Differential Expression of GRO-α and IL-8 mRNA in Psoriasis: A Model for Neutrophil Migration and Accumulation *In Vivo*

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Dense focal accumulation of neutrophils in the upper epidermis is a hallmark of psoriasis. Because the signals for neutrophil diapedesis and migration *in vivo* are not fully understood, psoriatic lesions with pronounced migration of neutrophils may serve as an important model for studying neutrophil chemotaxis. In this study, we present evidence for differential expression of the neutrophil chemotactic cytokines growth-related oncogene α, interleukin-8, and ENA-78 (epithelial cell derived and neutrophil-activating properties, 78 amino acids) in psoriatic lesions. *In situ* hybridization and immunohistochemistry of serial sections were employed to identify and microanatomically localize the cells producing these chemokines. High levels of focal interleukin-8 message were found to be expressed in the upper epidermis by keratinocytes and, most importantly, neutrophils themselves. Growth-related oncogene α transcripts were detected in clusters of keratinocytes of the upper epidermis at the same sites where interleukin-8 mRNA was abundant. In contrast to interleukin-8, growth-related oncogene α was also detected in the papillary dermis produced by vessel-associated cells. Sites of interleukin-8 and growth-related oncogene α mRNA expression were associated with infiltration of neutrophils. Interestingly, mRNA expression of the highly homologous chemokine ENA-78 was quiescent. In conclusion, our data indicate that growth-related oncogene α is an important chemoattractant for neutrophil diapedesis *in vivo*, whereas further migration of neutrophils and formation of micropustules appears to be influenced by the cooperative action of both growth-related oncogene α and interleukin-8. ***Key words: ENA-78. J Invest Dermatol 107:778–782, 1996***

Neutrophils are one of the dominant leukocyte subsets in many inflammatory conditions in the skin and other organs. In addition to their role as effector cells in the efferent limb of the immune system, there is a growing body of evidence that neutrophils synthesize and release a plethora of inflammatory cytokines characterizing them as important modulators of the immune response (Lloyd and Oppenheim, 1993). Mechanisms of neutrophil diapedesis and migration, with respect to chemotactic cytokines, have primarily been deduced from *in vitro* studies (Rot, 1992; Springer, 1994), leaving the *in vivo* relevance to be proved.

Psoriatic lesions are characterized by a focal dense infiltration of neutrophils that migrate from the vascular to the dermal compartment and through all layers of the epidermis up to the stratum corneum. High amounts of interleukin-8 (IL-8) (Schröder and Christophers, 1986; Walz *et al*, 1987; Matsushima *et al*, 1988; Yoshimura *et al*, 1988) and growth-related oncogene α (GRO-α; Anisowicz *et al*, 1987; Richmond *et al*, 1988) have been found in scale material from psoriatic lesions (Camp *et al*, 1986; Schröder and Christophers, 1986; Schröder *et al*, 1992). Both agents are chemokines, also known as intercrines (Oppenheim *et al*, 1991), a supergene family of small secreted proteins that mediate inflammation by inducing chemotaxis and activation of some inflammatory cells. Members of the so-called C-X-C subfamily of chemokines include IL-8, GRO-α, and ENA-78 (epithelial cell derived and neutrophil-activating properties, 78 amino acids), which are all potent chemoattractants for neutrophils (Oppenheim *et al*, 1991; Walz *et al*, 1991). The pronounced accumulation of neutrophils in the upper malphighian layer with formation of microabscesses in psoriatic lesions has previously been attributed to high levels of IL-8 expression in this area (Schröder and Christophers, 1986; Gillitzer *et al*, 1991). Because IL-8 is not detectable in papillary vessels of psoriatic lesions by *in situ* hybridization (Gillitzer *et al*, 1991), it is tempting to speculate that other neutrophil-specific C-X-C chemokines such as GRO-α and/or ENA-78 may be involved in the guidance of neutrophils through the endothelial junctions and underlying tissue. The microanatomic location of GRO-α and ENA-78 in psoriasis has thus far not been studied, and it is unknown which cell types produce these cytokines *in vivo*. In addition, it is important to know whether these chemokines are simultaneously expressed with IL-8 mRNA and/or other chemokines. To address this issues, we used *in situ* hybridization to identify and localize expression of the chemokines GRO-α, ENA-78, and IL-8.

