Acta Derm Venereol (Stockh) 1992; 72: 165–168

Interleukin-6 in Normal Skin and Psoriasis

A. CASTELLS-RODELLAS[1], J. V. CASTELL[2], A. RAMIREZ-BOSCA[3], J. F. NICOLAS[4], F. VALCUENDE-CAVERO[5] and J. THIVOLET[4]

[1]Service of Dermatology, Hospital General Vall D'Hebron, Barcelona, [2]Department of Biochemistry, Faculty of Medicine, Valencia, and [3]Dermatology Unit, Faculty of Medicine, Valencia, Spain; [4]Dermatology and Immunology Research Laboratory, Hôpital E. Herriot, Lyon, France; and [5]Service of Internal Medicine, Hospital Gran Vía, Castellón, Spain

Interleukin-6 (IL-6 or BSF-2/IFNβ_2) is a component of normal human skin. IL-6 was immunologically detected in basal keratinocytes, endothelial cells and in a number of mononucleated cells and fibroblasts in normal skin and sudoriparous ducts. In psoriasis, intense labelling of the cytoplasm in the vicinity of keratinocyte membranes was detected in all epidermal layers and other skin appendages. The fact that this interleukin acts synergistically with respect to IL-1 and Tumour Necrosis Factor (TNF) strengthens the hypothesis whereby IL-6 may contribute via its receptor action to EGF function in modulating cell hyper-proliferation in psoriasis. ***Key words: Inflammation; Immunohistochemistry.***

(Accepted September 9, 1991.)

Acta Derm Venereol (Stockh) 1992; 72: 165–168.

A. Castells-Rodellas, Service of Dermatology, Hospital General Vall D'Hebron, Passeig Vall d'Hebron s/n, 08035 Barcelona, Spain.

Interleukins (ILs) are inflammatory mediators and immunomodulators. Antigen-presenting cells (APCs) and macrophages produce (IL-1(1), while the epidermis produces Epidermal T-Activator Factor (ETAF) or IL-1-like (2). Additional cytokines have been detected in a number of dermatoses (3).

Interleukin-6 (IL-6), also known as B-C cell Stimulatory Factor-2 (BSF-2), is a lymphokine structurally identical to β_2-inteferon (IFN-β_2) (4); it is synthesized by fibroblasts, monocytes, T-cells and several other cell lines (5). In fibroblasts, IL-1 (6) and Tumour Necrosis Factor (TNF) (7) stimulate IL-6 production. In turn, IL-6 stimulates the synthesis of acute-phase proteins (8); immunoglobin production on the part of B-cells and transformed B-cells (9) induces the differentiation of T-cytotoxic cells (10), regulates T-cell proliferation (11) and increases the number of IL-2 receptors present on thymocytes (12). IL-6 activity is in some cases synergic with respect to IL-1 action (13), and IL-1 has in turn been shown to stimulate IL-6 production on the part of fibroblasts – this suggesting the existence of a possible amplification effect on the action of IL-1 (14). Recently, Sironi et al. (15) have shown IL-1 to stimulate endothelial cell synthesis of IL-6 that is not mitogenic to endothelial cells and does not induce PGI_2 or prostacyclin production.

Psoriasis involves a local immune alteration (16,17) with inflammatory reaction and the release of cytokines and mediators. In the present work we applied immunohistochemical techniques to study the presence and location of IL-6 in both normal skin and in lesional and non-lesional psoriasis.

MATERIAL AND METHODS

Patients

Fourteen patients (8 males and 6 females) aged 12–58 with untreated psoriasis confirmed both clinically and histologically were studied. Ten had psoriasis vulgaris, one flexural psoriasis, one psoriasis of the penis, and 2 had psoriasis guttata. Six normal skin biopsies (controls) were obtained from patients without familial or personal data of psoriasis but who were to undergo plastic and reconstructive surgery.

Antibody

Recombinant IL-6 was obtained by genetic manipulation of *E. coli* (9). Samples were initially provided by Dr Kishimoto (Japan).

