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Cellular Localization of Interleukin-8 and Its Inducer, Tumor Necrosis Factor-alpha in Psoriasis

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The importance of immunologic mechanisms in psoriasis has been deduced from the ability of immunosuppressive therapies to ameliorate this common and chronic skin disease. Certainly the histology of psoriatic lesions suggests a dialogue between the hyperplastic keratinocytes and infiltrating T lymphocytes and macrophages. To begin dissecting the cytokine network involved in the pathophysiology of psoriasis, the location, in both epidermal and dermal compartments, of tumor necrosis factor-alpha, interleukin-8, intercellular adhesion molecule-1, and transforming growth factor-alpha at the protein and/or mRNA levels were identified. Tumor necrosis factor-alpha was selected as a potentially key regulatory cytokine, first because it induces cultured keratinocyte interleukin-8, intercellular adhesion molecule-1, and transforming growth factor-alpha production, and second because intercellular adhesion molecule-1 expression by keratinocytes in psoriatic epidermis had been identified previously. Using immunohistochemical localization, tumor necrosis factor-alpha was identified in 12 psoriatic lesions as intense and diffuse expression by dermal dendrocytes (macrophages) in the papillary dermis (without significant staining of endothelial cells, mast cells, or dermal Langerhans cells), and focally by keratinocytes and intraepidermal Langerhans cells. Functional interaction between the dermal dendrocytes and keratinocytes was suggested by the presence of interleukin-8 expression of suprabasal keratinocytes immediately above the tumor necrosis factor-alpha–positive dermal dendrocytes. Interleukin-8 mRNA and transforming growth factor-alpha mRNA were detectable in the epidermal roof of psoriatic lesions, but neither was detectable at the protein or mRNA levels in any normal skin specimens. Treatment of cultured human keratinocytes with phorbol ester (which experimentally produces psoriasiform changes on mouse skin) or tumor necrosis factor-alpha also increased interleukin-8 and transforming growth factor-alpha mRNAs. Further elucidation of the cellular and molecular basis for the genesis and evolution of psoriasis will provide the framework for a better evaluation of the cause and treatment of this skin disease. (Am J Pathol 1991, 138:129–140)

Psoriasis is a chronic skin disease characterized histologically by prominent keratinocyte (KC) hyperplasia and an early inflammatory cell infiltrate that primarily includes T lymphocytes and macrophages (reviewed in Fry[1]). Most investigators studied the pathophysiology of psoriasis by focusing either on the growth-regulatory disturbances of the KC (emphasizing epidermal growth factor-receptor expression [EGFr] or transforming growth factor-α [TGF-α] production[2,3]) or delineation of the fluxes of mononuclear cell subsets during various phases (onset *versus* resolution) of psoriatic lesions.[4] We took a more holistic and integrative approach[5,6] by establishing that immunodulatory cytokines such as gamma interferon (IFN-γ) and tumor necrosis factor-alpha (TNF-α) not only affect KCs immunologically (eg, by induction of prostaglandin

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E2, class II major histocompatibility antigen, HLA-DR, and intercellular adhesion molecule-1 (ICAM-1) production) but also display growth-modulatory effects on KCs (such as increasing TGF-α production and altering EGFr surface expression). Tumor necrosis factor-α is known to accumulate in the epidermis *in vivo*[7] and potential cutaneous sources for TNF-α include the reticuloendothelial system,[7] mast cells,[8] and even KCs themselves.[9]

To establish a coherent sequence of molecular events responsible for the complex cellular associations in psoriasis involving T cells, Langerhans' cells (LCs), dermal dendrocytes, mast cells, and hyperplastic KCs, we studied the spatial distribution of interleukin-8 (IL-8), TNF-α, ICAM-1, and HLA-DR. Interleukin-8 and TNF-α are likely to be of particular importance following the observation that TNF-α is a potent inducer of IL-8 and also can induce ICAM-1 but not HLA-DR on cultured KCs.[10] The expression of IL-8 (a potent T-cell and neutrophil chemoattractant)[11] and ICAM-1 (the primary adhesion molecule allowing T-cell and monocyte/macrophage adhesion *via* lymphocyte function-associated antigen-1 [LFA-1]) is regulated coordinately by TNF-α in KCs at the transcriptional level.[10] Thus the spatial distribution of TNF-α, IL-8, and ICAM-1 would provide insight into the mechanism(s) responsible for the cellular pattern of trafficking within psoriatic lesions. A growing body of data supports the notion that the molecular basis for mononuclear cell trafficking in inflammatory and neoplastic skin diseases involves aberrant expression of various adhesion molecules, chemotactic factors, and their regulatory cytokines.[12] Because topical application of phorbol ester induces psoriasiform changes on mouse skin,[13] we determined the effects of treating cultured KCs with respect to IL-8 and TGF-α mRNA production.

The present report provides supportive evidence for reciprocal dermal–epidermal interactions involving activated KCs and lymphohistiocytic cell types mediated *via* TNF-α associated with increased expression of IL-8 and TGF-α in the pathophysiology of psoriasis.

Materials and Methods

Patients

Twelve psoriasis patients (age range, 24 to 59 years; 7 men, 5 women) with active lesions were biopsied (6-mm punch) after informed consent was obtained and the study approved by the University of Michigan Human Subjects Committee. All patients were untreated (or using emollients alone) for at least 3 weeks. The advancing, active margin of plaques on the lower back or buttocks were selected for biopsy. All patients had more than 70% total-body skin involvement (without arthritis or any other systemic illness) and reported a history of recent exacerbation of their psoriatic skin lesions. Six biopsies were obtained from normal healthy adult volunteers (age range, 35 to 68 years; 5 men, 1 woman) who were taking no prescription medications and who had no evidence of cutaneous or systemic illness. In our preliminary studies, keratome biopsies (two strips of skin measuring approximately $6 \times 2 \times 0.05$ cm) were taken from eight different psoriatic patients and eight different normal volunteers after informed consent was obtained.

Tissue Processing and Staining

Two 6-mm punch biopsies were obtained from each patient and one and a half of these were pooled for dermal–epidermal separation and RNA isolation, as described below; in two cases a small portion was fixed in 10% neutral buffered formalin before paraffin embedding. The remaining tissue was mounted on gum tragacanth (Sigma Chemical Co., St. Louis, MO), snap frozen in isopentane chilled in liquid nitrogen, and stored at −70°C. Five-micron–thick cryostat sections were stained using an avidin-biotin immunoperoxidase technique (Vectastain Kit-Vector Labs, Burlingame, CA), as previously described.[14] For double-labeling immunofluorescence studies, the cryostat sections were stained using both fluoroscein-conjugated and rhodamine-conjugated secondary antibodies (Tago Inc., Burlingame, CA) at 1:200 dilution, as previously described.[15] Texas-red conjugated avidin stain was purchased from Cappel (Malvern, PA) and used at 1:1000 dilution. All the histologic sections were examined using an Olympus BH-2 light microscope (Tokyo, Japan) equipped with an epifluorescence and photographic unit.