In this study, we demonstrate that GRO-α and IL-8 mRNA are

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Abbreviations: GRO-α, growth-related oncogene α (synonymous with melanoma growth-stimulating activity/MGSA); ENA-78, epithelial cell derived and neutrophil-activating properties, 78 amino acids; MCP-1, monocyte chemoattractant protein-1 (synonymous with monocyte chemoattractant and activating factor).

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differentially expressed in psoriatic lesions and are produced by different cell populations. GRO-α and IL-8 may, consequently, influence different steps of neutrophil migration and activation *in vivo*.

MATERIALS AND METHODS

Selection and Preparation of Skin Sections Fresh incisional spindle-shaped biopsy specimens (n = 21) from untreated patients containing 2/3 lesional and 1/3 nonlesional psoriatic skin were taken under local anesthesia. For control purposes, normal skin biopsies (n = 5) from healthy volunteers were used. The biopsies were placed in OCT compound (Tissue-Tek, Miles Scientific, Naperville, IL) immediately after excision and stored at −80°C until use. For immunohistochemistry and *in situ* hybridization, serial 5- to 6-μm sections were fixed in acetone (10 min, 4°C) for immunohistochemistry and 4% paraformaldehyde (20 min at room temperature) for *in situ* hybridization.

Immunohistology For immunohistologic staining, a three-step streptavidin–biotin peroxidase or alkaline phosphatase procedure was used as described elsewhere (Gillitzer *et al*, 1990). Briefly, after blocking Fc receptors with 20% sheep serum (for goat anti-GRO-α antiserum, 10% fetal bovine serum instead of sheep serum was used) in milk powder solution (blotto), containing 5% skim milk powder and 0.1% Tween 20 in phosphate-buffered saline at pH 7.4, sections were overlaid with the following antibodies at 4°C overnight: monoclonal antibody (MoAb) anti-CD45 (HLe, at 1:300, Becton-Dickinson, Sunnyvale, CA), MoAb anti-human neutrophil elastase (Dako-elastase, at 1:100; Dako, Copenhagen, Denmark), and goat antiserum against human GRO-α (at 1:200; R&D Systems, Abingdon, U.K.). Afterwards, sections were washed with Blotto and incubated for 1 h at room temperature with biotinylated sheep anti-mouse Ig (Amersham, Braunschweig, Germany) or mouse anti-goat Ig (Jackson ImmunoResearch Laboratories, West Grove, PA), respectively. Finally, after further washing, sections were incubated with a preformed streptavidin–biotin peroxidase or alkaline phosphatase complex (streptABComplex-POX or streptABComplex-AP, Dako) for 1 h at room temperature. Labeling was visualized using peroxidase-specific substrates (3-amino-9-ethylcarbazole, diaminobenzidine) (Sigma Chemicals, St. Louis, MO) or alkaline phosphatase–specific substrates (NAMP/Fast Red or Fast Blue, NABP/New Fuchsin; Sigma), respectively. For control purposes, the first MoAb was omitted and replaced by an irrelevant isotype-matched reagent. These experiments consistently yielded negative results.

