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Localization of tumour necrosis factor-alpha (TNF-α) and its receptors in normal and psoriatic skin: epidermal cells express the 55-kD but not the 75-kD TNF receptor

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SUMMARY

The distribution of TNF-α, p55 TNF receptor (TNF-R) and p75 TNF-R in normal skin and uninvolved and lesional skin from psoriasis patients has been investigated, using specific mono- and polyclonal antibodies. In normal skin, and uninvolved and lesional skin from psoriasis patients, p55 TNF-R is associated with epidermal keratinocytes and a network of upper dermal dendritic cells. This suggests that the actions of TNF-α on epidermal cells *in vivo* are mediated by binding to the p55 TNF-R. In lesional psoriasis skin, there was staining of the parakeratotic stratum corneum and increased expression of p55 TNF-R in association with upper dermal blood vessels. Staining for p75 TNF-R in normal skin was restricted to eccrine sweat ducts and dermal dendritic cells, and was absent from the epidermis. In lesional psoriasis skin, there was staining for p75 TNF-R in association with upper dermal blood vessels and perivascular infiltrating cells. TNF-α in normal skin was predominantly localized to the basal cell layers of the epidermis, and was seen in association with eccrine ducts and sebaceous glands. In lesional psoriasis skin, and to a lesser extent in uninvolved psoriasis skin, TNF-α was distributed throughout the epidermis, and was also specifically localized to upper dermal blood vessels. Up-regulation of TNF-α, p55 TNF-R and p75 TNF-R on dermal blood vessels in psoriasis may play an important role in the pathogenesis of this condition by promoting cutaneous recruitment of inflammatory cells.

Keywords TNF-α TNF receptors skin psoriasis

INTRODUCTION

TNF-α is a cytokine which plays an important role in host defences, including immune responses [1,2]. TNF-α up-regulates expression of intercellular adhesion molecule-1 (ICAM-1) and MHC class I antigen by human dermal microvascular endothelial cells in tissue culture [3]. It enhances adhesiveness of cultured porcine dermal microvascular endothelial cells for peripheral blood mononuclear cells (PBMC) [4], and promotes preferential attachment of memory T cells and natural killer (NK) cells from peripheral blood to cultured human umbilical vein endothelial cells [5]. TNF-α messenger RNA (mRNA) is induced in, and TNF-α protein is synthesized and released from, cultured human keratinocytes following stimulation with endotoxin, ultraviolet light, or the sensitizer urushiol which induces poison ivy dermatitis [6,7]. Moreover, TNF-α induces production of ICAM-1 by keratinocytes [8], as well as of the neutrophil chemotactic factor and activator IL-8, which is also chemotactic for lymphocytes, by keratinocytes [9,10], dermal fibroblasts [9], and endothelial cells [11]. TNF-α also increases the rate of secretion of the pro-inflammatory cytokines IL-1 and IL-6 by cultured human keratinocytes [12]. As a result of its immunomodulatory properties, it has been proposed that TNF-α is involved in the pathogenesis of a variety of inflammatory skin disorders [2]. TNF-α has been shown to be a critical mediator in the evolution of both allergic and irritant contact dermatitis reactions in the murine system [13]. In allergic contact dermatitis to rhus (poison ivy), keratinocytes express TNF-α, with resultant keratinocyte production of ICAM-1 and IL-8, and induction and augmentation of the endothelial cell adhesion molecules endothelial leucocyte adhesion molecule-1 (E-selectin), vascular cell adhesion molecule-1 (VCAM-1) and ICAM-1 [14].

TNF-α exerts its biological effects by binding to specific cell-surface receptors. Two distinct TNF-α-binding molecules, the 55-kD (p55 TNF-R) and the 75-kD (p75 TNF-R) TNF-α receptors, have recently been shown to be expressed by human cells, and have been cloned and sequenced [15–18]. These distinct receptor types are independently regulated [19], and have dissimilar cytoplasmic domains [20], indicating functional

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dichotomy of the two receptors. Differences in the cellular expression of p55 TNF-R and p75 TNF-R have become apparent with the availability of specific MoAbs. Both TNF-Rs are present on lymphocytic cell lines, peripheral blood monocytes and NK cells; resting lymphocytes have very few TNF receptors, but activated lymphocytes preferentially express p75 TNF-R [17,19,20]. In human thymus, p75 TNF-R is confined to medullary lymphoblasts and dendritic cells; in lymph nodes and other secondary lymphoid tissues, p75 TNF-R is expressed on activated lymphocytes and interdigitating reticulum cells of the T cell area, whereas the p55 TNF-R is confined to germinal centre dendritic reticulum cells [21]. In rheumatoid arthritis, both p55 and p75 TNF-Rs are expressed by synovial membrane cells and underlying vascular endothelial cells; lymphoid aggregate cells express predominantly p75 TNF-R [22]. Early studies using ^{125}I-labelled TNF-α indicated that the skin contains a very large number of TNF-α receptors [23]. The p55 TNF-R has been reported to be present on normal and transformed human keratinocytes in tissue culture, and mRNA for this receptor has been demonstrated in a transformed keratinocyte line [24].

There is confusion in the literature regarding the cutaneous localization of TNF-α even in normal skin [10,14,25–29], and there have been very few studies regarding its distribution in psoriasis. Furthermore, there is no information on the *in vivo* distribution of the TNF-Rs in normal and inflamed skin; this is a significant gap in our knowledge, since immunoregulatory effects of TNF-α could be mediated by modulation of receptor expression, in addition to any alteration in the production of the cytokine itself. We have therefore investigated the presence and distribution of TNF-α, as well as of its two receptors (p55 TNF-R and p75 TNF-R), in normal skin and in uninvolved and lesional skin from patients with psoriasis, using specific mono- and polyclonal antibodies.

