DETECTION OF A γ INTERFERON-INDUCED PROTEIN
IP-10 IN PSORIATIC PLAQUES

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The demonstration of activated T lymphocytes and HLA-DR+ keratinocytes in psoriatic plaques suggests that delayed cellular immune responses may play an important role in the pathogenesis of psoriasis (1-3). γ-Interferon (IFN-γ) is an important cytokine, produced by activated T lymphocytes, which elicits many features of delayed cellular immune responses in vivo. These include induration, mononuclear cell infiltration, keratinocyte proliferation, Langerhans cell redistribution, and induction of keratinocyte HLA-DR expression (4). Additionally, IFN-γ induces keratinocyte HLA-DR expression in vitro (5). IP-10 is a cytokine the expression of which, in keratinocytes and other cells, is induced by IFN-γ (6, 7). It is structurally homologous to a family of proteins with chemotactic and mitogenic activities (8). IP-10 has been detected during the development of cutaneous delayed cellular immune responses (9). To further study the role of these reactions in the pathogenesis of psoriasis, active plaques were tested for the presence of the IP-10 protein. We now report that IP-10 was detected in keratinocytes from active psoriatic plaques.

Material and Methods

Patients and Skin Biopsies. Biopsies of normal skin and of lesional and normal-appearing skin from 34 patients with psoriasis vulgaris, 2 patients with erythrodermic psoriasis, and 1 patient with pustular psoriasis of the von Zumbusch type were taken after informed consent was obtained in accordance with a protocol approved by the Rockefeller University Hospital Institutional Review Board. 16 patients received treatment with topical tar and ultraviolet B irradiation, 1 received etretinate (Hoffman-La Roche, Nutley, NJ) and 1 received topical corticosteroids. Treatment continued for 3-4 wk, with marked clinical improvement in scale, erythema, and thickness, before obtaining repeat biopsies (1).

Immunoperoxidase Studies. Immunoperoxidase studies of formalin-fixed frozen skin sections with an affinity-purified rabbit anti-IP-10 antibody (7) were performed as previously described (1). Absorption experiments were performed by incubating the anti-IP-10 antibody with gel-purified recombinant IP-10 protein coupled to CNBr-activated Sepharose 4 B (Pharmacia Fine Chemicals, Piscataway, NJ) (7) overnight at 4°C. For competition experiments, the anti-IP-10 antibody was mixed with soluble IP-10 protein or an equal volume of phosphate buffered saline (PBS) just before incubation with the skin sections.

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RNA Isolation and Northern Blotting. Skin biopsies were snap frozen in liquid nitrogen and stored at -70°C. Total cellular RNA was isolated from these biopsies by the guanidinium isothiocyanate-cesium chloride method (10). 4-μg samples of RNA per biopsy specimen were fractionated on a 1% agarose gel containing 2.2 M formaldehyde, transferred to nitrocellulose, and hybridized with a 32P-labeled 355 bp FNU4H1 fragment of the IP-10 cDNA pIFN-γ31.7 containing only coding sequence (6). The γ-actin cDNA was a generous gift from Dr. Larry Kedes, Stanford University, Stanford, CA (11).

Immunoprecipitation Analysis. Cultured normal human keratinocytes were grown in serum-free low-calcium medium as previously described (12). 500 μCi of 35S-methionine (New England Nuclear, Boston, MA) were added to each 75-cm2 flask containing a confluent monolayer of keratinocytes, and 10 ml of methionine-free RPMI-1640 (Flow Laboratories, Inc., McLean, VA) plus 10% dialyzed FCS (HyClone Laboratories, Inc., Logan, UT). Human IFN-γ (100 U/ml), purified from peripheral blood buffy-coat white blood cells (13), was added to selected flasks. All flasks were incubated overnight at 37°C in 5% CO2. The supernatants were aspirated and saved. After three washes in PBS, the cells were lysed with 0.5% NP-40 plus 100 mM iodoacetamide, and immunoprecipitations performed as described (14).

Results

As previously described (7), antibody to IP-10 immunoprecipitates a 6-10 kD band from 35S-labeled normal keratinocytes (Fig. 1). This molecule can be identified both in cell lysates of labeled keratinocytes and in cell supernatants provided these cells are exposed to IFN-γ. Thus, IP-10 is an IFN-γ inducible protein produced by normal keratinocytes.