In Situ Hybridization

Preparation of ^{35}S-Labeled RNA Probes The cDNA probes used for *in situ* hybridization were kindly provided by T. Yoshimura [NCI, Frederick, MD; MCP-1, monocyte chemoattractant protein-1 (synonymous with monocyte chemoattractant and activating factor)], C. Weissmann (University of Zürich, Zürich, Switzerland; IL-8), A. Anisowicz (Dana Farber Cancer Institute, Boston, MA; GRO-α), and A. Walz (Theodor Kocher Institute, Bern, Switzerland; ENA-78). Subcloning of specific DNA fragments in plasmids with SP6/T7 (Promega, Madison, WI) or T3/T7 initiation sites (Bluescript, Stratagene, La Jolla, CA) was performed according to standard protocols (Sambrook *et al*, 1989). For sense and anti-sense reaction products, SP6, T3, and T7 polymerase reactions were performed as previously described (Gillitzer *et al*, 1993). Briefly, after linearization of plasmid DNA with appropriate restriction enzymes, ^{35}S-labeled sense and anti-sense probes were obtained by *in vitro* transcription using SP6, T3, or T7 RNA polymerases (Boehringer Mannheim, Mannheim, Germany) together with ATP, guanosine triphosphate, cytidine triphosphate (Boehringer), and [^{35}S]uridine triphosphate (Amersham) as substrates. After elimination of the original linearized template cDNA with deoxyribonuclease (Pharmacia LKB Biotechnology, Munich, Germany) alkaline hydrolysis of labeled probes was performed for 30–50 min according to the formula t(min) = (Lo − Lf)/0.11 × Lo × Lf (Lo = initial length in kilobase pairs, Lf = final size in kilobase pairs) (Angerer *et al*, 1987) at 60°C in a carbonate buffer (pH 10.2). After several ethanol precipitation steps, the radioactive riboprobe was adjusted to a specific activity of 1×10^6 cpm/μl in 0.01 M Tris(hydroxymethyl)-aminomethane-HCl, pH 7.5, containing 1 mM ethylenediamine tetraacetic acid.

Hybridization Procedure The hybridization protocol described by Müller *et al* (1988) was used. Briefly, sections were treated with 1 μg proteinase K per ml (Boehringer, Mannheim, Germany) for 30 min at 37°C. Afterwards, sections were refixed in 4% paraformaldehyde, acetylated with acetic anhydride in 0.1 M triethanolamine (pH ajusted to 8.0) for 10 min, dehydrated in graded ethanol, and air-dried. Then, sections were overlaid with 20 μl of hybridization solution (50% formamide, 300 mM NaCl, 20 mM Tris(hydroxymethyl)-aminomethane-HCl, pH 8.0, 5 mM ethylenediamine tetraacetic acid, 1× Denhardt's solution, 10% dextran sulfate, 100 mM dithiothreitol, and 2×10^5 cpm of heat-denaturated radioactive sense or anti-sense probe per μl). The slides were coverslipped, sealed, and hybridized at 46–47°C for 12–16 h. Ribonuclease treatment was included as a control and consistently abrogated specific hybridization signals. After removing the coverslips, the nonhybridized RNA probe was removed by incubation with highly stringent washing solutions (50% formamide, 2× saline-sodium citrate, and 5 mM ethylenediamine tetraacetic acid at 54–57°C) under constant stirring. To further minimize nonspecific background, noncomplementary unhybridized single-stranded probe was digested with ribonuclease A (20 μl per ml) and ribonuclease T1 (1 U per ml) (Boehringer) for 30 min at 37°C. For autoradiography, slides were dipped in NTB-2 Kodak solution (1:2 in 800 mM ammonium acetate) and exposed for 1–5 wk at 4°C.

RESULTS

Psoriasis: IL-8 mRNA Expression in Upper Level Keratinocytes and Neutrophils of Microabscesses Using IL-8 anti-sense probes, a strong and abundant cell-associated silver grain accumulation in the upper malphighian layer of the acanthotic epidermis of psoriatic lesions was detected in 16 of 21 lesions studied. Three different signal patterns were usually identified in psoriatic lesions: i) a cluster of IL-8 mRNA+ cells in the viable layers of the epidermis but not in the parakeratotic stratum corneum **(Fig 1*A*)**; (ii) an exclusive focal expression of IL-8 message in the parakeratotic stratum corneum **(Fig 2*A*)**; (iii) a simultaneous expression in the upper viable epidermal layer and the parakeratotic stratum corneum. In contrast, control experiments with IL-8 sense probes were entirely negative and the MCP-1 anti-sense probe as an internal control revealed signals in the basal layer of the rete ridges as previously shown (Gillitzer *et al*, 1993) but no signals at sites of IL-8 expression. In normal or uninvolved skin, IL-8 message was entirely absent.