Anti-human IL-6 polyclonal antibody was obtained in rabbit after repeated immunization with recombinant human IL-6 (9) emulsified in Freund's adjuvant. Following the 5th and 6th booster immunization (about 4 months), the serum titre was found to be highest, as assessed by direct ELISA in IL-6 coated plates. Antibodies were purified by ammonium sulphate precipitation (50% saturation), then dialysed against 5 mM phosphate buffer (pH 7.2) and subjected to DEAE chromatography. The column was eluted with the same buffer. The non-retained fraction contained essentially IgGs. The antibody-containing solution was adjusted to the initial volume of the serum sample from which it was obtained and the precipitate stored as described elsewhere (18). Antibody was tested via a competitive RIA against human interleukin 1α, interleukin 1β, TNF α, and γ-interferon. None was able to displace [^{125}I]interleukin-6 (18) at molar concentrations 1000-fold higher. Pre-immune rabbit serum had no measurable titre against human IL-6.

Other reagents

Other reagents employed were phosphate-buffered saline (PBS: 50 mM phosphate, 0.9% NaCl, pH 7.4) (Bio-Merieux, France), avidin-biotin-peroxidase amplification system (Vestastain ABC Kit (Rabbit IgG), Vector, Burlingame, Calif., USA), and 3-amino-9-ethyl-carbazole (Sigma, St. Louis, Mo., USA).

Specimens

Biopsy specimens of lesional and non-lesional skin were obtained with a 6-mm punch. Each specimen was divided into two portions: one was frozen in liquid nitrogen and stored at −50°C, while the other was fixed in buffered isotonic 10% formaldehyde.

Techniques

The specimen fixed in formaldehyde was embedded in paraffin wax, after which 4-µm sections were cut and stained with hematoxylin-eosin.

Five-µm cryostat sections were cut from each frozen specimen. The sections were then fixed at −20°C in acetone before incubation with polyclonal antibody; some sections were incubated with preimmune rabbit serum purified in the same way as polyclonal anti-IL-6. Briefly, the following steps were then performed: (*a*) Incubation with hydrogen peroxide (0.3%, 30 min) to block endogenous peroxidase; (*b*) Washing with PBS for 15 min; (*c*) Incubation with normal horse serum for 20 min; (*d*) Incubation with polyclonal antibody with anti-IL-6 diluted to 1 : 20 in PBS for one hour, some sections being with either preimmune rabbit serum or with anti-IL-6 plus IL-6 treated serum diluted to 1 : 20 in PBS; (*e*) Washing with PBS for 15 min; (*f*) In-

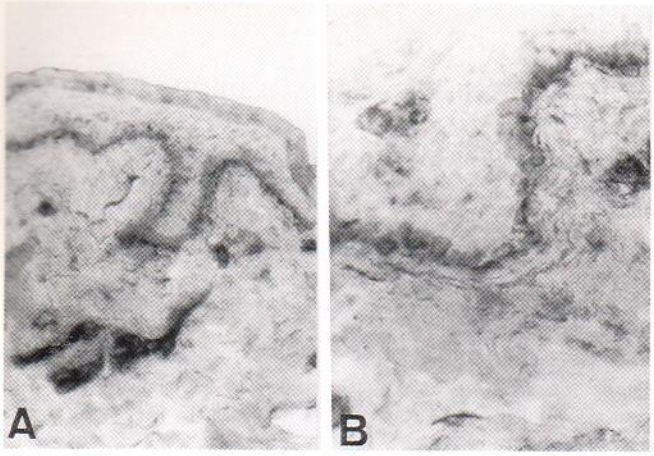

Fig. 1. Normal skin. Anti-human IL-6. The basal keratinocytes showed intense marking. Endothelial cells also showed marking. (*A*, original 200×), (*B*, original 600×).

cubation with biotinylated anti-rabbit antibody for 45 min; (*g*) Washing with PBS for 15 min; (*h*) Incubation with avidin-biotin-peroxidase complex for 45 min; (*i*) Washing with PBS for 15 min. Sections were then incubated with a solution containing 5 mg of 3-amino-9-ethylcarbazole and 200 µl of hydrogen peroxide per 10 ml of acetate buffer (pH 5.2) to visualize the site of antibody binding. After a final washing, the sections were counterstained with Mayer's hematoxylin and mounted in gelatin/glycerin medium.

RESULTS

Hematoxylin-eosin staining revealed the typical morphology and structure of normal skin. In all cases of psoriasis, histopathology was characteristic of the disease.