Keratinocytes in active plaques from 37 patients were reactive with the anti-IP-10 antibody by immunoperoxidase staining (Fig. 2a). The pattern of staining was cytoplasmic and basal keratinocytes were more intensely stained than were spinous keratinocytes. Varying percentages of cells in the dermal infiltrate were stained, but in all cases staining was less intense than that of the keratinocytes. This observation is consistent with quantitative immunoprecipitation studies, which demonstrated greater IFN-γ-induced IP-10 synthesis in keratinocytes as compared with monocytes.
FIGURE 2. Immunoperoxidase reactivity of lesional and uninvolved skin from a psoriasis patient with the anti-IP-10 antibody. (a) Plaque before treatment shows cytoplasmic staining of most of the keratinocytes. (b) Uninvolved skin from the same patient demonstrated staining of only the basal layer. (c) After 3-4 wk of topical tar and ultraviolet B therapy, only the basal epidermal layer of the plaque reacted with the anti-IP-10 antibody.

endothelial cells, and fibroblasts (9). Normal-appearing skin, taken from 20 of these psoriatic subjects before treatment, demonstrated staining of only the basal layer (Fig. 2b). In this respect, unaffected skin from psoriasis patients stained identically to skin from normal individuals.

In contrast with pretreatment plaques, only the basal epidermal layer of treated plaques reacted with the anti-IP-10 antibody in most cases (Fig. 2c). After 3-4 wk of therapy, all patients experienced marked improvement in clinical scaling, erythema, and thickness. Routine microscopy demonstrated significant reductions in epidermal acanthosis. As reported previously, treated psoriatic plaques had essentially no HLA-DR+ keratinocytes (1) and significantly fewer T lymphocytes bearing IL-2 receptors (1, 15) suggesting that treatment dampens the ongoing cellular immune response.

To confirm that staining with the anti-IP-10 antibody was specific, both absorption
and competition experiments were performed. Absorption with recombinant IP-10 protein removed all staining by the anti-IP-10 antibody of both psoriatic and nonlesional keratinocytes (Fig. 3). These observations were confirmed by competition experiments in which soluble IP-10 protein was added to the anti-IP-10 antibody just before incubation with the skin sections. IP-10 protein, but not an equal volume of PBS, removed reactivity of the antibody with both psoriatic and normal keratinocytes (data not shown).

Northern blot analysis was performed to determine if the increase in IP-10 staining is psoriatic plaques was associated with an increase in the steady-state level of the IP-10 mRNA. Total cellular RNA was isolated from patients' active psoriatic plaques, as well as from their normal-appearing skin. Identical amounts of RNA were subjects to RNA blot analysis using the IP-10 cDNA probe (Fig. 4). IP-10 mRNA was found in the RNA isolated from active plaques but was not detected in uninvolved skin from the same patients. When the identical blot was reacted again using a β-actin cDNA probe, there were similar levels of actin mRNA present in both active plaques and nonlesional skin. Similar results were obtained with biopsies of plaques and normal-appearing skin from three other patients. These findings suggest that the observed increase in IP-10 mRNA in lesional psoriatic skin could not be explained on the basis of a nonspecific increase in total mRNA content, and is instead the result of a specific increase in IP-10 mRNA in lesional skin.
FIGURE 4. Northern blot analysis of lesional and nonlesional skin from a psoriasis patient. Total cellular RNA was isolated from an active psoriatic plaque and from normal appearing skin from the same patient. Identical amounts of RNA were subjected to RNA blotting analysis using the IP-10 cDNA probe and a γ-actin cDNA probe. IP-10 mRNA was found in the RNA isolated from the active plaque (Plaque) but was not detected in uninvolved skin from the same patient (Normal). Similar levels of actin mRNA were present in both the plaque and nonlesional skin as can be seen when the identical blot was rehybridized with a γ-actin cDNA probe.

Discussion

IP-10, a cytokine the expression of which is induced by IFN-γ, is a member of a newly emerging family of soluble mediators, including chemotactic and platelet-granule proteins (platelet factor 4, β thromboglobulin, connective tissue-activating peptide III), proteins induced by transformation (9E3 and gro), proteins induced by growth factors (310c and KC), and known growth factors (melanocyte-stimulating factor and connective tissue-activating peptide III) (reviewed in 7, 8, 16). These proteins have chemotactic and mitogenic activities and are involved in inflammation and growth control.

IP-10 was detected in keratinocytes from active psoriatic plaques. Successful treatment of plaques by various means decreased expression of IP-10 in plaques. Activated T cells and HLA-DR+ keratinocytes have been detected in active psoriatic plaques (1–3). Since IP-10 is detected in delayed cellular immune responses (9), the present study further points to the role of ongoing cellular immune responses in the pathogenesis of psoriasis.