The expression of IL-8 mRNA in the upper viable layers of the epidermis was associated with an increased number of CD45+ neutrophil elastase (NE)+ infiltrating neutrophils in the dermis and epidermis. Most notably, the hybridization pattern was not identical with the staining pattern for neutrophils, suggesting that in these cases the keratinocytes produced IL-8. In contrast, expression of IL-8 in the parakeratotic horny layer was only present when there was an accumulation of neutrophils, as identified by immunohistologic staining with MoAb anti-human neutrophil elastase or MoAb anti-CD45 on serial sections **(Fig 2*A,B*)**, suggesting that neutrophils, rather than parakeratotic keratinocytes, produce IL-8 mRNA. A similar correspondence between the pattern of neutrophil elastase staining and IL-8 mRNA expression as shown in **Fig 2*A,B*** has not been found outside the parakeratotic layer. Our hybridization study, therefore, clearly shows that two different cell populations in psoriatic lesions express IL-8 mRNA, namely, keratinocytes of the upper viable epidermal layers and neutrophils residing in the parakeratotic stratum corneum.

GRO-α mRNA Expression and Immunoreactivity in Upper Level Keratinocytes and Vessel-Associated Cells of the Papillary Dermis *In situ* hybridization with GRO-α anti-sense probes, but not sense probes, revealed strong hybridization signals in the epidermis in 17 of 21 biopsies **(Fig 1*B,C*)**. GRO-α message was only detectable in lesional skin and was absent in perilesional skin as well as in normal skin of healthy volunteers. With the exception of some single cells in the lower epidermis, the mRNA expression was mainly clustered, with high signal densities in the upper epidermal layers, as observed with IL-8 anti-sense probes. In contrast to IL-8, GRO-α mRNA expression was seldom detected in the parakeratotic layers of the stratum corneum, demonstrating the specificity of the hybridization procedure with no cross-reactivity between the two highly homologous chemokines. The most noteworthy finding of this study was the detection of strong cell-associated hybridization signals in the papillary dermis in 16 of 21 lesions tested **(Figs 1*B,C*, 3*A*, 4*A,B*)**. Interestingly, in two specimens, there was only GRO-α message in the dermis, but not in the epidermis. These sections were clinically regarded as early

