Co-Localized Overexpression of GRO-α and IL-8 mRNA Is Restricted to the Suprapapillary Layers of Psoriatic Lesions

Reinhard Kulke, Iris Tödt-Pingel, Dirk Rademacher, Joachim Röwert, Jens-M. Schröder, and Enno Christophers

Department of Dermatology, Clinical Research Unit, University of Kiel, Kiel, Germany

Epidermal infiltration by polymorphonuclear cells is a prominent feature in psoriatic lesions. Expression of neutrophil-specific chemoattractants by lesional keratinocytes could play an important role in the regulation of this infiltration process. We therefore examined the mRNA expression of GRO-α, a well-characterized peptide with neutrophil-specific activation profile in psoriatic lesions by *in situ* hybridization. Clusters of clearly detectable and in some cases highly abundant GRO-α hybridization signals could be demonstrated in the differentiated layers of psoriatic epidermis. The signals were clearly associated with keratinocytes, with no nearby neutrophils detectable by microscopic examination. When additional tissue sections of GRO-α–expressing lesions were examined with an interleukin-8/neutrophil-activating peptide–8 (IL-8)–specific anti-sense probe, IL-8 expression was detectable and confined to areas also expressing GRO-α. Expression of both GRO-α and IL-8 is focally upregulated by an as yet unknown mechanism in lesional psoriatic keratinocytes, ultimately leading to neutrophil tissue infiltration. We suggest that the focal expression of GRO-α and IL-8 in the epidermal layers above the dermal papillae may be involved in the "squirting papilla" reaction described as a characteristic feature of psoriatic plaque-type lesions. *Key words: psoriasis/squirting papilla.*
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A typical feature of psoriasis is epidermal hyperplasia, which is accompanied by an inflammatory infiltrate located in the vicinity of blood vessels consisting mainly of lymphocytes, macrophages, and neutrophils. Collections of polymorphonuclear leukocytes migrate from the dermal papillae into the epidermis, where characteristic microabscesses are formed [1]. The nearly selective accumulation of neutrophils could be caused by the local generation of neutrophil-specific chemoattractants, leading to the hypothesis that lesional psoriatic keratinocytes play an important role in eliciting neutrophilic inflammatory response mechanisms.

Of all chemokines examined so far, only interleukin-8/neutrophil-activating peptide–8 (IL-8) and GRO-α [2–6] have been detected in significant amounts in psoriatic scales. Both are basic heparin-binding peptides that display prominent neutrophil- and to a lesser extent lymphocyte-activating properties. They share a cysteine-X-cysteine motif near the amino terminus of the processed polypeptide and belong to the chemokine-α subfamily [7]. The human members of this group, which also includes platelet factor 4, β-thromboglobulin, and ENA-78, an epithelial cell–derived, neutrophil-activating peptide of 78 amino acids, are clustered on chromosome 4 [8]. Expression of both IL-8 and GRO-α mRNA has been described for a variety of cells, among them monocytes, fibroblasts, endothelial cells, and keratinocytes. Expression can be induced by so-called primary inflammatory mediators like interleukin-1 or tumor necrosis factor–α, and IL-8 and GRO-α have been shown to be important mediators of physiological as well as pathological immune reactions (for review see [9,10]).

GRO-α was originally described as a gene selectively overexpressed in tumorigenic Chinese hamster embryo fibroblasts. A human equivalent was isolated from a bladder carcinoma cell line [6]. The human gene is identical to a growth factor termed MGSA, which was isolated from a melanoma cell line [11]. Subsequently, GRO-α was shown to be identical to a neutrophil chemotactic protein secreted by fibroblasts and monocytes [12]. Recently, two additional GRO genes, GRO-β and GRO-γ, were identified with 80–90% overall homology at the DNA and protein level [13].

An analysis of neutrophil chemotactic proteins in lesional psoriatic scales revealed GRO-α as well as different IL-8 forms as major chemotaxins [14,15]. By immunohistochemistry, GRO-α/MGSA–like immunoreactivity has been reported to be localized within psoriatic epidermis [16]. GRO-α mRNA expression has been demonstrated by Northern blot hybridization and semiquantitative polymerase chain reaction analysis in lesional psoriatic skin [17]. In contrast to IL-8 [18], the cellular origin of the GRO-α expression has not yet been identified. Furthermore, it is currently unknown whether expression of both chemokines *in situ* is regulated in a coordinated fashion. We therefore examined the expression of the IL-8 and the GRO-α gene in psoriatic lesions by *in situ* hybridization.