MATERIALS AND METHODS

Reagents and cell lines

Human recombinant TNF-α was a gift from Genentech (San Francisco, CA). Human recombinant soluble p55 TNF-R was prepared as described [18]. A specific polyclonal rabbit anti-TNF-α antibody (10 μg/ml) was isolated from serum of rabbits immunized with human recombinant TNF-α, as previously described [30]. The specificity of this antibody for TNF-α, and not for other cytokines including lymphotoxin, IL-1α and β, IL-6 and IL-8, was confirmed by demonstrating binding to TNF-α, but not to the other cytokines, attached to ELISA plates. Mouse MoAbs htr-9 (15 μg/ml) directed against the p55 TNF-R and utr-1 (20 μg/ml) directed against the p75 TNF-R were kindly provided by Dr Manfred Brockhaus (Hoffmann-La Roche Ltd., Basel, Switzerland) [31]. Two additional mouse MoAbs directed against the p55 TNF-R, TBP-1 (10 μg/ml) and TBP-2 (20 μg/ml), were the gift of Dr Günther Adolf (Bender & Co, Vienna, Austria) [32]. The murine monoclonal 4D6 F4 directed against keratin 14 was the gift of Dr David Hudson (Imperial Cancer Research Fund). Normal mouse IgG1 (of the same isotype as the monoclonals) (Sigma, Poole, UK) served as a control to establish the background for the TNF-R immunostaining. Rabbit anti-von Willebrand factor antibody, FITC swine anti-rabbit IgG, polyclonal goat anti-mouse IgG, monoclonal mouse anti-rabbit IgG, and alkaline phosphatase, anti-alkaline phosphatase (APAAP) complex were obtained from Dako (Copenhagen, Denmark). Normal goat serum (NGS) was obtained from Sigma. Streptavidin-conjugated Texas red was obtained from Amersham (Aylesbury, UK), and biotinylated goat anti-mouse IgG was obtained from Sigma. The HaCat spontaneously immortalized, non-tumorigenic human skin keratinocyte cell line, which expresses amongst others basal keratins 5

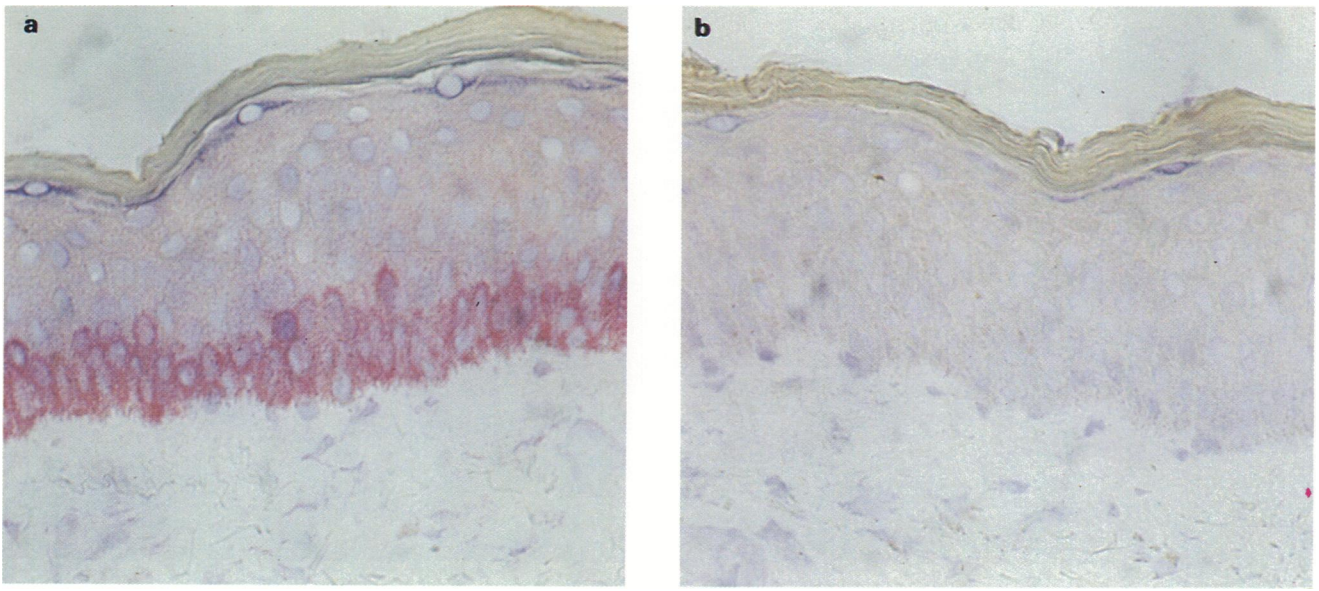

Fig. 1. Distribution of immunoreactive TNF-α in uninvolved psoriasis skin. (a) TNF-α is localized to the basal layers of the epidermis. (b) Adjacent serial section showing complete abrogation of staining following pre-absorption of anti-TNF-α antibody with recombinant TNF-α (×350).