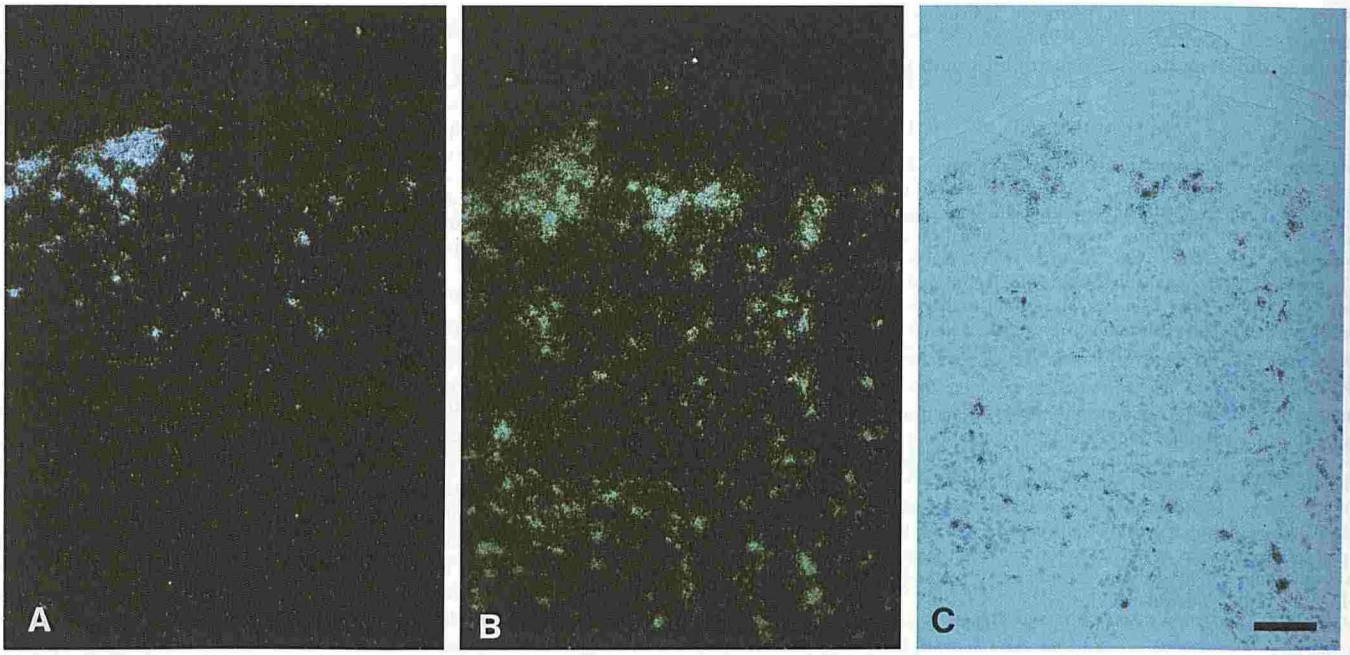

Figure 1. IL-8 mRNA expression is restricted to the upper epidermal layer whereas GRO-α is also expressed in the papillary dermis of psoriatic lesions. *In situ* hybridization with [^{35}S]uridine triphosphate-labeled anti-sense probes of IL-8 *(A)* and GRO-α *(B,C)* on serial sections of a psoriatic lesion as detailed in *Materials and Methods*. IL-8 mRNA expression is focally clustered in the viable layer of the upper epidermis, but no message is detectable in the dermis even under dark-field illumination. GRO-α is coexpressed with IL-8 in the same area of the epidermis. In addition, GRO-α is strongly expressed in single scattered cells of the dermal compartment. *(A,B)* Dark-field illumination; *(C)* bright-field illumination. *Scale bar*, 50 μm.

lesions that had developed only a few days prior to excision. To further quantify the distribution of GRO-α expressing cells in dermis *versus* epidermis, the total number of positive cells was counted in both compartments in sections of 21 different biopsies. In seven of these, the number of positive epidermal cells was greater than or equal to the number of positive dermal cells (ratio epidermis/dermis = 1.8 ± 0.7; mean ± SD). In 14 sections, the overall number of dermal GRO-α mRNA expressing cells was higher than in the epidermis (ratio epidermis/dermis = 0.4 ± 0.2; mean ± SD). Dermal cells strongly expressing GRO-α mRNA were either closely associated with papillary vessels (50–70% of dermal GRO-α mRNA+ cells) or singly distributed in the papillary space **(Fig 4*A,B*)**. Using GRO-α–specific antiserum, we could demonstrate strong immunoreactivity in papillary vessels as well as

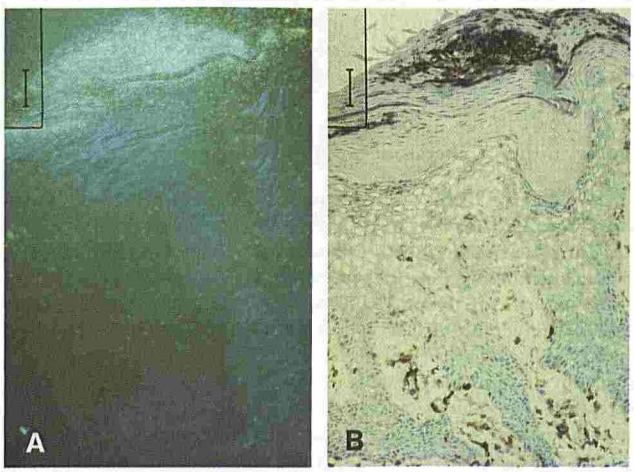

Figure 2. Neutrophils of microabscesses in psoriasis express high levels of IL-8 message. *In situ* hybridization of a psoriatic lesion with [^{35}S]uridine triphosphate-labeled IL-8 anti-sense probe *(A)* and immunohistochemical labeling of a serial section with MoAb anti-CD45 using a three-step streptABC method with 3-amino-9-ethylcarbazole as substrate *(B)*. In this section, *in situ* signals are exclusively visible in the parakeratotic stratum corneum *(A)* where CD45+ neutrophils are clustered *(B)*. *(A)* Epipolarization illumination; *(B)* bright-field illumination. *Scale bar*, 25 μm.