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Reprint requests to: Dr. Reinhard Kulke, Hautklinik der CAU, Schittenhelmstrasse 7, 24105 Kiel, Germany.

Abbreviations: GRO-α, GRO/melanoma growth–stimulating activity; GRO-β, GRO-γ, GRO-α–related genes; IL-8, interleukin-8/neutrophil-activating peptide–8; ENA-78, epithelial cell–derived, neutrophil-activating peptide of 78 amino acids.

MATERIALS AND METHODS

Preparation of Skin Specimens Lesional and nonlesional skin specimens from randomly selected patients with untreated and long-standing psoriasis ($n = 12$) were taken by incisional biopsy with written consent of the patients. Normal skin biopsies ($n = 3$) were obtained from nonpsoriatic volunteers. The material was snap-frozen and stored at −70°C until it was further processed.

Construction of Specific Probes Parts of the GRO-α (nucleotides 52–377) and IL-8 (nucleotides 54–427) cDNAs were polymerase chain reaction amplified starting with cDNA prepared from tumor necrosis factor–stimulated dermal fibroblasts. An E-cadherin–specific cDNA probe (nucleotides 13–375) was generated from cDNA prepared from human foreskin-derived keratinocytes. The amplified cDNA fragments were cloned into the *Sal* I site of the vector pGEM1 (Promega, Madison, WI). The identity and orientation of all fragments was confirmed by restriction enzyme analysis. For synthesis of GRO-α or IL-8 anti-sense probes (opposite polarity as mRNA), the RNA expression vectors were linearized with *Sph* I or *Xba* I respectively, each cutting in the multiple cloning site distal from the T7 (GRO-α) or Sp6 (IL-8) bacteriophage promoters used for transcription. The E-cadherin RNA expression vector was cut with *Bam* HI, allowing the synthesis of anti-sense RNA probes using the T7 RNA polymerase. Synthesis of sense and anti-sense RNA probes was performed in the presence of ^{35}S-UTP, resulting in probes with a specific activity of approximately $1–2 \times 10^8$ cpm/μg. *In vitro* RNA synthesis was followed by DNase treatment to eliminate the cDNA template and limited alkaline hydrolysis for reduction of probe length to approximately 150 nt. The quality of the transcribed and hydrolyzed RNAs was tested by agarose gel electrophoresis before applying the probes at a concentration of 5×10^7 cpm/ml of hybridization mixture.

RNA *in Situ* Hybridization Serial cryostat sections of biopsy material, mounted on aminopropylsilane-coated slides, were fixed in freshly prepared 4% paraformaldehyde in phosphate-buffered saline and dehydrated. The sections were then acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0) for 10 min, washed in 0.2 × sodium citrate/sodium chloride buffer and incubated for 2 h at 45°C covered by prehybridization solution (50% formamide, 0.6 M NaCl, 2.5 × Denhardt's solution, 10 mM Tris-HCl [pH 7.5], 1 mM ethylenediamine tetraacetic acid, 0.1% sodium dodecyl sulfate, 0.15 mg of tRNA/ml). After removal of this solution, the tissue sections were overlayed for 12–18 h with hybridization solution (prehybridization solution containing 10% dextran sulfate and ^{35}S-labeled riboprobes). The incubated slides were washed in 50% formamid–1 × sodium citrate/sodium chloride buffer at 50°C, treated with RNase A (10 μg/ml), further washed in 0.1 × sodium citrate/sodium chloride buffer at 60°C, dehydrated, and coated with Kodak NTB-2 emulsion. Exposure was for 2–4 wk, followed by hematoxylin and eosin counterstaining after development. For evaluation a Zeiss Axiophot microscope equipped with dark- and bright-field condenser was used. The specific detection of chemokine mRNA by *in situ* hybridization was confirmed by using sense riboprobes, which gave only background signals, or by pretreatment of the tissue sections with DNase free RNase, which completely abrogated specific signals.