Dermal–Epidermal Separation, RNA Isolation, Northern Blotting, and KC Culture

The punch biopsies were immediately placed in 0.15 mol/l (molar) NaCl and warmed to 60°C for 2 to 4 minutes, as previously described by Jensen and coworkers,[16] to produce a dermal–epidermal 'split.' The epidermal roof was separated easily as a thin, near-translucent sheet from the bulky dermal fragment and placed in 2 ml of cold (4°C) guanidine hydrochloride (5.7 mol/l) containing potassium acetate (0.1 mol/l) followed immediately by tissue homogenization, sonication (60 seconds), and freezing to −80°C. The volume was increased to 5 ml by adding 3 ml of guanidine HCl solution, followed by the addition of 2.5 ml 100% ethanol, as previously described.[16] Nucleic acids were pelleted by centrifugation at 6000 rpm for 45 minutes in a JS 13.1 rotor. The pellets were redissolved in 0.9 ml of guanidine hydrochloride (5.7 mol/l)/potassium

acetate (0.1 mol/l)/EDTA (0.025 mol/l) in 1.5 ml polypropylene microfuge tubes, vigorously vortexed, and passed through a 20-gauge spinal needle 10 times to shear DNA. RNA was precipitated by adding 1:2 volume of cold ethanol, frozen at −80°C, and centrifuged at 4°C in a microfuge. Before electrophoresis, RNA was treated by phenol:chloroform:isoamyl alcohol extraction two times, reprecipitated in ethanol, pelleted, and dissolved in water. Ten micrograms of RNA was loaded into each well, electrophoresed, and electroblotted onto Nytran filters (Schleicher and Schuell, Keenne, NH), as previously described.[10] Northern blot analysis was performed subsequently using ^{32}P-labeled cDNA probes for IL-8,[17] TGF-α, and TNF-α mRNA (American Type Culture Collection, Rockville, MD), with cyclophilin cDNA reactivity used as the reference gene.[10,17] The blots were hybridized overnight at 42°C, washed at high stringency, and submitted to autoradiography.

In preliminary studies, keratome biopsies of skin, which contain both dermis and epidermis, were snap frozen immediately by immersion in liquid nitrogen and processed exactly as previously described.[18] Multipassaged human KCs obtained from normal skin were cultured in a serum-free medium containing epidermal growth factor, insulin, and bovine pituitary extract (Clonetics Corp., San Diego, CA) at 37°C, 5% CO_2, as previously described.[5] Keratinocytes were grown to confluency in 10-cm diameter plastic petri dishes and either phorbol ester (12-0 tetradecanoylphorbol-13-acetate, TPA-10 nmol/l [nanomolar], Sigma) combined with calcium ionophore (A23187, Sigma) or recombinant TNF-α (Genentech, S. San Francisco, CA) was added for various time intervals before extraction of RNA, as described above.[10]

Antibodies

Polyclonal rabbit antisera to human recombinant TNF-α and IL-8 were prepared each with a neutralization titre of more than 1:25,000. The ideal dilution for each antibody providing optimal staining was found to be 1:2000 using PBS/5% fetal calf serum as diluent. Absorptions using the 1:2000 dilution were performed using either 4 μg/ml recombinant TNF-α (Genentech) or recombinant IL-8 (Sandoz Pharmaceutical Corp., Vienna, Austria) for overnight incubation at 4°C with continuous agitation. Cross-addition experiments revealed that only the recombinant TNF-α (and not the recombinant IL-8) abolished the anti-TNF-α immunoperoxidase staining and vice versa for the IL-8 anti-sera. Preimmune rabbit serum also was used as a control at equivalent dilutions.

A panel of antibodies was used to detect other relevant antigens and cell types, including anti-CD-1a (Leu 6, Becton-Dickinson, Mountain View, CA) for Langerhans cells, anti-CD-14 (Leu M3, Becton- Dickinson) for monocyte/macrophages, and anti-HLA-DR (L-243, Becton-Dickinson) for class II major histocompatibility antigen. Anti-factor XIIIa (Calbiochem Corp., La Jolla, CA) identified dermal dendrocytes and RR 1/1 (gift from Dr. T. Springer, Center for Blood Research, Boston, MA) identified ICAM-1.

Results

Normal Skin

Immunohistochemical staining of all six normal skin specimens was identical (Figures 1a and b). There was no detectable discrete reactivity of any epidermal cells for either TNF-α or IL-8. In the dermis, the only strongly positive reactivity for both TNF-α and IL-8 was confined to the eccrine duct epithelium (Figure 1, inset) and acrosyringium. A rare papillary dermal dendritic cell was TNF-α positive, but all other dermal cell types were negative for TNF-α and IL-8. Staining normal skin with normal rabbit preimmune serum revealed no positive epidermal/dermal cells. Preabsorption of the anti-TNF-α and anti-IL-8 antisera with their respective recombinant cytokines completely abolished all specific staining of both normal and psoriatic skin specimens. There was no detectable IL-8 mRNA in any of the eight keratome biopsies of normal skin (50 μg total RNA loaded/lane; data not shown).

Psoriatic Plaques

Staining of the psoriatic plaques using normal rabbit preimmune serum revealed no positive epidermal or dermal cells, with only a mild diffuse staining of the basement membrane zone. Immunohistochemical staining of the 12 psoriatic plaques for TNF-α revealed similar cellular localization with minor interpatient variation in the relative number of positive epidermal and dermal cell types. The most conspicuous expression of TNF-α was found in dermal dendrocytes within the papillary dermis (Figure 2). These positively stained cells were present around blood vessels and were prominent particularly at or near the dermal–epidermal junction. Macrophage ontogeny of these dendritic cells was confirmed by double-immunofluorescence labeling in which the FITC-stained Leu M3 (CD-14)-positive cells (a marker for dermal dendrocytes)[19] coincided with the rhodamine-stained TNF-α cells (Figure 3). Anti-Leu M3 antibody (mouse) was used to identify the epidermal dendrocytes rather than factor XIIIa antibody (rabbit) because we used an indirect staining procedure for the detection of TNF-α (which is a rabbit anti-serum). Mast cells are another potentially important elongated