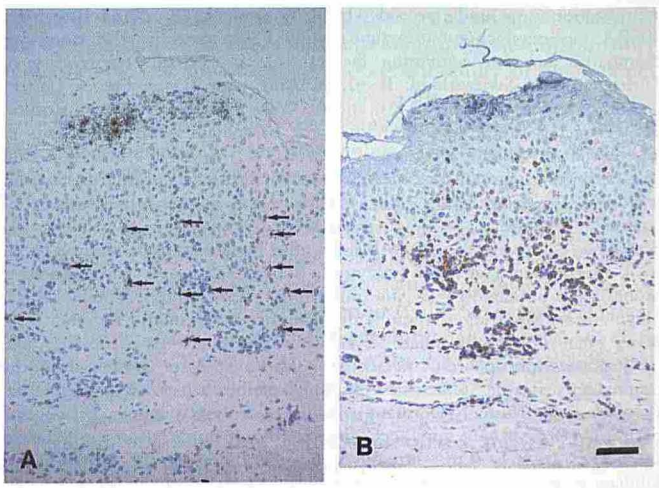

Figure 3. Expression of GRO-α mRNA in the psoriatic epidermis is associated with increased infiltration of neutrophils. *In situ* hybridization with [^{35}S]uridine triphosphate-labeled GRO-α anti-sense probe *(A)* and three-step streptABC–horseradish peroxidase immunolabeling for neutrophils with MoAb anti-neutrophil elastase (NE) and 3-amino-9-ethylcarbazole substrate as described in *Materials and Methods (B)*. GRO-α mRNA is strongly expressed in the upper epidermis and some scattered cells in the dermis *(arrows)*. In the area of strong GRO-α mRNA expression, there is pronounced infiltration of neutrophils as revealed by staining for neutrophils on a serial section *(B)*. The pattern of GRO-α and NE-expression on serial sections is different, indicating that neutrophils are not a major source for GRO-α. Bright-field illumination. *Scale bar*, 50 μm.

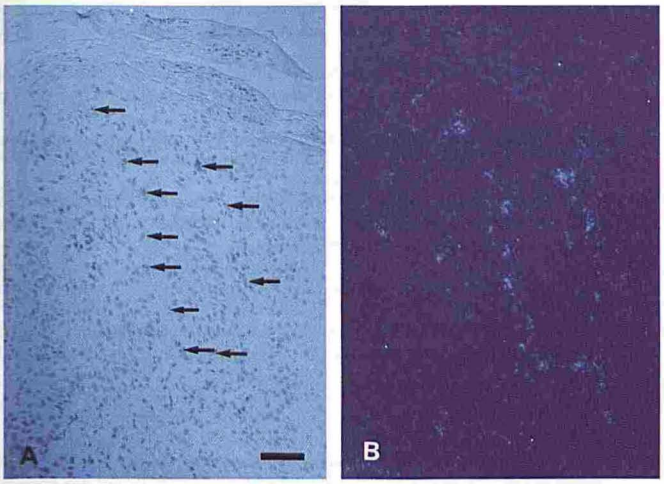

Figure 4. GRO-α mRNA is strongly expressed in vessel-associated cells of the papillary dermis in psoriatic lesions. *In situ* hybridization with GRO-α anti-sense probe. In this section GRO-α mRNA is preferentially expressed in cells of the papillary dermis associated with papillary vessels (arrows). In contrast to **Fig 1**, epidermal GRO-α mRNA expression is low. *(A)* Bright-field illumination; *(B)* dark-field illumination. *Scale bar*, 25 μm.

in scattered cells of the papillary dermis **(Fig 5)**. The intensity of dermal and/or epidermal GRO-α mRNA expression was mainly multifocal and highly variable even within one section. Taken together, three different expression patterns for GRO-α could be defined: (i) an exclusive expression of GRO-α in the upper dermis (in two cases of 21 investigated); (ii) an exclusive epidermal expression, mainly in the viable layers of the upper epidermis (three cases); (iii) a concomitant expression of GRO-α mRNA in the dermis and epidermis (14 of 21 cases).

To exclude that infiltrating neutrophils produce GRO-α, we performed *in situ* hybridization and immunohistologic labeling of neutrophils with neutrophil elastase–specific MoAb on serial sections. We found that the GRO-α hybridization pattern did not correspond to the distribution pattern of neutrophils **(Fig 3*A,B*)**, indicating that neutrophils were not a major source for GRO-α. Neutrophils, however, were detected in close proximity to GRO-α signals, suggesting that the latter exhibits chemoattractant activity for neutrophils in psoriatic lesions.