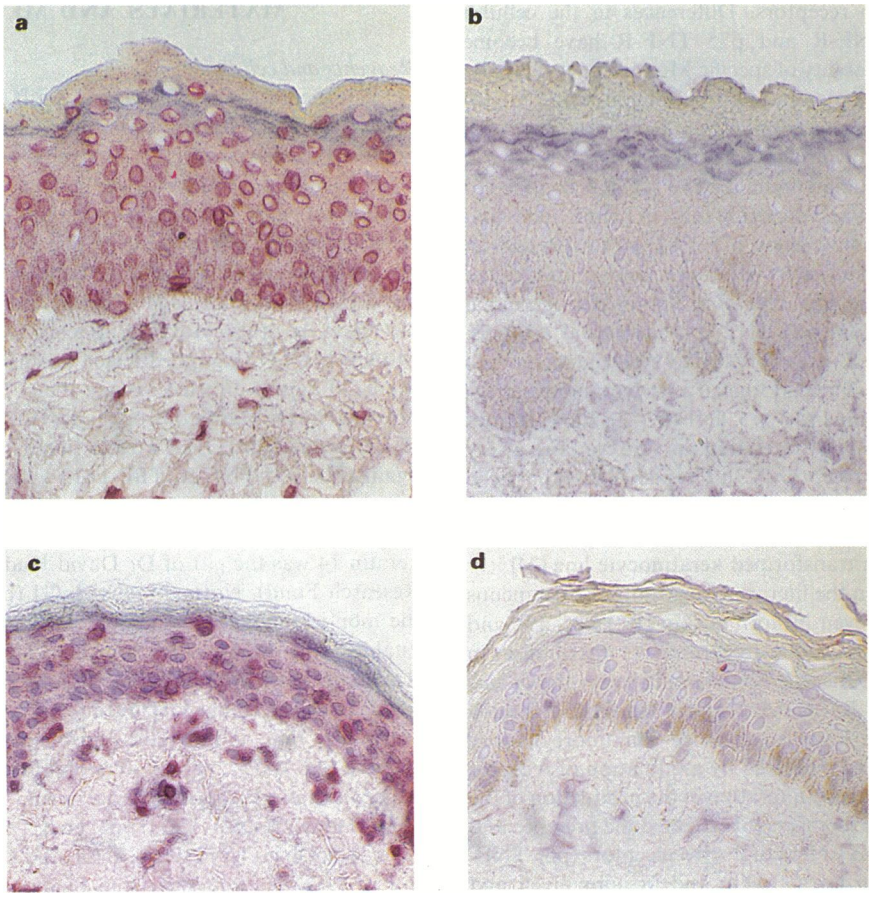

Fig. 2. Distribution of immunoreactive p55 TNF-R in normal human skin as detected using htr-9 antibody. (a) p55 TNF-R is present throughout the viable epidermis and in association with dendritic upper dermal cells; note perinuclear accentuation of staining. (b) Adjacent serial section of normal skin incubated with murine IgG1 as control. (c) Epidermal and dermal cell staining with anti-p55 TNF-R. (d) Adjacent serial section of normal skin showing complete abrogation of staining following pre-absorption of htr-9 antibody with recombinant p55 TNF-R (× 300).

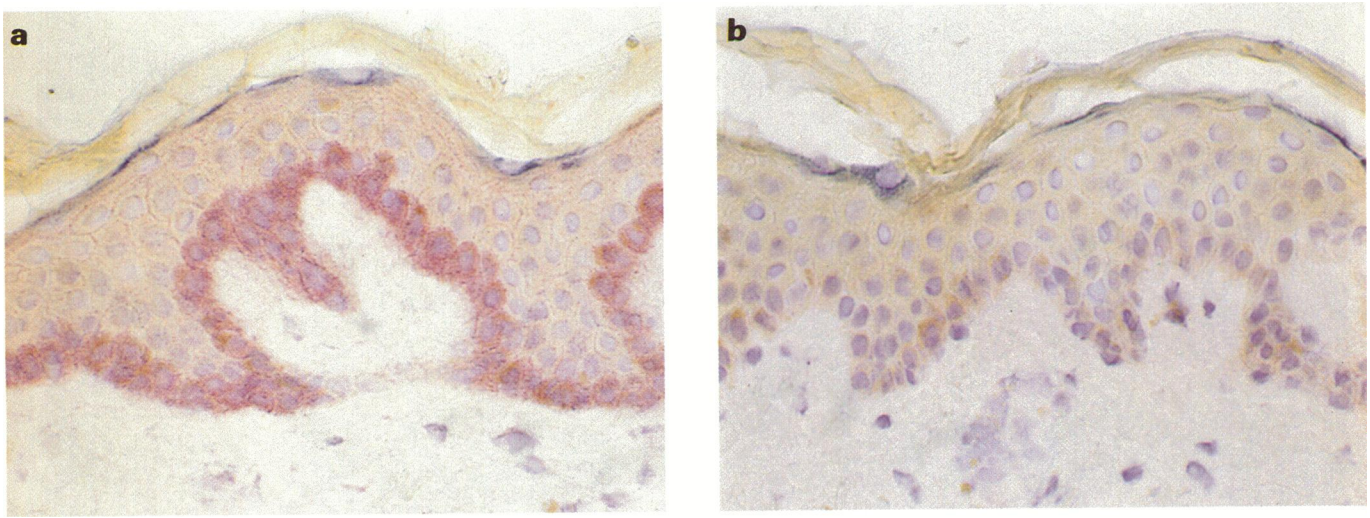

Fig. 3. Distribution of immunoreactive TNF-α in normal skin. (a) TNF-α is localized to the basal layers of the epidermis. (b) Normal skin incubated with preimmune normal rabbit serum as a control.

and 14, was the gift of Professor Norbert Fusenig (Deutsches Krebsforschungszentrum, Heidelberg, Germany).

Skin biopsies

Samples of normal skin ($n=8$) were obtained from normal healthy volunteers. In addition, paired biopsies of involved and lesional skin were obtained from patients ($n=7$) with stable plaque psoriasis. The psoriasis patients were not on systemic therapy, and had not used topical therapy on the biopsy sites for at least 7 days. Informed consent was obtained from all patients. The biopsies were snap frozen and stored at −70°C until required.