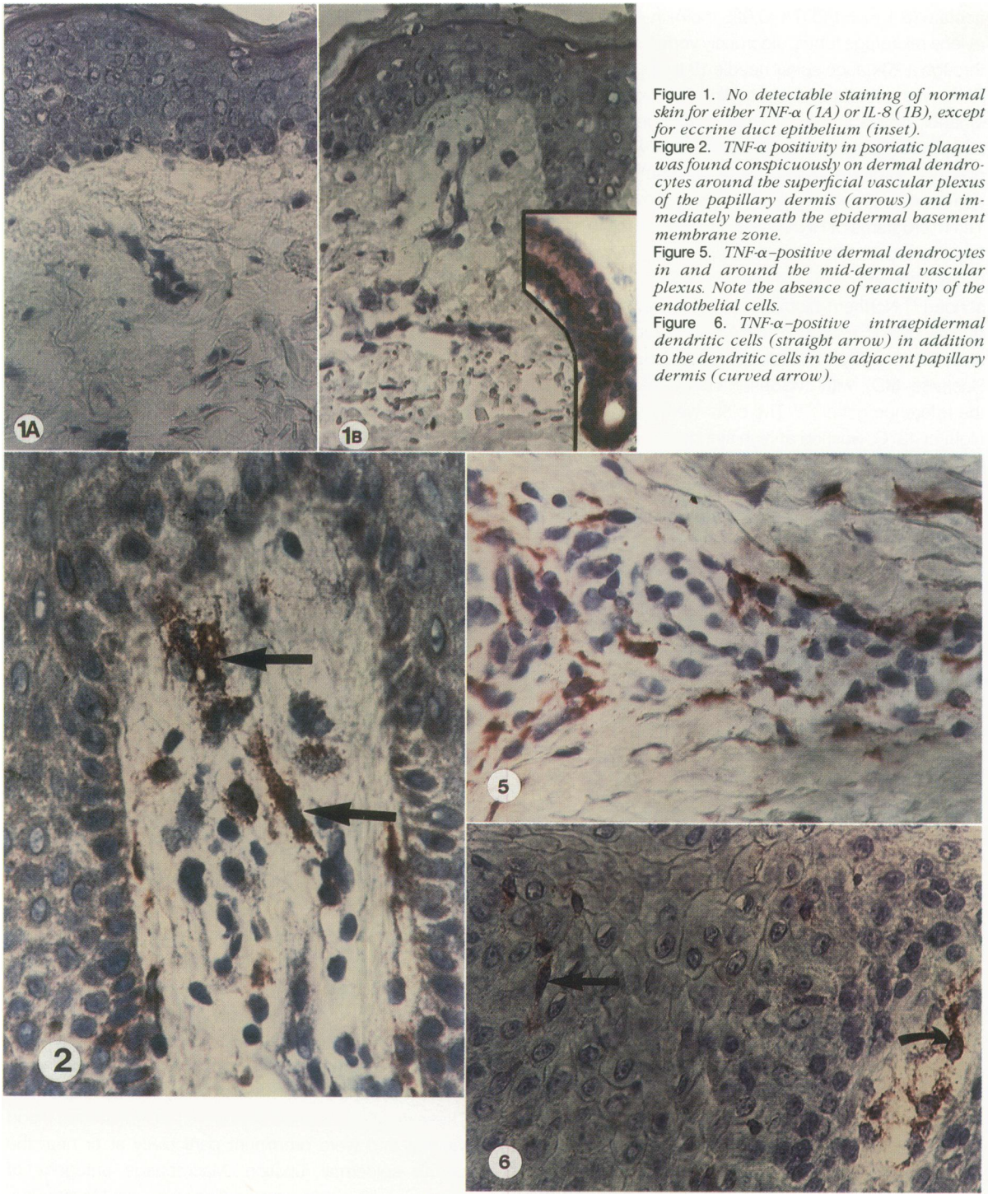
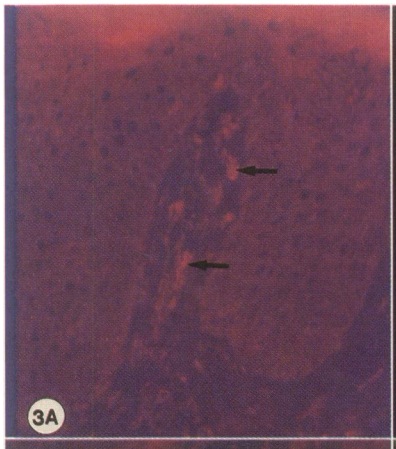


Figure 1. *No detectable staining of normal skin for either TNF-α (1A) or IL-8 (1B), except for eccrine duct epithelium (inset).*
Figure 2. *TNF-α positivity in psoriatic plaques was found conspicuously on dermal dendrocytes around the superficial vascular plexus of the papillary dermis (arrows) and immediately beneath the epidermal basement membrane zone.*
Figure 5. *TNF-α–positive dermal dendrocytes in and around the mid-dermal vascular plexus. Note the absence of reactivity of the endothelial cells.*
Figure 6. *TNF-α–positive intraepidermal dendritic cells (straight arrow) in addition to the dendritic cells in the adjacent papillary dermis (curved arrow).*

dermal cell population in addition to the dermal dendrocyte, which also have been reported to be increased in psoriatic lesions.[20] We did not observe double labeling of avidin-positive mast cells and TNF-α.[21] There were, however, many areas in which mast cells were adjacent to TNF-α–containing dermal dendrocytes (Figure 4). In the mid-dermis, perivascular dermal dendrocytes (but not endothelial cells) were positive for TNF-α (Figure 5). Occasional TNF-α–positive lymphocytes were seen in both the dermis and epidermis, while eccrine duct epithelium and acrosyringium also were TNF-α positive (data not shown).

Within the epidermal compartment, KCs focally expressed TNF-α in the mid and upper layers, and some intraepidermal dendritic cells were TNF-α positive (Figure



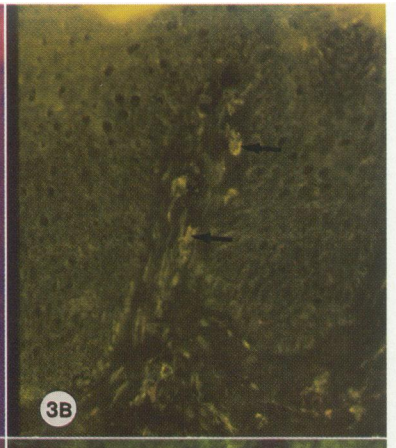
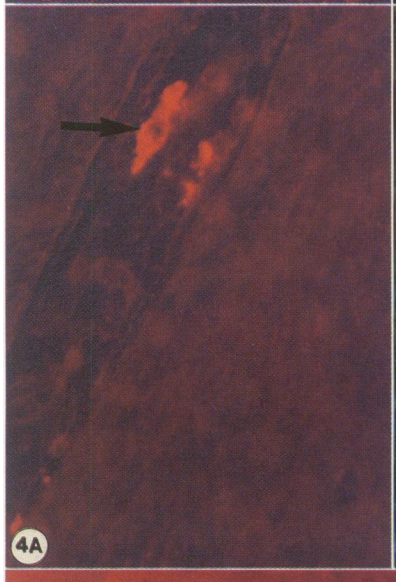