RESULTS

Suprapapillary GRO-α mRNA Expression in Psoriasis To generate probes specific for the C-X-C chemokines IL-8 and GRO-α, parts of their cDNAs were cloned into plasmid vectors with flanking bacteriophage promoters, starting with specific reverse transcriptase–polymerase chain reaction–amplified cDNA prepared from tumor necrosis factor–α–stimulated fibroblasts. The E-cadherin [19] probe used to demonstrate the intactness of epidermal RNA in tissue sections was amplified and cloned from cDNA prepared from foreskin-derived keratinocytes.

Using these chemokine probes, we analyzed the RNA expression patterns in serial cryostat sections of psoriatic lesions. The specimens were obtained from distinct areas of the trunk and thighs of 12 patients suffering from long-standing psoriasis who had not received any local or systemic treatment for at least 3 wk. For control purposes, three specimens from uninvolved skin of psoriatic patients and three biopsies from nonpsoriatic volunteers were included in our study.

Nine of the twelve psoriatic skin specimens examined with the MGSA/GRO-α–specific probe showed clearly detectable and in some cases highly abundant hybridization signals in the upper layers of the acanthotic psoriatic epidermis, which were usually located above the dermal papillae **(Fig 1)**. The GRO-α mRNA expression pattern was focal with clusters of up to several dozen cells exhibiting high expression levels dispersed between areas of intervening epidermis with no detectable GRO-α mRNA expression.