Immunohistochemical techniques

Cryostat sections (6 μm) from each biopsy were fixed in acetone/methanol (1:1) for 10 min at −20°C, washed in 0·05 mM Tris-buffered saline (TBS) for 2 min at 4°C, then blocked for 20 min with 20% NGS. Sections were incubated with the primary antibody (polyclonal rabbit anti-TNF-α, the various anti-p55 TNF-R MoAbs, or monoclonal anti-p75 TNF-R) diluted in 2% v/v NGS/TBS overnight at 4°C. The rabbit polyclonal antibody was detected with mouse anti-rabbit IgG diluted 1:30 in 2% NGS/TBS for 30 min at room temperature, then with goat anti-mouse IgG diluted 1:30 in 2% NGS/TBS for 30 min, and finally with the APAAP complex diluted 1:40 in 2% NGS/TBS for 30 min. The mouse TNF-R MoAbs were detected with the goat anti-mouse IgG followed by the APAAP complex, as described above. To enhance the staining, the goat anti-mouse IgG and APAAP steps were repeated on all sections. The sections were then incubated with naphthol AX-MX phosphate (Sigma) and fast red TR salt (Sigma), and the reaction was stopped with distilled water when optimum colour had developed after the elapse of standard time intervals. For staining using anti-TNF-α and anti-p55 TNF-R antibodies, this was always after 10 min; for the anti-p75 TNF-R antibody, the corresponding time was after 20 min. Control specimens, and paired specimens from uninvolved and involved sites in psoriasis patients, were processed simultaneously. All tissue sections were counterstained with haematoxylin. Positive staining was identified by bright pink colouration. HaCat cells grown on coverslips were stained with the antikeratin antibody 4D6 F4 similarly. The distribution and intensity of immunohistochemical staining with the various antibodies was assessed under the light microscope.

Double immunofluorescence staining of sections of normal skin, and of uninvolved and lesional psoriasis skin, was also carried out using antibodies to von Willebrand factor and p55 TNF-R. In brief, the sections were incubated overnight with anti-p55 TNF-R (htr-9) and rabbit anti-human von Willebrand factor MoAbs (1:100). Following washing, sections were incubated with biotinylated goat anti-mouse IgG (1:100), followed by FITC swine anti-rabbit (1:40), and then streptavidin Texas red (1:50). The sections were then examined using a Leitz Orthoplan immunofluorescence microscope.

Additional specificity controls

Specificity controls for the immunostaining were carried out as follows. Immunostaining of normal and of psoriatic skin by the TNF-α antibody was totally abrogated following preincubation of the antibody with 10 μg/ml of recombinant human TNF-α (Fig. 1). The specificities of p55 TNF-R (htr-9) antibody and the p75 TNF-R (utr-1) antibody have been well characterized; in particular, staining with these antibodies is reduced to background when the sections are preincubated with recombinant TNF-α [31], and in the case of htr-9 following preabsorption of the antibody with recombinant soluble p55 TNF-R [18]. Immunostaining of normal skin by the p55 TNF-R antibodies htr-9 (Fig. 2c, d) and TBP-1 and TBP-2 (data not shown) was also totally abrogated following preincubation of the antibodies with 9 μg/ml of recombinant human p55 TNF-R.

RESULTS

Distribution of TNF-α, p55 TNF-R and p75 TNF-R in normal human skin

Staining with the anti-TNF-α antibody was almost exclusively distributed to the basal layers of the epidermis in all eight biopsies of normal skin (Fig. 3). When present in the sections, sebaceous glands, eccrine sweat glands, hair follicles, and arrector pili muscles also stained positively. The anti-TNF-α antibody did not stain HaCat cells (in contrast to the 4D6 F4 antikeratin 14 antibody), indicating that the basal distribution of staining with antibody was not the result of epitope cross-reactivity with keratins 5 or 14 (data not shown).

Specific staining for the p55 TNF-R with htr-9 antibody was distributed throughout all the viable layers of the epidermis in all eight biopsies; staining was cytoplasmic with marked perinuclear accentuation (Fig. 2). A similar staining pattern was obtained with TBP-1 and TBP-2 antibodies directed against p55 TNF-R (data not shown). A network of upper dermal dendritic cells also stained positively with all three antibodies directed against p55 TNF-R (Fig. 2); in addition, staining was detected in eccrine ducts (Fig. 4), sebaceous glands, and hair follicles. There was, by contrast, no staining of the epidermis, follicular epithelium, or sebaceous glands with antibody to p75 TNF-R in any of the biopsies examined. Upper dermal dendritic cells and eccrine ducts did, however, stain weakly with anti-p75 TNF-R (data not shown).

Distribution of TNF-α, p55 TNF-R and p75 TNF-R in psoriasis skin

In uninvolved psoriasis skin, TNF-α was again predominantly distributed to the basal layers (Fig. 1), but there was faint staining in some biopsies of upper epidermal layers (not shown). In involved psoriasis skin, the anti-TNF-α antibody stained strongest in the basal cell layers of the epidermis, but weaker staining was also distributed to the upper epidermal layers in all seven biopsies examined (Fig. 5). In four of the seven lesional skin biopsies, there was staining associated with upper dermal blood vessels with anti-TNF-α antibody (not shown). However, elsewhere in the dermis there were very few infiltrating cells which expressed TNF-α.

In uninvolved psoriasis skin, staining with the anti-p55 TNF-R antibody was not qualitatively different from that in normal skin. In lesional psoriasis skin, immunostaining with anti-p55 TNF-R antibody was present throughout all layers of the epidermis, including the parakeratotic stratum corneum (Fig. 6). The histology of psoriasis is characterized by the presence of prominent, tortuous, dilated capillary blood vessels within the tips of the dermal papillae. There was marked staining associated with these blood vessels in the dermal papillae (Fig. 6), and with blood vessels in the reticular dermis, with the anti-p55 TNF-R antibody. Staining was not just of