Based upon its structural homology to a number of proteins with chemotactic and growth promoting activities, one can speculate that IP-10 may have similar functions that play a role in the pathogenesis of psoriatic plaques. For instance, IP-10 may be a chemotactic stimulus for the neutrophils, monocytes and T lymphocytes that are abundant in active plaques. In addition, IP-10 might promote keratinocyte growth and be responsible in part for the keratinocyte hyperproliferation seen in psoriatic plaques. IFN-γ-induced increases in IP-10 synthesis may, in part, be responsible for the flaring of psoriasis that is associated with a number of conditions, e.g. streptococcal infection, reaction to certain drugs, and the acquired immunodeficiency syndrome.

The immunocytochemical detection of IP-10 in normal basal keratinocytes suggests that it plays a role in normal growth regulation. However, IP-10 mRNA is not detected in nonlesional skin. Additionally, IP-10 protein is not immunoprecipitated
from cultured normal human keratinocytes in the absence of IFN-γ, despite the fact that these cells are stained by the same antibody in immunoperoxidase studies (data not shown). Thus, the anti-IP-10 antibody may react with a crossreactive epitope expressed in basal keratinocytes that is unrelated to IP-10 protein. Alternatively, IP-10 mRNA expression in normal skin may be below the limits of detection by Northern blot analysis due to the limited expression of IP-10 in basal keratinocytes which make up only a small fraction of the biopsy samples.

Soluble mediators of inflammation, such as IFN-γ, produced in psoriatic lesions and during delayed cellular immune responses, may generate activated keratinocytes. We postulate that the principal functions of these cells are increased cell division, secretion of cytokines, and increased motility. Cytokines could influence keratinocyte growth in an autocrine manner, as has been described for IL-1 (17) and transforming growth factor α (18, 19). Cytokines released from activated keratinocytes could also promote growth and differentiation of neighboring epidermal cells, e.g., melanocytes, Langerhans cells, and Thy-1⁺ dendritic cells. For instance, keratinocyte granulocyte/macrophage colony-stimulating factor (GM-CSF) increases Langerhans cell differentiation in vitro (20, 21). Additionally, secretory products of keratinocytes such as IL-1 (22–24) and IL-6 (25–27) could activate monocytes and T cells in the underlying dermis. It is well known that increased keratinocyte motility is an integral part of the wound-healing process. After wounding, basal keratinocytes migrate to the injured area, and mitoses can be seen at the edge of the defect.

The active psoriatic plaque can be viewed as an aberrant local overproduction of activated keratinocytes. Thus increased levels of cytokines, specifically IP-10 protein and arachidonic acid metabolites (28) have been detected in active plaques. Keratinocyte HLA-DR expression has been demonstrated in psoriatic plaques as well as in other inflammatory cutaneous lesions (reviewed in 1), and in the cutaneous delayed-type hypersensitivity reaction to the purified protein derivative of tuberculin (29). Psoriatic plaques also exhibit altered epidermal growth factor receptor expression (30), keratin expression (31), plasminogen-activator distribution (32), 4F2/EL-2 expression (33), lectin reactivity (34), and increased protein kinase C activity (35). Moreover, keratinocytes isolated from psoriatic plaques demonstrate increased RNA content and increased numbers of cells within the S, G₂, and mitotic phases of the cell cycle when compared with uninvolved skin from the same patients or skin from normal individuals (33).

Thus, the elucidation of how inflammation and keratinocyte growth are interrelated has direct application to the understanding of a large number of inflammatory and hyperproliferative cutaneous diseases.

Summary

The pathologic features of psoriatic plaques are inflammation and increased epidermal turnover. IP-10, a cytokine the expression of which is induced by γ-interferon, is a member of a family of soluble mediators with inflammatory and growth-promoting activities. IP-10 protein was detected in keratinocytes and the dermal infiltrate from active psoriatic plaques using an affinity-purified rabbit anti-IP-10 antibody in immunoperoxidase studies. Successful treatment of active plaques decreased IP-10 expression in plaques. These results were corroborated by Northern blot analysis with an IP-10 cDNA probe. We have previously detected activated T cells and HLA-
DR⁺ keratinocytes in active psoriatic plaques. Since IP-10 is detected in delayed cellular immune responses, the present study further points to the role of ongoing cellular immune responses in the pathogenesis of psoriasis.

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