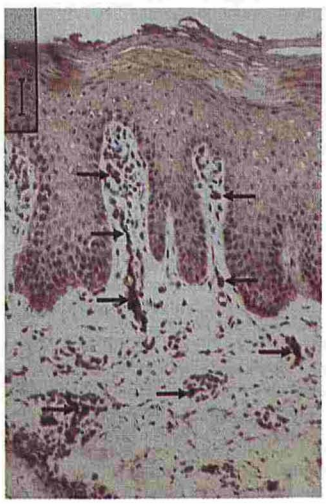

Figure 5. GRO-α immunoreactivity in a psoriatic lesion is confined to papillary vessels and single scattered cells of the papillary dermis. Three-step streptABC–horseradish peroxidase method using goat anti–GRO-α serum and a three-step streptABC–horseradish peroxidase method and 3-amino-9-ethylcarbazole as substrate. Strong immunoreactivity is observed in vessels of the papillary dermis *(arrows)* and some infiltrating cells. In addition, there is homogeneous although weaker immunoreactivity in the epidermis. *Scale bar*, 50 μm.

When we performed additional *in situ* hybridizations on serial sections with IL-8 anti-sense probes, we found that in all lesions GRO-α and IL-8 were coexpressed in the same focal areas of the upper stratum spinosum **(Fig 1*A,B,C*)**. Despite the overlap of GRO-α and IL-8 hybridization signals in serial sections, one cannot determine from our data whether individual cells synthesize both GRO-α and IL-8 mRNA simultaneously.

As opposed to the strong and focal coexpression of IL-8 and GRO-α, only a negligible number of epidermal cells expressed ENA-78 mRNA. In addition, ENA-78 mRNA expression was quiescent in uninvolved as well as healthy skin.

DISCUSSION

Neutrophils in psoriatic lesions focally constitute a major portion of the papillary infiltrate and are the dominant leukocyte subpopulation in the epidermis. Because neutrophils also actively participate in the immunoregulatory network by secretion of various cytokines upon stimulation (Lloyd and Oppenheim, 1993), they have to be considered as an important inflammatory cell component in the pathogenesis of psoriasis. In this *in vivo* study, we directed our attention to mechanisms leading to neutrophil diapedesis from the blood and their subsequent migration to the upper epidermal layer by studying the *in situ* mRNA expression of the neutrophil chemoattractant C-X-C chemokines ENA 78, GRO-α, and IL-8 in psoriasis lesions. The following observations were made: (i) along with keratinocytes, a major portion of lesional IL-8 message is produced by neutrophils residing in microabscesses in the stratum corneum; (ii) as opposed to IL-8, GRO-α is also highly expressed by single cells in the papillary dermis (vessel-associated cells and infiltrating cells); (iii) GRO-α mRNA expression is highly variable being predominantly epidermal in some regions and dermal in others; (iv) in the upper epidermis, GRO-α as well as IL-8 mRNA are typically coexpressed in clusters of keratinocytes; (v) focal expression of GRO-α and IL-8 in the epidermis is associated with a focal infiltration of neutrophils; (vi) ENA-78 mRNA message is only detectable in a few epidermal cell and does not appear to play an important role for neutrophil migration in psoriasis.