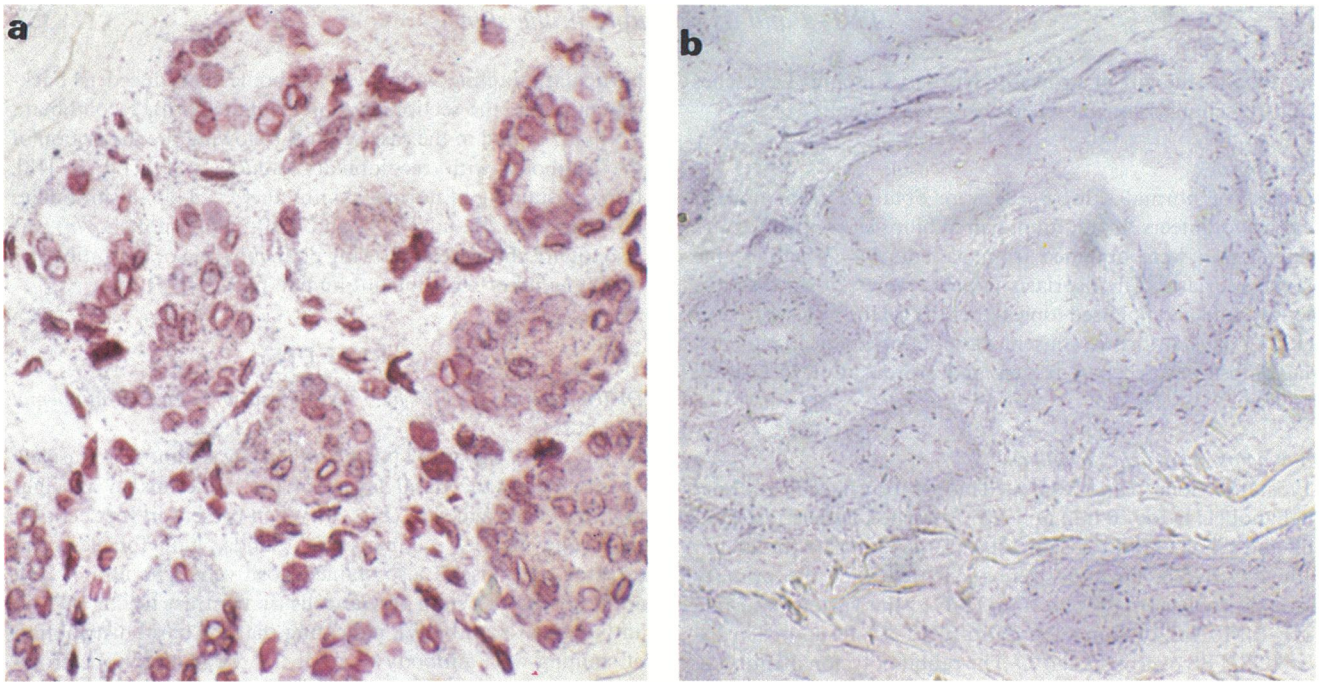

Fig. 4. (a) Immunoreactive p55 TNF-R (htr-9 antibody) is also present in dermal eccrine sweat ducts in normal skin. (b) Adjacent serial section incubated with murine IgG1 as control (× 500).