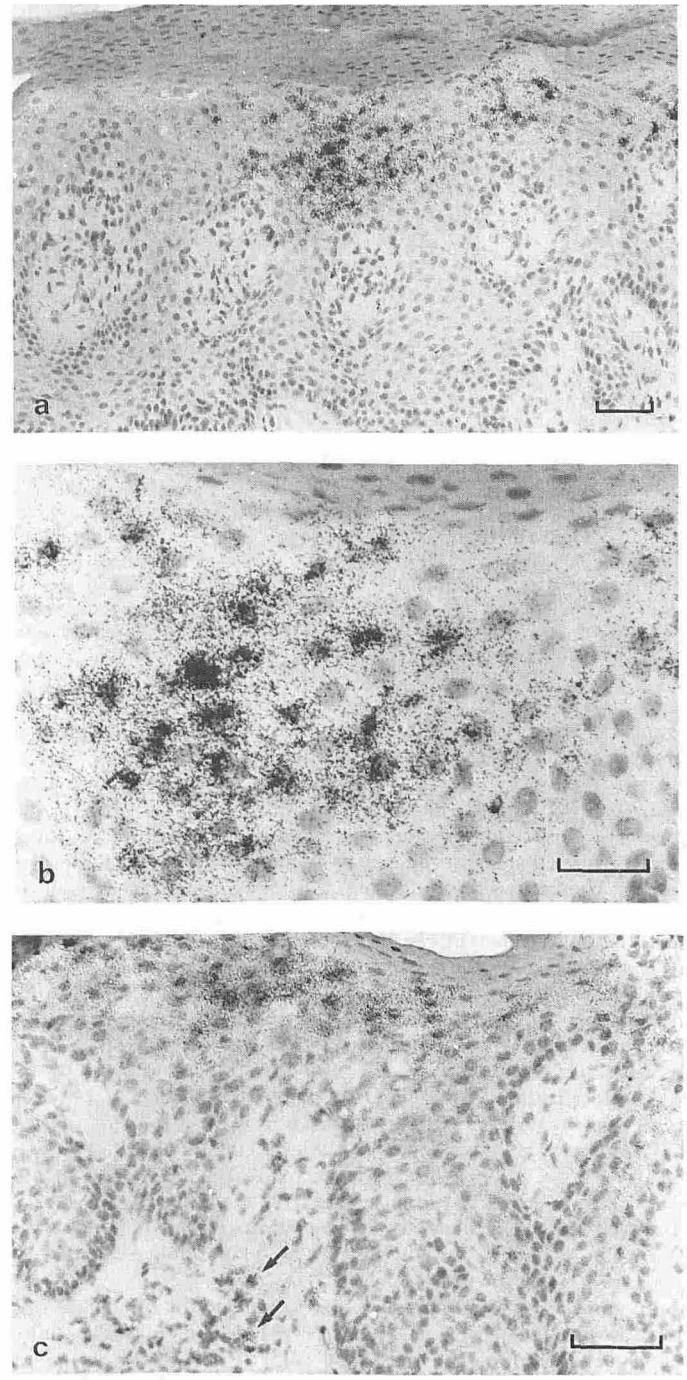

Figure 1. GRO-α mRNA expression in psoriatic lesions is restricted to suprapapillary areas. *In situ* hybridization with ^{35}S-UTP–labeled GRO-α anti-sense RNA probe and emulsion autoradiography on cryostat sections of two different chronic plaque-type psoriatic lesions was performed as described in *Materials and Methods*. *a,b*) In bright field illumination, strong keratinocyte-associated silver grain signals are focally clustered above the rete ridges in the differentiated layers of this psoriatic lesion. *c*) Lesion displaying a similar epidermal GRO-α–specific signal pattern. In addition, some dermal cells (→) also express smaller amounts of GRO-α mRNA. *Scale bars*: *a,c*) 200 μm; *b*) 100 μm.

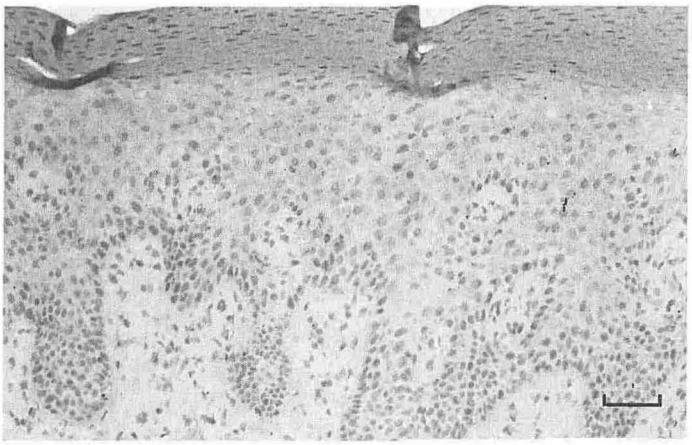

Figure 2. *In situ* hybridization with GRO-α–specific sense probe. *In situ* hybridization with ^{35}S-UTP–labeled GRO-α sense RNA probe and emulsion autoradiography on a serial cryostat section of the psoriatic lesion shown in **Fig 1*a*** and ***b*** to express high levels of GRO-α signals. Lack of detectable *in situ* signals in this bright field illumination photomicrograph supports the interpretation that the signals seen after hybridization with ^{35}S-UTP–labeled GRO-α anti-sense RNA probes on the same lesion **(Fig 1*a,b*)** are not due to unspecific background hybridization. *Scale bar*, 200 μm.

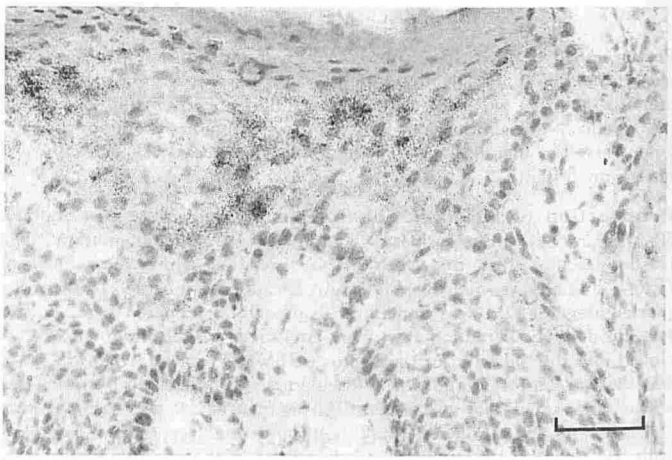

Figure 3. IL-8 mRNA co-localizes to GRO-α–expressing suprapapillary areas in psoriatic lesions. *In situ* hybridization with ^{35}S-UTP–labeled IL-8 anti-sense RNA probe and emulsion autoradiography on a serial cryostat section of the psoriatic lesion shown in **Fig 1*c*** to express high levels of GRO-α signals. The signals obtained with the IL-8 RNA probe are clustered in the same suprapapillary epidermal areas where GRO-α mRNA expression could be detected. Bright field illumination; *scale bar*, 200 μm.