Normal skin

The basal keratinocytes, and particularly those contacting with the dermoepidermal junction showed intense marking (Fig. 1A, B); this was hardly appreciable in the cytoplasm of keratinocytes within the spiny layer. The keratinocyte nuclei were apparently not marked.

In all sections of the dermis we noted a strong marking of the cytoplasm of endothelial cells in the papillar vessels, e.g., in the mid- and deep dermis; a number of round mononucleated cells distributed in an anarchic and irregular fashion were also seen in the dermis, together with other, elongated and equally scarce cells compatible with fibroblasts. Some intraepidermal sudoriparous ducts also showed marking, although of less intensity than the endothelial cells.

Psoriatic skin

Lesional skin. The pattern observed was common to all forms of psoriasis: all keratinocytes, regardless of their level within the epidermis, revealed IL-6 within the cytoplasm and membrane with a much greater intensity than in normal skin (Fig. 2A, B). Only in some sections (3/15), and in addition to this pattern, were basal keratinocytes seen to stain in a fashion similar to normal skin. Some keratinocytes of the parakeratotic layer exhibited nuclear reactivity.

Within the dermis, the endothelial cells were intensely marked both in the periphery and in the cytoplasm (Fig. 3A, B). In the infiltrate, a very small number of round cells distributed throughout the dermis presented a dyed cytoplasm. Some elongated forms, corresponding to fibroblasts, were seen distributed at different levels in the dermis, exhibiting cytoplasmic staining. Some dyed sudoriparous ducts were also noted in these sections.

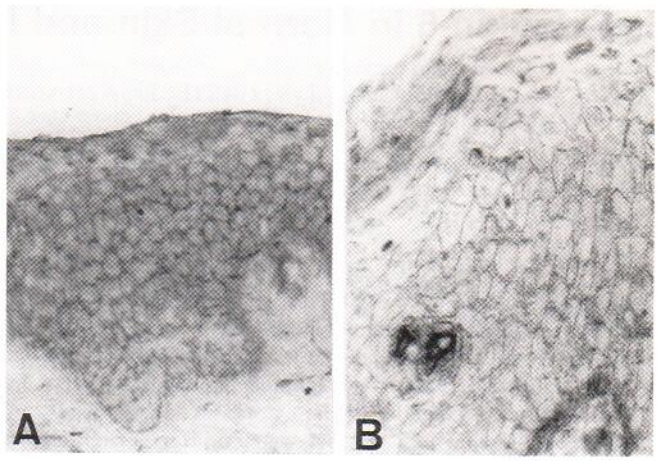

Fig. 2. Psoriatic skin. Anti-human IL-6. All keratinocytes revealed IL-6 within the cytoplasm. (*A*, original 400×), (*B*, original 600×).

Non-lesional skin. In the non-lesional areas of psoriatic skin, IL-6 distribution was very similar to that observed in normal skin.

The sections incubated with purified preimmune rabbit serum, and also the anti-IL-6 serum preincubated with IL-6, proved negative or else showed very weak immunostaining.

DISCUSSION

Psoriasis involves epidermal hyperproliferation related to inflammatory and immune phenomena within the dermis and epidermis. In an earlier study (16), we hypothesized the possible existence of a balance between an immune mechanism and inflammatory reaction, involving the release of mediators that induce an increase in keratinocyte mitotic index.

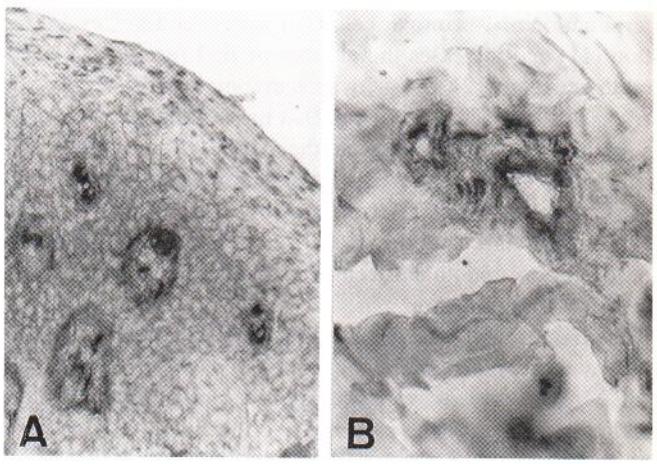

Fig. 3. Psoriatic skin. Anti-human IL-6. The endothelial cells in the papillar vessels were intensely marked. (*A*, original 400×), (*B*, original 600×).