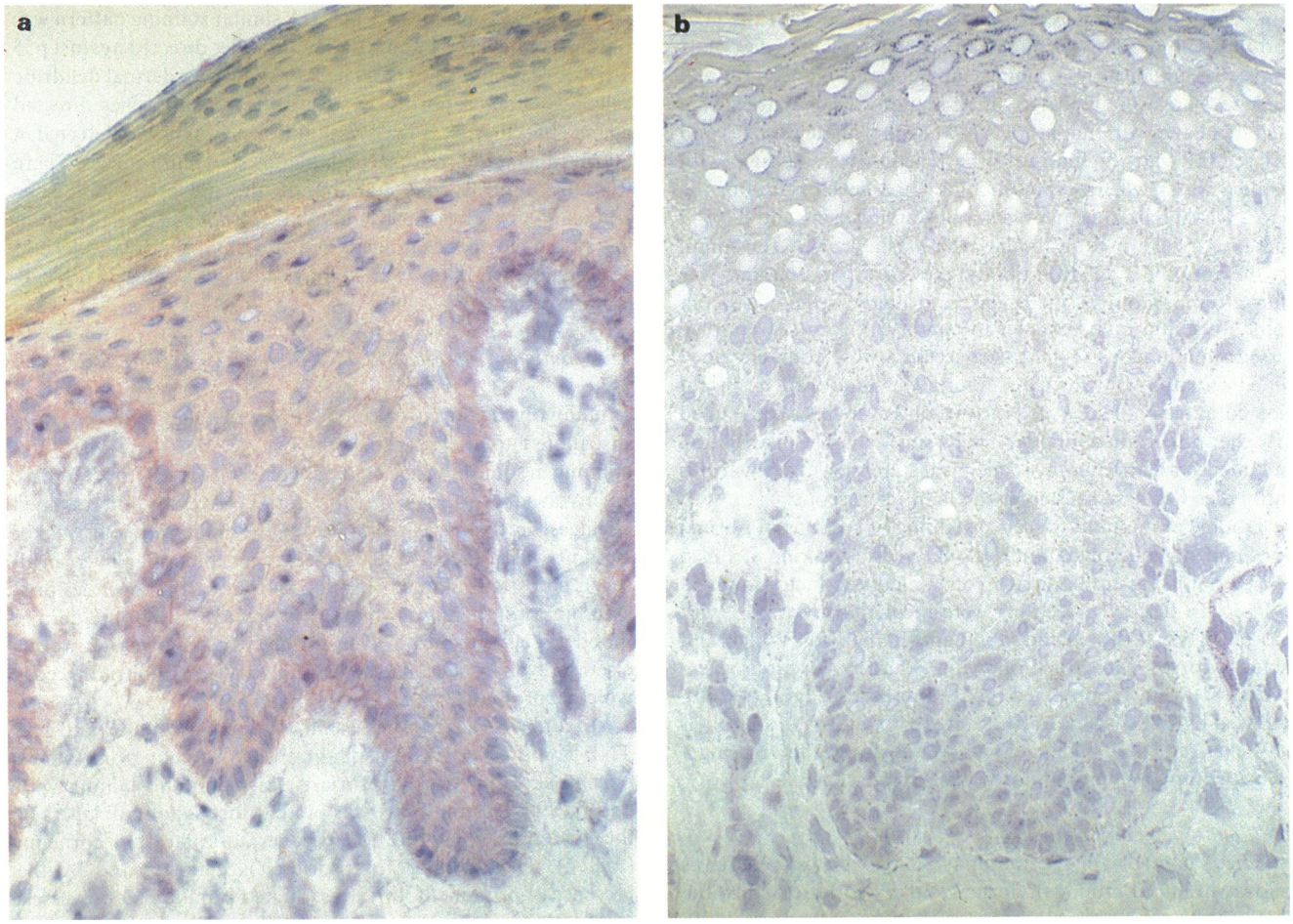

Fig. 5. Distribution of immunoreactive TNF-α in lesional psoriasis skin. (a) TNF-α immunoreactivity is strongest in the basal cell layers of the epidermis, but weaker staining is also distributed to the upper epidermal layers. (b) Adjacent serial section incubated with preimmune rabbit serum as control (× 350).

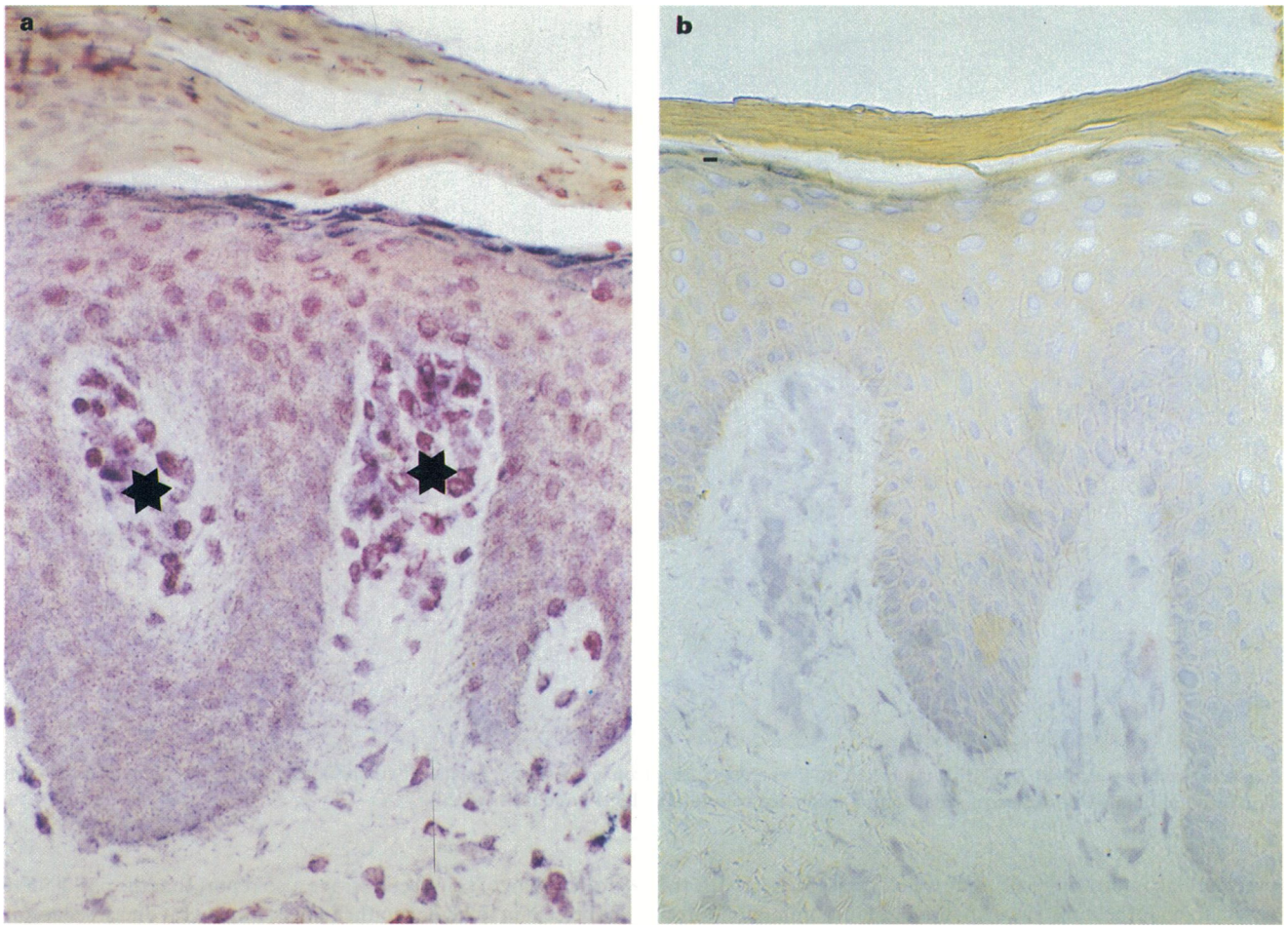

Fig. 6. (a) Immunoreactive p55 TNF-R is distributed throughout the epidermis, including the parakeratotic stratum corneum, in involved psoriatic skin (htr-9 antibody). Note perivascular cell-associated staining within the papillary dermis at the sites typically occupied by dilated tortuous blood vessels in psoriasis (*). (b) Adjacent serial section incubated with murine IgG1 as control (× 350).