Removal of up to several 100 μm of psoriatic tissue by sequential cryostat cutting resulted in disappearance of the expression signals, further supporting the concept that GRO expression is focally restricted. Within the clusters of expression, our GRO-α probe gave rise to an inhomogeneous labeling pattern, and in some heavily labeled areas strong signals appeared to be localized to the nuclei. The limited resolution of ^{35}S-labeled probes does not allow a precise subcellular localization and could result in a signal distribution as shown in **Fig 1*b***, especially if the mRNA is localized to paranuclear cytoplasmic areas, as shown for IL-8 mRNA in stimulated fibroblasts by high resolution FISH [20]. No specific mRNA signals were detectable in the tissue sections after hybridizing with GRO-α sense probes **(Fig 2)** or after predigestion with RNase (data not shown).

The localization of the GRO-α–specific signals detected by our riboprobe corresponds to the localization of IL-8–specific mRNA to the upper malpighian layers as published earlier [18]. In contrast to IL-8, however, we did not detect GRO-α expression in the parakeratotic layers of psoriatic lesions. GRO-α expression therefore seems to be restricted to viable keratinocytes and dermal cells, and is not detectable in fully differentiated keratinocytes or microabscesses formed by neutrophils.

In some lesions, GRO-α–specific signals could clearly be observed to be located in the dermis **(Fig 1*c*)**. The autoradiography signals were confined to individual cells or very small groups of cells, probably located in the vicinity of dermal vessels. This indicates that either endothelial cells or other vessel-associated cells express GRO-α mRNA in some psoriatic lesions.

IL-8 mRNA Expression Co-Localizes to GRO-α When additional sections of four of the lesional psoriatic specimens expressing GRO-α were hybridized with an IL-8–specific antisense probe, IL-8 mRNA expression was detectable in all of these specimens. Most interestingly, IL-8 expression was confined to the same focal areas of the epidermis also expressing the GRO-α gene **(Fig 3)**. With our method of examining serial tissue sections with different probes, however, we cannot tell whether individual cells co-express IL-8 and GRO-α or whether expression of these two chemokines occurs in neighboring but separate cells or groups of cells.

Serial hybridizations of some of the sections with probes specific for MGSA/GRO-α and E-cadherin, a cellular adhesion molecule known to be expressed by basal as well as differentiated epidermal keratinocytes [21,22], were performed to demonstrate the intactness of the mRNA within the tissue sections. In all cases, a homogeneous epidermal hybridization signal was obtained with the E-cadherin probe **(Fig 4)**, excluding degradation of mRNA in the lower epidermal layers or in between the focal clusters as a possible cause for the restricted GRO-α mRNA expression pattern.

DISCUSSION

Lesional psoriatic skin is characterized by altered keratinocyte proliferation and differentiation in addition to a pronounced epidermal infiltrate dominated by neutrophils and T lymphocytes. Detailed biochemical analysis of neutrophil chemotactic proteins detected in psoriatic scales allowed the isolation and characterization of the two most abundantly present neutrophil-attracting peptides, identified as different forms of IL-8 and MGSA/GRO-α [14,15]. Both are also secreted by cytokine-stimulated cells, such as cultivated fibroblasts [23,24], monocytes [4,25], or umbilical vein endothelial cells [26,27].

IL-8 and GRO-α are potent neutrophil chemotaxins causing half-maximal chemotactic responses at about 0.1 nM [23,25]. The efficacy (percentage of migrating input cells) of neutrophil chemotactic responses is higher with IL-8 than with GRO-α [12,23,25]. Additional functions such as release of lysosomal enzymes and superoxide anions [12,23] or activation of the cellular arachidonate 5-lipoxygenase [28] are less efficiently elicited by GRO-α than by IL-8. The localization of IL-8 mRNA expression in psoriatic lesions has recently been described [18], but the cellular localization of the GRO-α expression in psoriasis is not yet known. Keratinocytes are a potential cellular source, because GRO-α peptide has been detected in addition to IL-8 in supernatants of cytokine-stimulated cultured keratinocytes [29].

