was minimal (i.e., in basal medium), addition of rIL-6 (10 units/ml) caused a 7-fold increase in tritiated thymidine incorporation. Enhancement of cell proliferation was confirmed in experiments in which the number of cells in the culture was determined 48 hr after addition of rIL-6. Enhancement of keratinocyte proliferation was seen by using rIL-6 expressed in either *E. coli* or yeast. When keratinocytes were grown in a medium rich in other growth factors, rIL-6 (100 units/ml) had little additional growth-promoting effect. Addition of γ interferon (500 units/ml) inhibited tritiated thymidine incorporation in these experiments. These experiments suggest that under certain experimental conditions, IL-6 preparations can enhance keratinocyte proliferation.

Table 1. IL-6 stimulates the proliferation of growth-arrested cultured human keratinocytes

Culture condition	^{3}[H]Thymidine incorporation, cpm
Prior growth in basal medium	
Basal	7,020 ± 630
Basal + rIL-6 (10 ng/ml)	54,310 ± 15,130
Complete	69,920 ± 8,460
Prior growth in complete medium	
Complete	160,490 ± 14,630
Complete + IFN-γ (20 ng/ml)	59,430 ± 6,470
Complete + rIL-6 (10 ng/ml)	168,400 ± 26,710

Experiments were done in triplicate. Basal medium was keratinocyte basal medium (Clonetics); complete medium was basal medium plus epidermal growth factor (10 ng/ml), insulin (5 μg/ml), hydrocortisone (0.5 μg/ml), and bovine pituitary extract (0.4% vol/vol); IFN-γ, γ interferon. Results represent mean ± SEM.

Increased Plasma IL-6 Levels in Psoriasis Patients. Plasma IL-6 levels were measured in active psoriasis patients by using two different bioassays for IL-6 (Table 2). With the hepatocyte-stimulating factor assay the average IL-6 level in eight patients was 3 ng/ml; this is comparable to levels seen in patients with acute bacterial infection or in those administered lipopolysaccharide (10). In three patients, IL-6 levels were also measured using the B9 hybridoma proliferation assay. There was close agreement between the levels determined by using the hepatocyte-stimulating factor assay and the B9 cell proliferation assay.

DISCUSSION

Psoriasis is a disease characterized by increased epidermal thickness (acanthosis), extremely rapid proliferation of keratinocytes, altered keratinocyte differentiation, an abnormal collection of polymorphonuclear leukocytes in the epidermis, and an activated mononuclear cell infiltrate in the underlying dermis (1–4). To explain the molecular pathology of this disease, one needs to consider not only regulation of epidermal growth and differentiation, but potential roles for cytokines that both influence epidermal growth and regulate cellular immune activation and inflammation.

Table 2. Elevated IL-6 bioactivity in the plasma of psoriatic patients

Patient	IL-6 bioassay, ng/ml: Hepatocyte stimulation in Hep3B2 cells	IL-6 bioassay, ng/ml: Plasmacytoma proliferation in B9 cells
PG	4	5
LC	2	2.5
JH	2	2.8
AM	2.5	ND
KW	7.5	ND
RB	3	ND
GB	1.5	ND
JP	1.5	ND

Plasma and serum samples from normal volunteers had no detectable IL-6 bioactivity; these assays detect IL-6 at concentrations >1.5 ng/ml (6, 9, 10). ND, IL-6 levels could not be determined because of nonspecific inhibitory activity in these samples.

The essential histological features of psoriasis are maintained in transplanted tissue (2), suggesting that local factors in a psoriatic plaque are sufficient for its maintenance. Many of the cellular features of psoriasis could be generated by growth factors or cytokines produced in psoriatic skin. IL-6, transforming growth factor α, and IL-1_α are autocrine growth factors that are synthesized by keratinocytes, as well as other cell types, and that stimulate the growth of cultured human keratinocytes (6, 12, 15–18). Our observation that IL-6 enhances keratinocyte proliferation under appropriate experimental conditions raises the possibility that IL-6 may contribute to the epidermal hyperplasia seen in the psoriatic lesion.

Apparent overexpression of IL-6 in hyperplastic psoriatic tissue may explain features of psoriasis that link keratinocyte proliferation with immune activation and tissue inflammation. Thus, IL-6 could help activate the cellular elements in the local inflammatory infiltrate (T cells, macrophages, and polymorphonuclear leukocytes), leading to an exacerbation of the local lesion (8). The observations that IL-6 is a major inducer of C-reactive protein gene expression in the hepatocyte (8, 19) and is highly pyrogenic (8, 19) suggest that characteristic systemic features of erythrodermic and Von Zumbusch types of psoriasis, such as fever and elevated plasma levels of C-reactive protein, may be at least partially mediated by IL-6.

Transforming growth factor α is expressed at high levels in hyperplastic psoriatic skin (12, 20). Although transforming growth factor α is a well-documented mitogen for keratinocytes and other cell types, it has no known function in regulating the immune response or tissue inflammation. Previous studies have shown that IL-1 bioactivity is decreased in hyperplastic psoriatic tissue (21).

Elevated expression of IL-6 mRNA and protein was observed in epidermal and dermal cells in psoriatic plaques. Elevated IL-6 levels were also seen in the plasma of psoriasis patients. The observed increase in IL-6 levels in the peripheral circulation is most likely due to enhanced production of IL-6 in psoriatic lesions. The number of plasma samples studied was too small to determine whether there was any correlation between total lesion mass and plasma level of IL-6. Although the stimulus for the observed increase in IL-6 expression in psoriatic plaques is unknown, several cytokines, such as IL-1, tumor necrosis factor, and IL-4 enhance IL-6 gene expression in keratinocytes (6, 8, 22).

The observation that IL-6 stimulates keratinocyte growth may have relevance to other cutaneous disorders characterized by epidermal hyperplasia. Epidermal growth activation in wound healing produces a keratinocyte phenotype similar to that seen in psoriatic tissue (23). Within hours to days after epidermal wounding, mononuclear and polymorphonuclear cellular elements accumulate in the underlying dermis and are present throughout the healing response (24). We have seen increased IL-6 levels in the hyperplastic epidermis at the edges of nonhealing wounds when compared with that seen in uninvolved skin from the same patients. It is tempting to speculate that other skin disorders associated with epidermal hyperplasia and dermal mononuclear cell activation, such as lichen planus, may be linked, in part, by expression of IL-6 or other cytokines. It appears likely that complex keratinocyte–lymphohistiocytic cytokine regulatory interactions are involved in cellular activation and growth factor/cytokine expression in psoriasis and a number of cutaneous diseases.

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