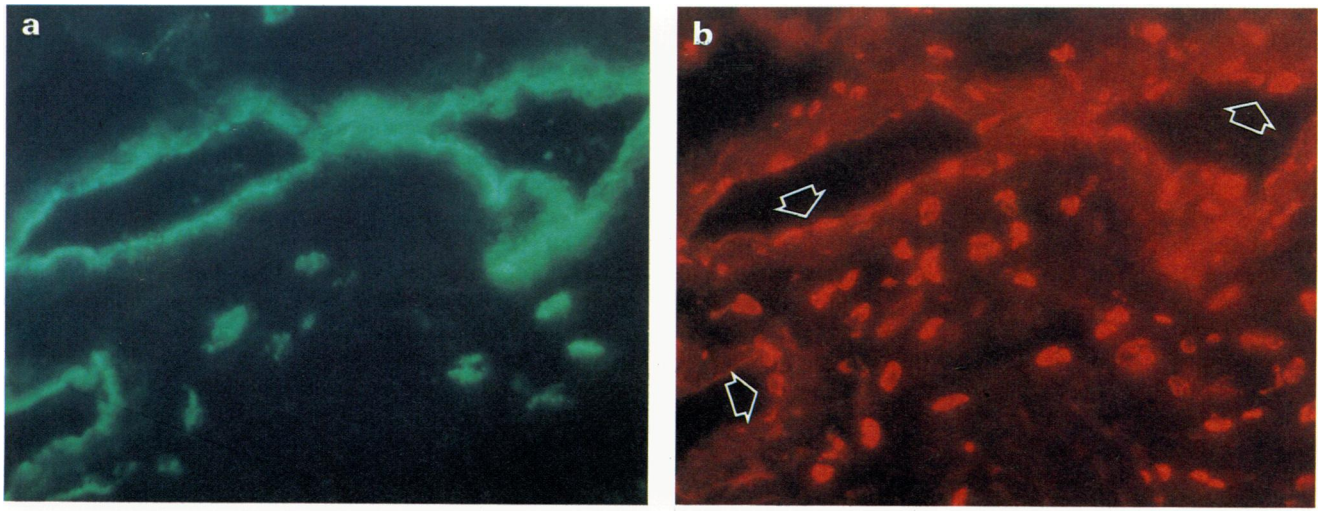

Fig. 7. Colocalization of staining on papillary dermal blood vessels from lesional psoriasis skin, with antibodies to (a) the endothelial cell marker von Willebrand factor, and (b) p55 TNF-R (htr-9). Note staining of individual cells arranged in a linear array at the site of the endothelial lining (arrowed), as well as of inflammatory cells outside the vessel wall, with htr-9 antibody (× 385).

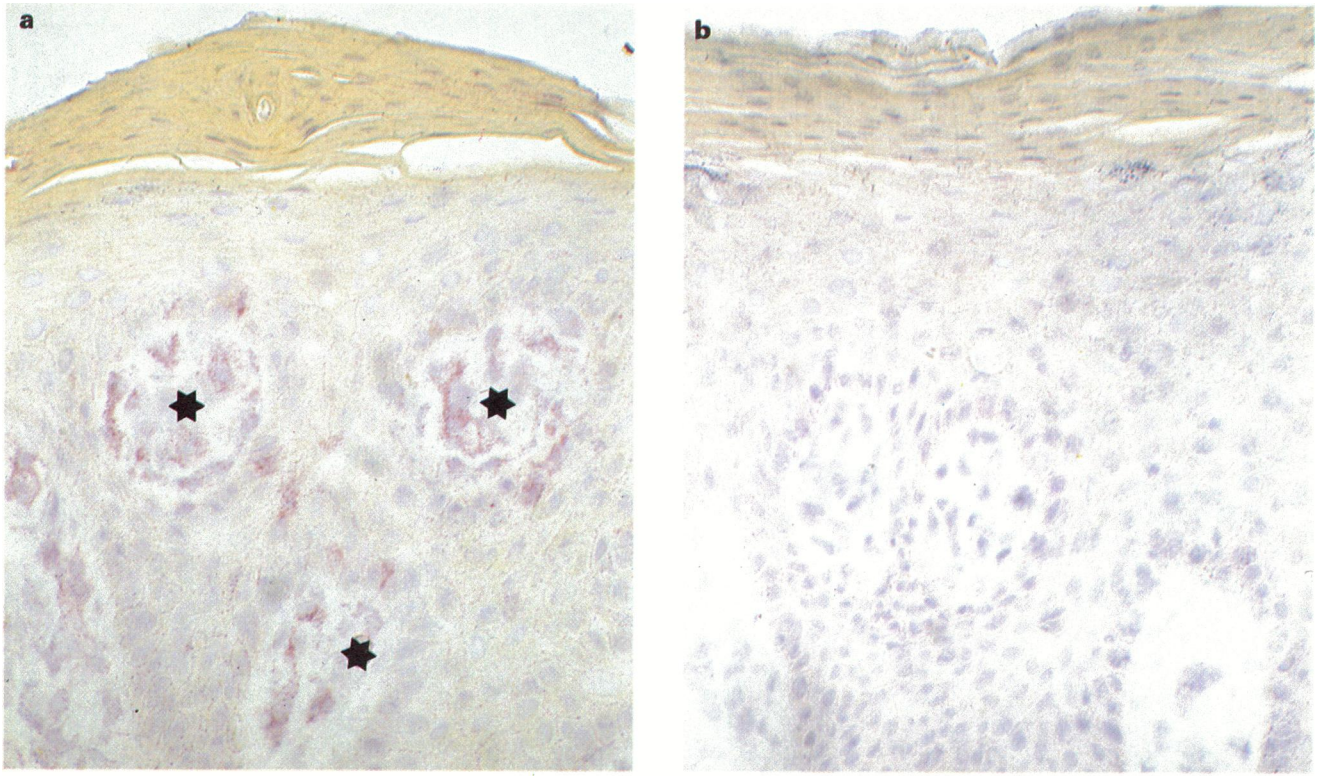

Fig. 8. (a) Immunoreactive p75 TNF-R is localized to the perivascular region in the dermal papillae (*) in this tangential section of involved psoriatic skin (utr-1 antibody). (b) Adjacent serial section incubated with murine IgG1 as control (× 350).

perivascular infiltrating cells, but was also associated with the vascular endothelium, since in double label immunofluorescence studies individual p55 TNF-R-positive cells were present in a linear array around the blood vessel lumen at the site of the endothelial lining, as shown by co-localization with staining for von Willebrand factor (Fig. 7).