Figure 3. *TNF-α positivity (rhodamine stained red, 3A) elongated cells in the papillary dermis are dermal dendrocytes (arrows) because they are also CD-14 (Leu M3) positive (fluorescein stain green, 3B-arrows).*

Figure 4. *A dermal mast cell in psoriasis (4a) is identified by its ability to bind Texas-red conjugated avidin (arrow) but is not colabeled by the fluorescein-stained green TNF-α–positive (arrows) dermal dendrocytes (4B). However the mast cells are immediately adjacent to the dermal dendrocytes.*



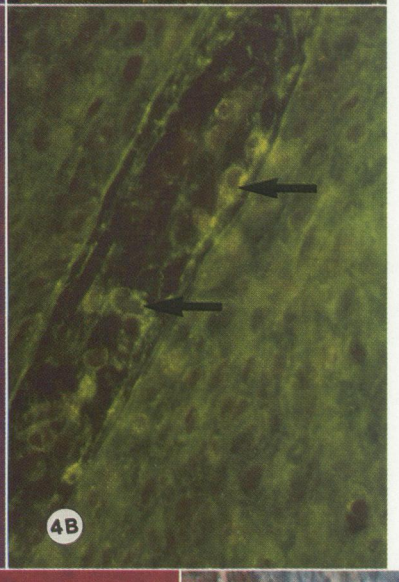
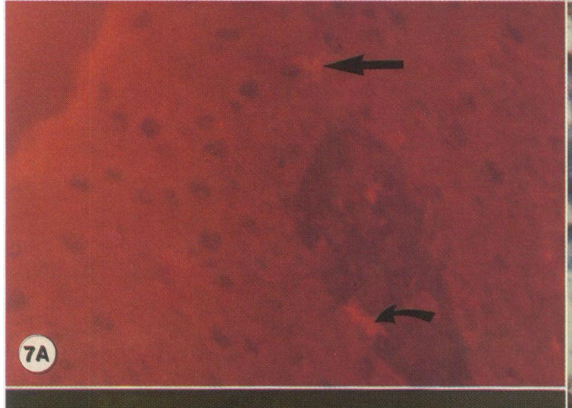
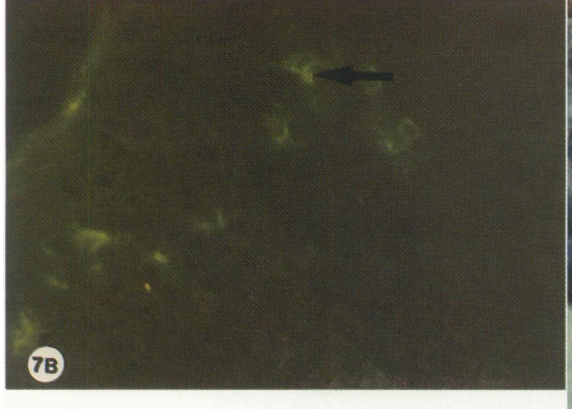
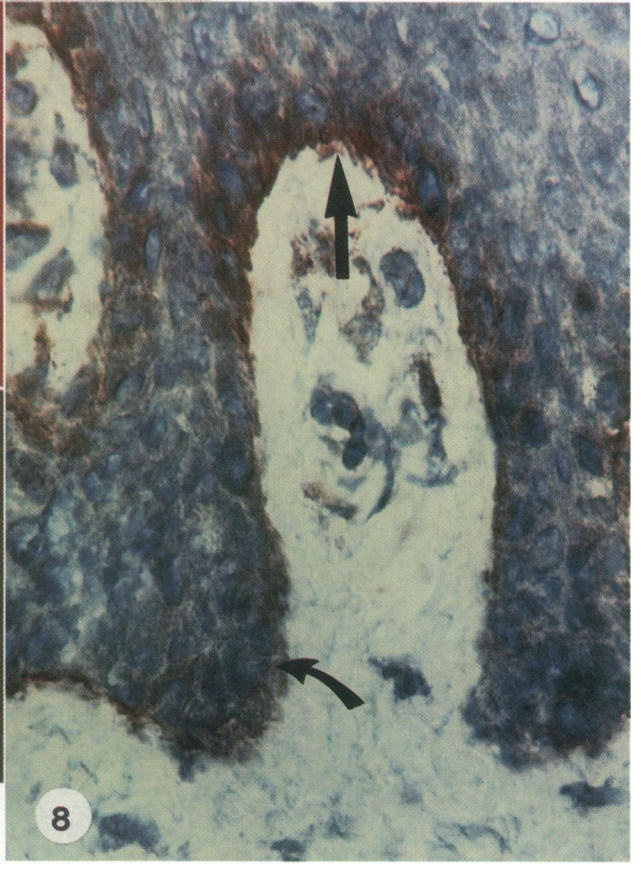


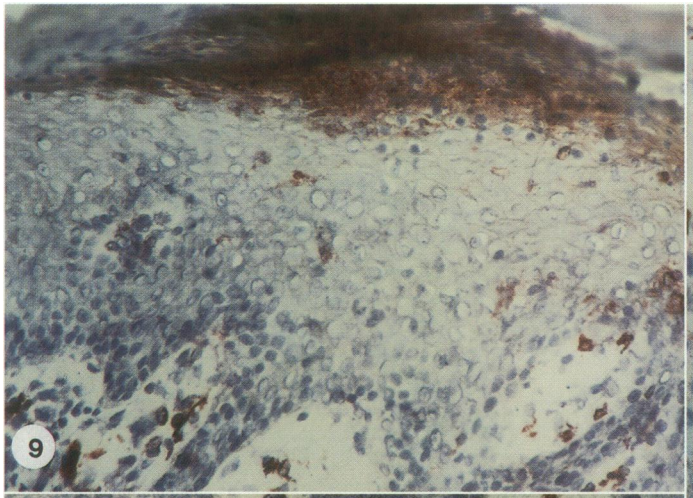
Figure 7. *A TNF-α–positive (rhodamine stained red; straight arrow) intraepidermal dendritic cell (7A) is identified as a Langerhans cell because it is also stained with anti–CD-1 (7B) antibody (fluorescein stained green; arrow). Note that a single TNF-α–positive dermal dendritic cell (curved arrow) is negative for CD-1 and that only a rare epidermal Langerhans cell is TNF-α positive compared to the total intraepidermal population.*

Figure 8. *Intense basal KC IL-8 positivity seen immediately above (straight arrow) or adjacent to (curved arrow) the dermal papilla.*