IL-6 is believed to mediate or amplify the tissue effects of IL-1; thus, a number of effects previously attributed to IL-1 might in reality be originated by IL-6 or by both interleukins (19). As a counterpart, the presence of an IL-1 inhibiting factor in psoriatic lesions (20) might cause a corresponding increase in epidermal IL-6.

In a preliminary communication, Reitamo et al. (21) reported the presence of IL-6 in all layers of the normal epidermis, and mRNA analysis suggested that keratinocytes are unable to produce IL-6. Nevertheless, it has recently been shown that epidermal keratinocytes are able to synthesize IL-6 (22) induced by IL-1 and TNF-α (23), and one cytokine influences expression of the other. In sections of both normal and psoriatic skin, we noted cytoplasmic staining along the cell membrane, thus confirming that keratinocytes secrete IL-6 and, like hepatocytes, may possess receptors on which IL-6 fixes – thus triggering autocrine activity.

Krueger et al. (24) reported that the number of mitoses observed in keratinocytes increases considerably when the cells are cultured in a defined medium without serum or epidermal growth factor (EGF) but to which IL-6 is added. However, if EGF or γ-interferon is added, cell proliferation decreases. This may be interpreted as IL-6 causing an internalization of receptors for EGF – a process in which protein-kinase C was not found to be involved (24). The multifunctional cytokines IL-1, IL-6 and TNF stimulate phosphorylation of the receptor for EGF (EGF-R), which as a result either reduces its activity or modifies its function; although this effect has not been demonstrated for IL-6, the hypothesis of IL-6 binding to the membrane of keratinocytes and down-regulating EGF-R is attractive (25). Nanney et al. (26) reported the presence of these receptors in all layers of the psoriatic epidermis. This suggests that perhaps the function of IL-6 bound to the keratinocyte cell membrane may be intended to reduce the number of EGF-Rs or to block the latter. In the normal epidermis, IL-6 is located in the basal keratinocytes (facing basal – where the presence of EGR-R is greatest), which reinforces this hypothesis.

The cytoplasmic staining of the endothelial cells reveals the stimulation of IL-6 synthesis – most likely as a result of the continuous release of IL-1 (14). The mononucleated cells, in whose cytoplasm immunoreactive IL-6 is observed, might be considered to be macrophages that synthesize small amounts of IL-6. The same may be said of the stained fibroblasts.

The presence of immunoreactive IL-6 in the membranes of cells of the sudoriparous duct could be the result of either synthesis or capture.

Castell et al. (27) in an 'in vivo' animal model, observed that the injection of exogenous recombinant human IL-6 labelled with ^{125}I was followed by its deposition, or by deposition of a degradation product of IL-6, in the collagen bundles of the dermis – thus reinforcing the hypothesis that skin may be the site of IL-6 catabolism. In both normal and psoriatic skin, IL-6 is observed in the epidermal keratinocytes and endothelial cells of the dermis, although it is not found in collagen – thus leading us to think that IL-6 is synthesized, and performs its activity 'in situ'.

Although further studies are required regarding IL-6 activity and metabolism, our results already show it to be involved in the immune and inflammatory reactions of the skin.