Immunostaining with anti-p75 TNF-R in uninvolved psoriasis skin did not differ from that in normal skin. In involved psoriasis skin, there was weak but distinct staining in the perivascular region in the dermal papillae in all biopsies (Fig. 8); studies to differentiate between endothelial and inflammatory cell staining were not carried out.

DISCUSSION

We document here for the first time the distribution of the p55 TNF-R and the p75 TNF-R in normal and inflamed skin. We report the novel finding that the p55 TNF-R is distributed throughout the viable layers of the epidermis in normal and psoriasis skin, and in the parakeratotic stratum corneum in lesional psoriasis epidermis. In both normal and psoriatic skin, staining was predominantly intracellular, with perinuclear accentuation. A similar staining pattern was obtained with all three antibodies directed against independent epitopes on the p55 TNF-R. Cytoplasmic and perinuclear staining with htr-9 antibody to p55 TNF-R has also been reported in synovial membrane cells from patients with rheumatoid arthritis [22]. This contrasts with a report of cell surface staining of cultured keratinocytes with htr-9 antibody to p55 TNF-R [24], but the latter study was based on flow cytometric staining of intact cells, rather than of skin sections. We also report that the p55 TNF-R is present in follicular epithelium, eccrine sweat ducts, sebaceous glands, and in association with arrector pili muscles and a network of upper dermal dendritic cells. In lesional psoriasis skin, there was up-regulation of expression of the p55 TNF-R in association with upper dermal blood vessels, confirmed by double label immunostaining with anti-p55 TNF-R and anti-von Willebrand factor antibodies. In contrast, the p75 TNF-R was not expressed by either normal or psoriatic epidermis. The p75 TNF-R was detected only in association with eccrine sweat glands and localized to a network of upper dermal dendritic cells in normal skin; in lesional psoriasis skin, p75 TNF-R was localized to upper dermal blood vessels and to the perivascular inflammatory infiltrate. Our findings are consistent with a recent report that human keratinocytes in culture express the p55 TNF-R but not the p75 TNF-R [24], and strongly suggest that TNF-α effects on the epidermis are mediated by binding to the p55 TNF-R.

Studies of the distribution of TNF-α in normal skin have produced conflicting results. In some reports, neither mRNA for TNF-α nor immunoreactive or bioactive TNF-α could be detected in normal human skin [25,26]. We found by immunostaining that TNF-α was localized to the basal layers of the epidermis, follicular epithelium, and eccrine sweat ducts. This localization agrees with that reported by one group using *in situ* hybridization for TNF-α mRNA [27]. Another group noted that only eccrine duct epithelium and perivascular dermal dendrocytes stained positively with antibodies to TNF-α [10,14]. Other authors have reported variously the distribution of TNF-α in normal human skin as localized to upper, but not basal,

epidermal cells [32], or to dermal mast cells and basal keratinocytes only [29]. It is hard to explain these disparate findings, but they probably result from use of different techniques and antibodies.

TNF-α induces production by cultured keratinocytes of transforming growth factor-alpha (TGF-α) [10], which promotes keratinocyte division. This effect of TNF-α, taken together with its effects on the recruitment of inflammatory cells, might suggest a role for this cytokine in the development of psoriasis. Moreover, continuous hypodermic infusion of recombinant murine TNF-α into mice induces local proliferation of fibroblasts, capillaries and epidermal cells, which are features of the histopathology of psoriasis [33]. However, TNF-α inhibits proliferation of cultured human keratinocytes [34], TNF-α treatment of mice results in a lowered mitotic index and induction of a granular layer in the mouse tail model of psoriasis [35], and systemic administration of TNF-α may induce remission in patients with extensive psoriasis [36,37]. The consequent uncertainty regarding the role of TNF-α in psoriasis is matched by conflicting reports on the distribution of TNF-α in psoriatic lesional skin. TNF-α mRNA could not be detected in either the epidermis or dermis of psoriatic skin in one study [25], TNF-α was reportedly absent from suction blister fluids and stratum corneum in psoriasis patients in another study [38], and serum levels of TNF-α have been reported not to be altered in psoriasis [39]. However, immunoreactive, but not bioactive, TNF-α was detected in extracts of psoriatic lesions by one group [26]. The only previous study reporting on immunolocalization of TNF-α in psoriasis lesions noted intense diffuse expression by papillary dermal macrophages, and focal keratinocyte and epidermal Langerhans cell TNF-α expression [10]. We found that, in lesional psoriatic skin, TNF-α expression was predominantly expressed by the basal layer, but was also detectable at higher levels of the epidermis; staining of upper dermal blood vessels was present in several biopsies.

Although epidermal TNF-α expression in lesional psoriasis skin, in contrast to normal skin, was not restricted to the basal layer, our results do not support a major role for an alteration in the level of epidermal expression of this cytokine in the pathogenesis of psoriasis. However, we sampled only lesions of stable plaque psoriasis, and the findings might have differed had we biopsied acute lesions. Similarly, there was no marked difference in the level of expression of the TNF-R p55 within the epidermis between normal, uninvolved and lesional psoriasis skin. By contrast, in view of the action of TNF-α in inducing adhesion molecules on vascular endothelium [3–5], our findings of up-regulation of expression of TNF-α, p55 TNF-R and of p75 TNF-R in association with upper dermal blood vessels and perivascular inflammatory cells would be consistent with an important role for TNF-α in promoting recruitment of inflammatory cells into inflamed skin in psoriasis.

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