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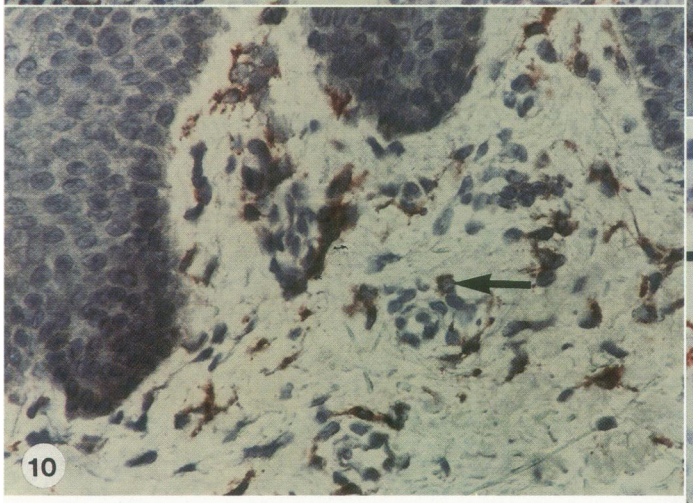
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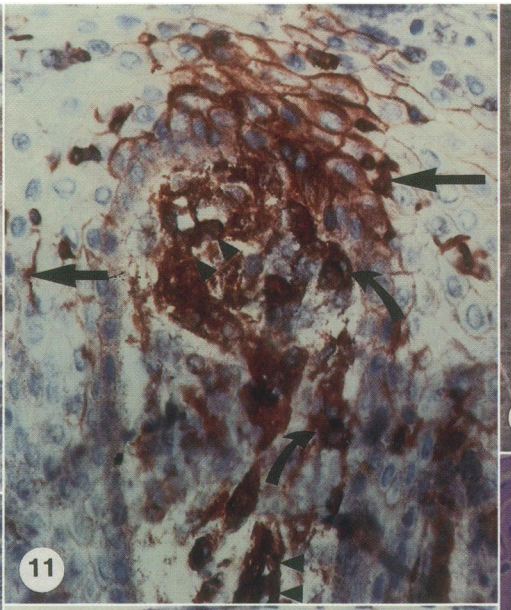
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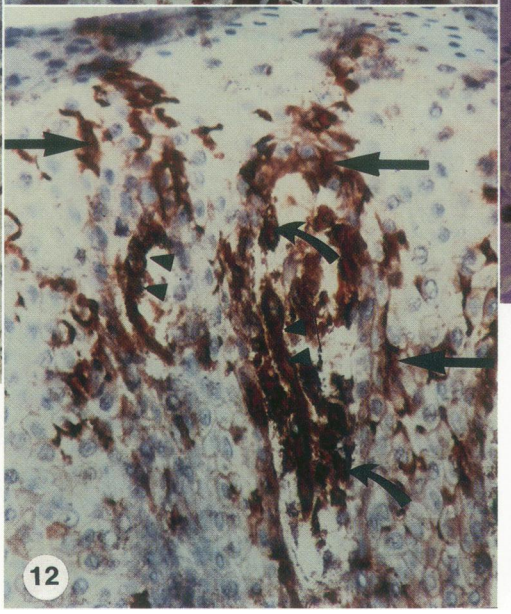
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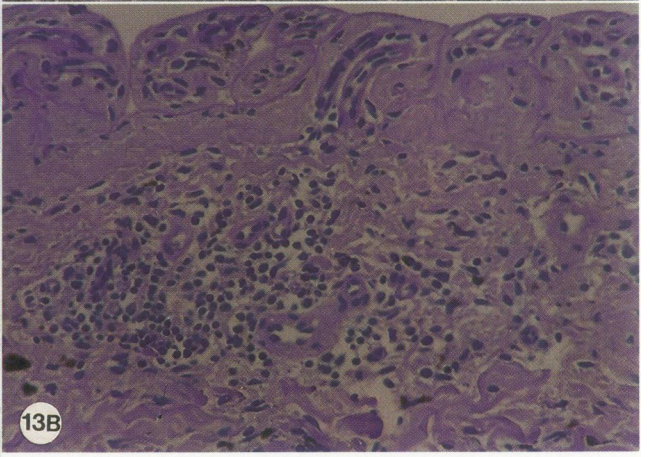
13B

Figure 9. *Prominent IL-8 positivity expressed by KCs in the outermost portion of the epidermis with extension into the stratum corneum.*
Figure 10. *IL-8–positive dermal cells include predominantly dermal dendrocytes with occasional lymphocytes (arrow), but not endothelial cells.*
Figure 11. *ICAM-1 expression is prominent by KCs in the zone of the squirting papillae (producing a chicken-wire pattern) and is seen also on intraepidermal dendritic Langerhans cells (straight arrows), dermal mononuclear cells (curved arrows), and vascular endothelium (arrow heads).*
Figure 12. *HLA-DR is not significantly expressed by KCs in the 'squirting papillae' zone but is present on epidermal LCs (straight arrows), dermal mononuclear cells (curved arrows), and vascular endothelium (arrow heads).*
Figure 13. *The saline heat-induced separation produces an excellent split between the dermis and epidermis of* **A**. *Normal intact (far right) skin visualized clinically by the pigment containing melanocytes in the basal epidermal layer being in thin epidermal sheet (far left) separated from the larger dermal fragment (middle), which is whitish due to the collagen.* **B**: *Psoriatic dermal residual fragment after separation demonstrating microscopically that the split occurs at the dermal–epidermal junction. Note that some of the prominent dermal papillae collapsed partially after removal of the overlying epidermis.*

6). These cells were identified as LCs by double-immunofluorescence staining (Figure 7). It should be noted that only rare dermal CD-1–positive LCs[22] exhibited TNF-α positivity and the vast majority of LCs in the epidermis and dermis were negative for TNF-α.

Immunohistochemical staining of 12 psoriatic plaques for IL-8 revealed relatively similar cellular localization patterns within the dermis, but essentially two different epidermal KC patterns. The first epidermal IL-8 staining pattern included predominantly basal KCs either immediately above or adjacent to the dermal papillae in the zone called the 'squirting papillae' (Figure 8). The second pattern included intense epidermal staining of the upper-most portion of the epidermis in the vicinity of the spongiform pustule zone of the psoriatic lesion.[9] Interleukin-8 reactivity extended into the stratum corneum layer as well. As can be seen in Figures 9 and 10, IL-8–positive cells in the dermis also were identified predominantly in dendritic cells. The dermal IL-8–positive cells included an occasional lymphocyte but not the microvascular endothelium (Figure 10). By double-immunofluorescence labeling, as described and illustrated for TNF-α, the greatest population (more than 95%) of IL-8–positive cells were Leu M3 positive (dermal dendrocytes). No significant colocalization for IL-8 with either dermal LCs or mast cells (avidin) was identified consistently.