In view of the peculiar topobiology of GRO-α and IL-8 gene expression and the frequent accumulation of neutrophils in close proximity or at the same sites, we propose the following model of neutrophil diapedesis and migration. Neutrophils encounter various adhesion-promoting glycoproteins (*e.g.*, intercellular adhesion molecule, vascular cell adhesion molecule, and E-selectin) in the papillary vessels of psoriatic lesions as previously shown (Gillitzer *et al*, 1991; Groves *et al*, 1993). This initiates a series of events characterized by neutrophils rolling along the vessel wall and ultimately adhering to the endothelium (Springer, 1994). GRO-α, at least in the *in vivo* model of psoriasis rather than IL-8 as previously discussed (Rot, 1992; Huber *et al*, 1993), may preferentially be synthesized by vessel-associated cells and presented via surface proteoglycans to neutrophils expressing the IL-8 receptor A and IL-8 receptor B (Holmes *et al*, 1991; Murphy and Tiffany, 1991). Thus, GRO-α may enhance activation of neutrophils and their extravasation and access to the cutaneous milieu. Because we never detected significant levels of IL-8 mRNA in the papillary space in various inflammatory skin disorders by *in situ* hybridization (U. Spandau, A.Toksoy, E.B. Bröcker, and R.Gillitzer, unpublished observations), GRO-α, rather than IL-8, has to be regarded as a pivotal chemotactic factor for neutrophil diapedesis in skin inflammation. Assuming unrestricted translation of high levels of GRO-α and IL-8 mRNA into functional peptides due to the transcriptional regulation of chemokine production, there is a focally higher density of neutrophil-specific C-X-C chemokines in the epidermis as compared to the dermis. Redistribution of these chemokines from the epidermis to the dermis could generate an

ascending chemotactic gradient that would further direct neutrophils to the epidermal compartment. Because IL-8 stimulates neutrophils not only via the IL-8 receptor B but also via the IL-8 receptor A (Lee *et al*, 1992), possible receptor desensitization or occupancy through initially bound GRO-α may be overcome and may explain why neutrophils sequentially and rapidly cross several compartment borders (vessel–dermis, dermis–epidermis). In addition, other chemoattractant substances like LTB4 and C5a may accelerate the trafficking of neutrophils (Hammarström *et al*, 1975; Ford Hutchinson *et al*, 1980). Those mediators, however, do not show leukocyte subtype specifity and therefore do not explain the restricted distribution of these subsets in psoriasis.

Stimulated neutrophils secrete some other potent immunomodulatory cytokines including IL-1, IL-6, granulocyte-macrophage colony stimulating factor, granulocyte-colony stimulating factor, and tumor necrosis factor α and IL-8 (Bazzoni *et al*, 1991; Lloyd and Oppenheim, 1993). Neutrophils, therefore, most likely contribute actively to the cytokine network of psoriasis pathogenesis. Despite the fact that IL-8 and GRO-α mRNA expression are triggered by the same cytokines *in vitro* (*e.g.*, tumor necrosis factor α, IL-1, and interferon-γ) (Golds *et al*, 1989; Barker *et al*, 1990; Schröder *et al*, 1990), their different expression in psoriasis implies different mechanisms of mRNA induction or suppression for IL-8 and GRO-α *in vivo*.

Due to the variable expression patterns of GRO-α as well as IL-8 mRNA and the periodic infiltration of neutrophils, one can speculate that within a psoriatic plaque a multifocal dynamic process occurs with a shift of GRO-α production starting at the dermal compartment and later ascending to the upper epidermal compartment. This is supported by the observation that in two early lesions GRO-α mRNA was exclusively expressed in the dermis. The transformation of GRO-α mRNA into protein has been demonstrated by GRO-α immunoreactivity on dermal cells either associated with vessels or scattered within clusters of infiltrating cells. These vessel-associated cells are most likely microvascular endothelial cells that have been demonstrated to produce GRO-α *in vitro* after stimulation with tumor necrosis factor α and IL-1 (M. Goebeler, A. Toksoy, U. Ritter, E.B. Bröcker, and R. Gillitzer, unpublished observations). In the epidermis, immunoreactivity was rather homogeneously distributed and did not correlate with the focal hybridization profiles. This is very reminiscent of what has been observed with MoAb against IL-8, where immunoreactivity in the epidermis (Sticherling *et al*, 1991; Antilla *et al*, 1992) did not correlate with the mRNA expression pattern (Gillitzer *et al*, 1991).

Our comparative analysis on IL-8 and GRO-α expression may very well explain neutrophil trafficking from the vessel compartment to the upper epidermis and may be valid for other inflammatory processes with neutrophil participation. We, however, cannot exclude that additional, as yet undetected, members of the rapidly growing chemokine family participate in this rhythmical event of neutrophil infiltration in psoriasis. Psoriatic lesions, nevertheless, represent an excellent model for studying chemokine action and chemotaxis *in vivo* and thus may help to develop therapeutic strategies that target chemokine action directly.

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