REFERENCES

1. Sauder DN, Dinarello CA, Morhenn VB. Langerhans cell production of Interleukin-1. J Invest Dermatol 1984; 82: 605–607.
2. Luger TA, Stalder BM, Katz SI, Oppenheim JJ. Epidermal cell (keratinocyte) derived thymocyte-activating factor (ETAF). J Immunol 1981; 127: 1493–1498.
3. Luger TA, Danner M, Schwartz T, et al. Epidermal cell derived mediators of immunity and inflammation. In: Caputo R, ed. Immunodermatology. Rome: CIC Edizioni Internazionali, 1987; 63–66.
4. Van Damme J, Van Beeumen B, Decock J, et al. Separation and comparison of two monokines with lymphocyte activating factor activity: IL-1β and hynridoma growth factor (HGF): Identification of leukocyte-derived HGF as IL-6. J Immunol 1988; 140: 1534–1540.
5. Kishimoto T, Hirano T. Molecular regulation of B lymphocyte response. Annu Rev Immunol 1988; 6: 485–512.
6. Van Damme J, Cayphas S, Opdenakker G, et al. Interleukin 1 and poly (rI). poly (rC) induce production of a hybridoma growth factor by human fibroblasts. Eur J Immunol 1987; 17: 1–5.
7. Kohas M., Hendricksen-DeStefano D, et al. Induction of β2-interferon by tumor necrosis factor: a homeostatic mechanism for the control of cell proliferation. Cell 1986; 45: 659–661.
8. Castell JV, Gomez-Lechón MJ, David M, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Letts 1989; 242: 237–239.
9. Hirano T, Yasukawa K, Harada H, et al. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature (Lond) 1986; 324: 73–75.
10. Okada M, Kitahar M, Kishimoto S, et al. IL-6/BSF-2 functions as a killer helper factor in the in vitro induction of cytotoxic T cells. J Immunol 1988; 141: 1543–1549.
11. Garman RD, Jacobs KA, Clark SC, Raulet DH. B-cell-stimulatory factor 2 (β2 interferon) functions as a second signal for interleukin 2 production by mature murine T cells. Proc Natl Acad Sci USA. 1987; 84: 7629–7631.
12. Le J, Frederickson G, Reis LFL, et al. Interleukin 2-dependent and interleukin 2-independent pathways of regulation of thymocyte function by interleukin 6. Proc Natl Acad Sci USA 1988; 85: 8643–8647.
13. Takai Y, Wong GG, Clark SC, et al. B cell stimulatory factor-2 is involved in the differentiation of cytotoxic T lymphocytes. J Immunol 1988; 140: 508–512.
14. May LT, Ghrayeb J, Santhanam U, et al. 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 1988; 263: 7760–7765.
15. Sironi M, Breviario F, Proserpio P, et al. IL-1 stimulates IL-6 production in endothelial cells. J Immunol 1989; 142: 549–553.
16. Ramirez-Boscá A, Martinez-Ojeda L, Valcuende-Cavero F, Castells-Rodellas A. A study of local immunity in psoriasis. Brit J Dermatol 1988; 119: 587–595.
17. Voorhees JJ. Leukotrienes and other lipoxygenase products in the pathogenesis and therapy of psoriasis and other dermatoses. Arch Dermatol 1983; 119: 541–547.
18. Montoya A, Castell JV. Long-term storage of peroxidase labelled immunoglobulins for use in enzyme immunoassay. J Immunol Meth 1987; 99: 13–20.
19. Elias JA, Trichieri G, Beck JM, et al. A synergistic interaction of IL-6 and IL-1 mediates the thymocyte-stimulating activity produced by recombinant IL-1-stimulated fibroblasts. J Immunol 1989; 142: 509–514.
20. Hammerberg C, Fisher G, Baadsgaard O, Voorhees JJ. Characterization of IL-1 and inhibitor in psoriatic skin. J Invest Dermatol 1989; 92: 439. Abstract.

21. Reitamo S, Asikainen KE, Lauerma AI, et al. Interleukin-6 in normal human skin. J Invest Dermatol 1989; 92: 506. Abstract.
22. Grossman RM, Krueger J, Yourish D, et al. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA, 1989; 86: 6367–6371.
23. Akira S, Hirano T, Taga T, Kishimoto T. Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). FASEB 1990; 4: 2860–2867.
24. Krueger J, Krane J, Gottlieb AB, et al. Cytokine modulation of epithelial function: Contrasting effects of interleukin-6/β2-interferon and γ-interferon on human keratinocyte growth and differentiation. J Invest Dermatol 1989; 92: 464. Abstract.
25. Bird TA, Saklatvala J. IL-1 and TNF transmodulate epidermal growth factor receptors by a protein kinase C-independent mechanism. J Immunol 1989; 142: 126–133.
26. Nanney LB, Stosheck ChM, Magid M, et al. Altered (^{125}I)Epidermal Growth Factor binding and receptor distribution in psoriasis. J Invest Dermatol 1986; 86: 260–265.
27. Castell JV, Geiger TH, Gross V, et al. Plasma clearance organ distribution and target cells of interleukin-6/hepatocyte-stimulating factor in the rat. Eur J Biochem 1988; 177: 357–361.