To identify further the potential importance of TNF-α in the pathophysiology of psoriasis, the cellular distribution of ICAM-1 and HLA-DR was studied. The two principal inducers of KC ICAM-1 are IFN-γ and TNF-α,[14,23] and they can be distinguished further because IFN-γ, but not TNF-α, induces HLA-DR. Thus, while we have not found a suitable direct detection method for IFN-γ, we can infer its presence or absence by the degree of KC HLA-DR expression. As previously described,[14] epidermal KC ICAM-1 positivity was observed in psoriatic plaques (Figure 11), without significant KC HLA-DR expression (Figure 12). These immunohistochemical staining results support the presence of TNF-α in the psoriatic plaque and demonstrate that TNF-α is an important cytokine responsible for the induction of ICAM-1 and IL-8 expression.

To supplement the protein localization of IL-8 in the psoriatic epidermis, we used a recently described[16] physical separation technique and devised an RNA isolation protocol to detect IL-8 mRNA. This technique extends our earlier study using keratome slices of skin (which contain both epidermis and dermis) in which we detected IL-8 transcripts in seven of eight different psoriatic plaques but none in any of eight normal skin keratomes.[24] Using this separation technique, we also were interested in specifically localizing TGF-α mRNA in the psoriatic lesions because TGF-α has been reported to be elevated in psoriasis.[3,18] The saline, heat-induced separation produced a remarkably good dermal–epidermal split of normal skin and psoriatic plaques (Figure 13). The epidermal sheets produced approximately 20 to 100 μg total RNA. Loading of 10 μg/lane of RNA followed by Northern blotting revealed IL-8 and TGF-α mRNAs (Figure 14) and cyclophilin mRNA (data not shown), which was detectable in psoriatic epidermal specimens. Seven of twelve psoriatic epidermal sheets were positive for IL-8, and 8 of 12 psoriatic epidermal sheets were positive for TGF-α.

Cultured Keratinocytes

The treatment of cultured KCs with TNF-α (250 U/ml; 18 and 36 hours) increased TGF-α mRNA production (Figure 15a). Treatment of cultured KCs with TPA (10 ng/ml) and ionophore (1 μg/ml) for 4 and 8 hours also induced IL-8 and ICAM-1 mRNAs (Figure 15b), as well as TGF-α mRNA (data not shown). Ionophore treatment alone did not influence IL-8 or ICAM-1 mRNA expression (Figure 15b, lane D).

Discussion

A plausible immunopathogenetic mechanism for psoriasis involves the reciprocal interactions between mononuclear cells within the dermis, including activated T cells, macrophages (LCs and dermal dendrocytes), and the overlying hyperplastic KCs. Such a complex and reciprocal interaction between KCs and immunocytes was studied

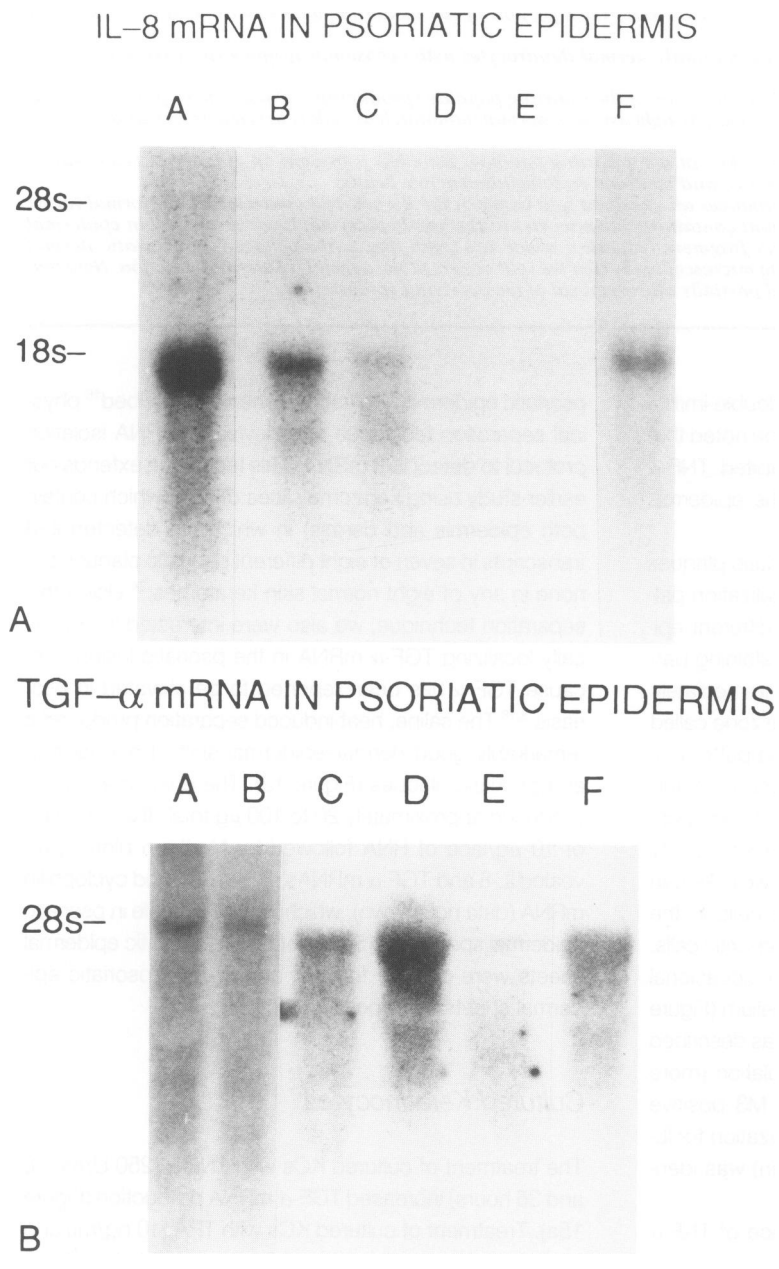

Figure 14. **A:** *Representative survey of epidermal IL-8 mRNA (2 Kb) detected in psoriatic plaques. Lanes A and B have strong expression, lanes C and F have moderate expression, with weak expression in lane E and no expression in lane D.* **B:** *Representative survey of epidermal TGF-α mRNA (4 Kb) detected in psoriatic plaques. Lanes A and B reveal expression of TGF-α as do lanes C, D, and F from a separate experiment.*

previously *in vitro*,[25] but the present study focuses on *in vivo* tissue reactions. The basis for these interactions between immunocompetent cells and KCs, which produces the psoriatic phenotype, probably includes the cytokines TNF-α, IL-8, and the adhesion molecule ICAM-1. The current *in vivo* results combined with our previous *in vitro* studies[10,25] clearly delineate important spatially coordinated cytokine-controlled interactions between TNF-α–producing dermal dendrocytes and overlying KCs, which have increased IL-8, ICAM-1, and TGF-α expression. The KC-derived IL-8 is a potent T-cell chemotactic factor and the KC-derived adhesion molecule ICAM-1 could mediate the recruitment and retention of T cells,[12] as characteristically observed in the squirting papilla zone of the psoriatic epidermis. The increased KC production of TGF-α induced by TNF-α may be an important contribution to the KC hyperplasia because TGF-α is a potent mitogen for KCs.[18]

Previously we suggested an important central role for the dermal dendrocyte in cutaneous inflammatory reactions[26,27] because it is anatomically situated immediately between the basal KC layer of the epidermis and adjacent to the endothelial cells, permitting the monitoring of the external and internal microenvironmental milieu. The relatively focal and minor TNF-α expression by epidermal

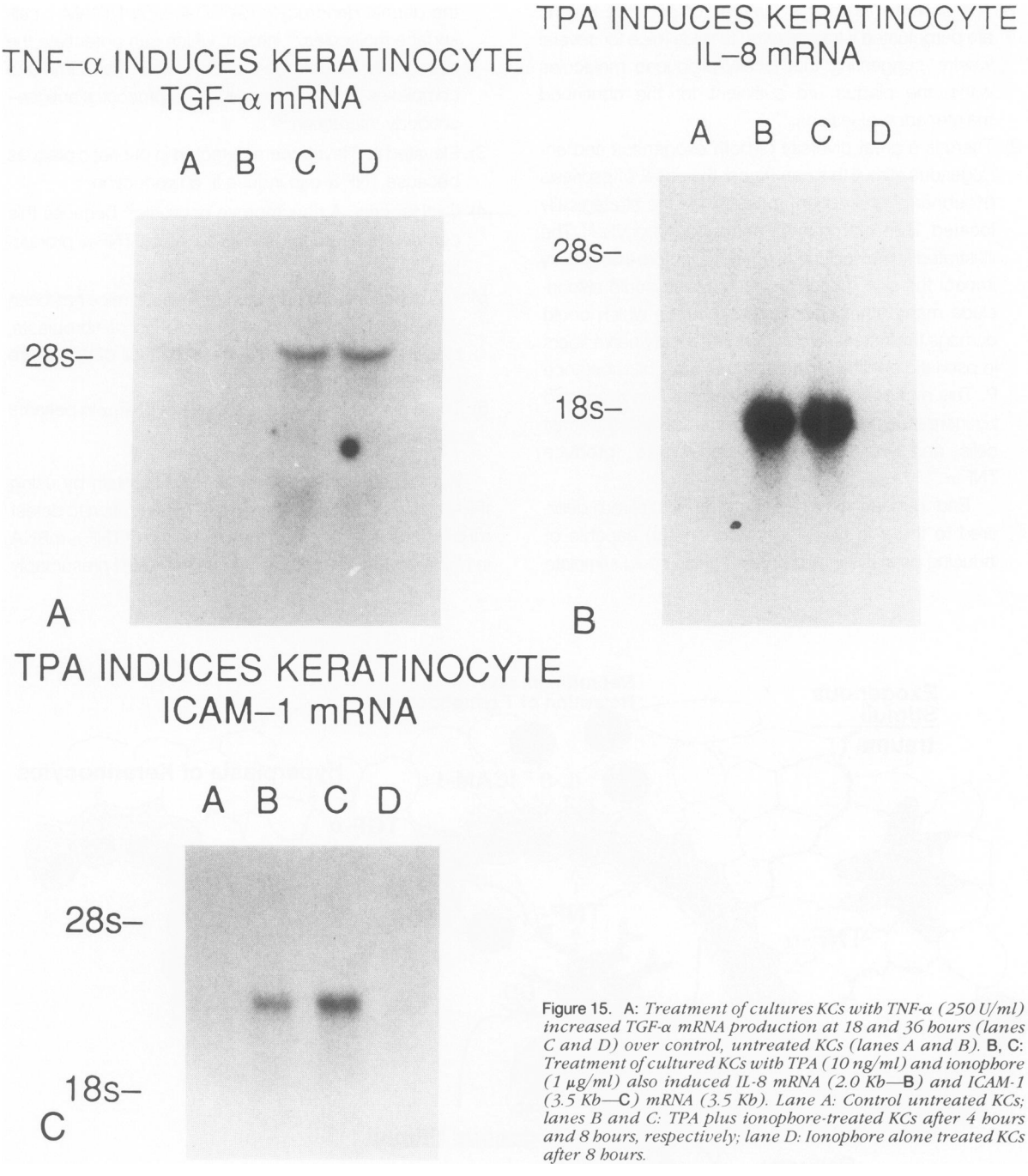

Figure 15. A: *Treatment of cultures KCs with TNF-α (250 U/ml) increased TGF-α mRNA production at 18 and 36 hours (lanes C and D) over control, untreated KCs (lanes A and B).* B, C: *Treatment of cultured KCs with TPA (10 ng/ml) and ionophore (1 μg/ml) also induced IL-8 mRNA (2.0 Kb—*B*) and ICAM-1 (3.5 Kb—*C*) mRNA (3.5 Kb). Lane A: Control untreated KCs; lanes B and C: TPA plus ionophore-treated KCs after 4 hours and 8 hours, respectively; lane D: Ionophore alone treated KCs after 8 hours.*

LCs is in marked contrast to the diffuse and strong expression by dermal dendrocytes or cultured activated LCs,[28] which suggests that LCs may not be stimulated maximally in the epidermal microenvironment.[29,30] Dermal mast cells, while increased in psoriatic lesions, did not appear to produce TNF-α or IL-8. However the dermal mast cells were intimately associated with TNF-α–containing dermal dendrocytes. This close cellular association may have functional significance because stimulation of dermal mast cells in short-term skin organ cultures has been observed to induce TNF-α–mediated adhesion molecule expression on endothelial cells.[31]

Thus the cytokine network envisaged above for psoriasis involves production of TNF-α and IL-8 and expression of ICAM-1 by resident and recruited cells. The current results may help explain several clinical and pathologic observations involving psoriasis (Figure 16), including the following:

1) The essential histologic features of psoriatic lesions are perpetuated if transplanted to nude mice for several weeks, suggesting that locally produced molecules within the plaque are sufficient for the continued maintenance of lesions.[32]
2) There is a great diversity of both exogenous and endogenous stimuli that can trigger the onset of psoriasis (Koebner phenomenon), possibly *via* the strategically located TNF-α–producing dermal dendrocyte.[27] The illustrated stimuli include exogenous factors that directly impact the skin,[33] such as trauma (and could also include many other environmental cues), which could damage the increased numbers of sensory nerve fibers in psoriatic skin,[34] leading to the release of substance P. This neuropeptide, which also could be produced by increased stress, then may interact with the mast cells and, *via* the dermal dendrocyte, produce TNF-α.[31]

Endogenous stimuli (ie, these indirect stimuli delivered to the skin from the blood stream) capable of inducing psoriasis include HIV-1, which could stimulate the dermal dendrocyte *via* CD-4, LFA-1/ICAM-1 cell-surface molecules,[27] lithium, which can potentiate the effects of macrophage-derived TNF-α,[35] and immune complexes, perhaps formed by streptococcal antigen–antibody interaction.[36]
3) Elevated IL-6 levels were detected in psoriatic plaques because TNF-α can induce IL-6 production.[37]
4) Cyclosporine A can improve psoriasis[38] because this compound has been shown to inhibit TNF-α production.[39]
5) The subcutaneous perfusion of TNF-α in mice has been associated with the local proliferation of fibroblasts, blood vessels, and epidermal KCs,[40] all of which are features seen in psoriasis.
6) There are increased serum levels of TNF-α in patients with severe psoriasis.[41]

We focused on detection of TNF-α protein by using the rabbit antisera rather than *in situ* hybridization to detect mRNA transcripts because the production of TNF-α mRNA in macrophages is extremely transient[42] and presumably

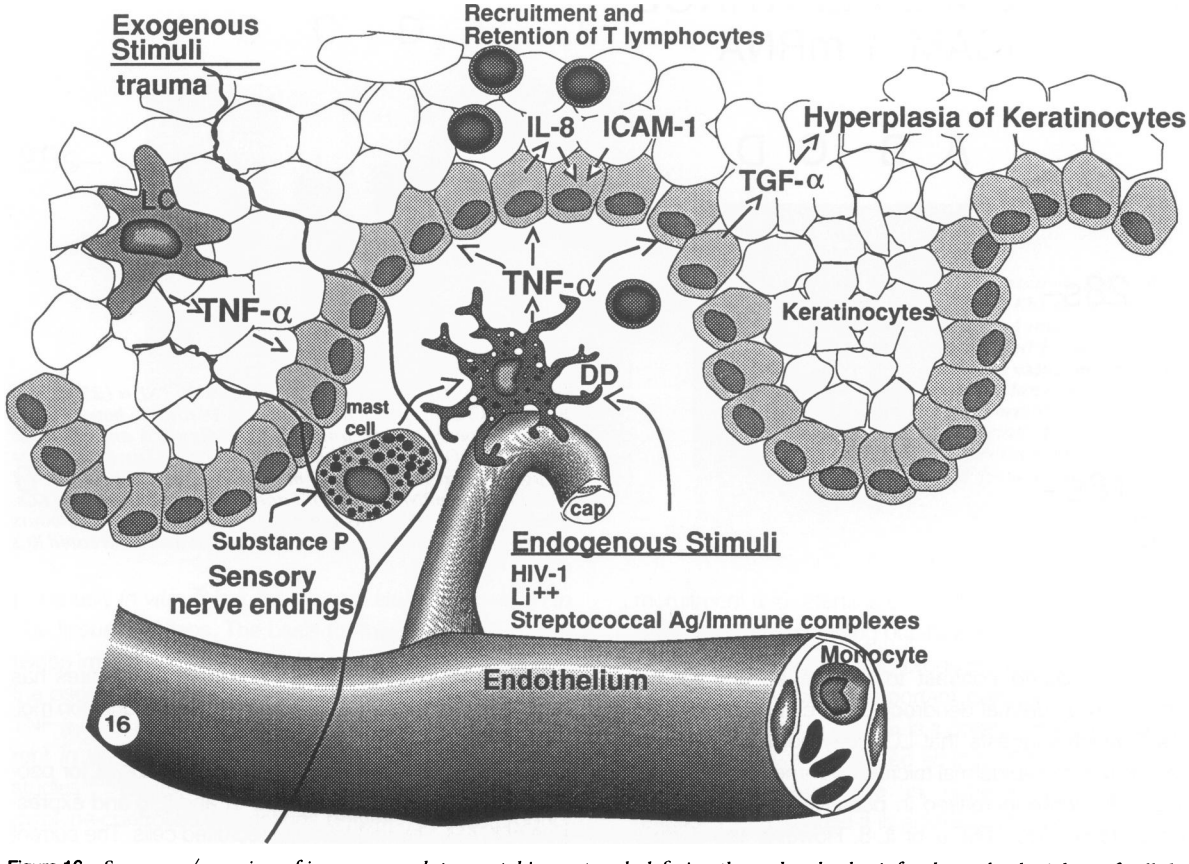

Figure 16. *Summary/overview of immunoregulatory cytokine network defining the molecular basis for the pathophysiology of cellular immune mechanisms in psoriasis. Central to this schema is the production of TNF-α by dermal dendrocytes (in response to a variety of endogenous and exogenous stimuli), which induces surrounding KCs to produce IL-8, ICAM-1, and TGF-α. DD, dermal dendrocyte; Cap, capillary loop; Ag, antigen.*

regulated by post-transcriptional events.[39] The immunohistochemical identification of IL-8 in the superficial portion of the epidermis in psoriasis is consistent with the previous demonstration of IL-8 protein in psoriatic scales.[43] The current identification of IL-8 mRNA in epidermal sheets of psoriatic lesions is consistent with our earlier report using keratome biopsies.[24] Furthermore, using the saline heat-separation technique, we could localize definitively the increased TGF-α in psoriasis to the epidermis. While initially we hoped to compare directly and contrast the profile of transcripts of epidermal *versus* dermal cells in psoriatic lesions to further refine the compartmentalization of various cytokines and growth factors, we could not isolate consistently sufficient mRNA from the dermal portion of the biopsies. By wet weight, there was at least 50 times more dermis than epidermis in the punch biopsies, but the dermal cells appeared to be less transcriptionally active than the epidermal KCs. Recently we observed that the epidermal location of T cells is more conducive to active participation in the cell cycle compared to the dermal location,[44] and this phenomenon may be more widespread than is now appreciated. Work is underway to use the polymerase chain reaction in our punch biopsy material to amplify further the dermal transcripts and thereby allow direct comparisons between the dermis and epidermis.[45] While phorbol esters can have many diverse cellular effects, the ability of TPA to induce IL-8, ICAM-1, and TGF-α may explain partially the hyperplastic and inflammatory response of epidermis to phorbol ester treatment.[13,45,46]

While a complete molecular understanding of the growth regulatory disturbances, immune cell trafficking, and inflammatory pathology seen in psoriasis will undoubtedly be more complex than one solely involving TNF-α, IL-8, ICAM-1, and TGF-α, this line of inquiry will serve as an important framework for the further evaluation of the cellular and molecular basis for the genesis and evolution of psoriasis.

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