In the present study, we have analyzed GRO-α m-RNA expression in psoriatic lesions by *in situ* hybridization, demonstrating abundant expression in clusters of keratinocytes located in the granular layer. This indicates that keratinocytes indeed seem to be the major cellular source of GRO-α. In principal, we cannot exclude potential cross-hybridization of our GRO-α probe, which covers the complete coding region, with the GRO-β or the GRO-γ mRNAs. Based on our previous observation that different forms of GRO-α protein but no GRO-β and GRO-γ protein can be detected in psoriatic scales [15], and based on the data presented by Kojima *et al* [17] showing that GRO-α is by far the most abundant GRO transcript detectable in psoriatic skin samples when

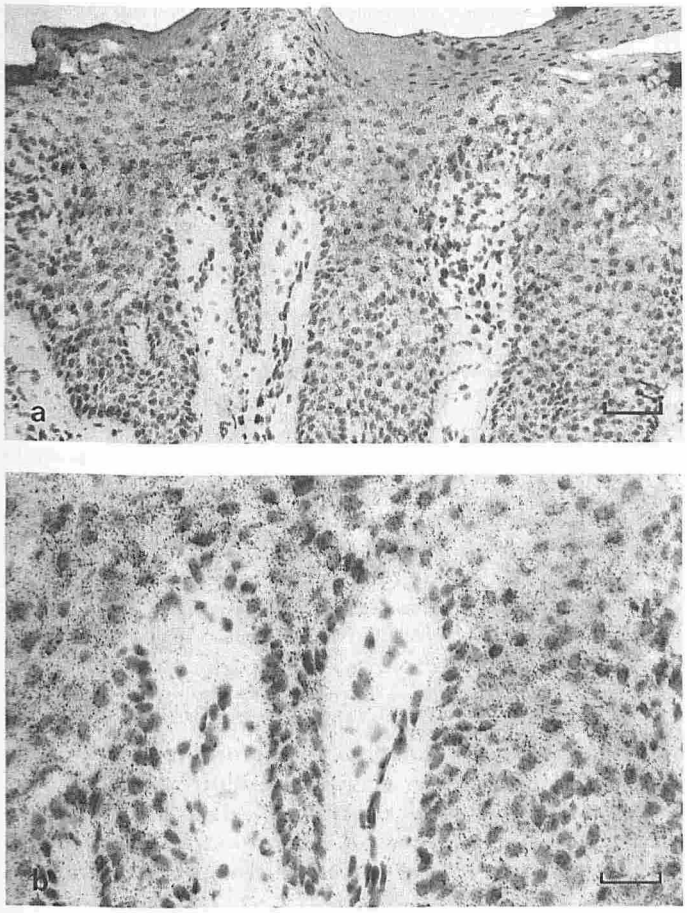

Figure 4. E-cadherin mRNA can be detected in all psoriatic epidermal layers. *In situ* hybridization with ^{35}S-UTP–labeled E-cadherin anti-sense RNA probe and emulsion autoradiography on a serial cryostat section of the psoriatic lesion shown in **Figs 1*c*** and **3** to express GRO-α and IL-8 mRNA. *a,b*) A homogenous E-cadherin mRNA expression pattern throughout all viable layers of the epidermis, demonstrating intactness of tissue RNA, as observed at different magnifications. Bright field illumination; *scale bars*: *a*) 200 μm; *b*) 100 μm.

analyzed by semiquantitative polymerase chain reaction, we suggest that most if not all of the signals detected by our riboprobe *in situ* represent GRO-α mRNA.

The possibility that a few solitary neutrophils or T lymphocytes also express the GRO-α mRNA cannot be ruled out, but most if not all of the signals were clearly associated with keratinocytes of the upper malpighian layer, and no neutrophils were detectable by standard or interference contrast microscopy.

The infiltration of psoriatic epidermis by neutrophils as well as the alternation of parakeratosis and orthokeratosis can be interpreted as evidence of rhythmical events within the seemingly static psoriatic plaque. The migration of neutrophils from the dermal papillae into the epidermis is limited in time but periodically repeated, leading to the concept of the squirting papilla [30]. It is tempting to speculate that focal and pulsed chemokine mRNA expression within the psoriatic epidermis overlaying the dermal papillae is involved in papilla activation. Transcriptional activation may be strong but relatively short lived and no longer detectable at the time when tissue infiltration becomes obvious.

The focal co-localization of GRO-α and IL-8 mRNA expression suggests that both genes are activated by a common mechanism. None of the known chemokine inducers like interleukin-1 or tumor necrosis factor–α show a similar focal mRNA or protein expression pattern in psoriatic skin, and it is presently not obvious which factors are responsible for lesional chemokine overproduction. We did not detect reproducible and significant GRO-α mRNA expression in the basal or suprabasal layers of psoriatic epidermis. It therefore seems unlikely that an epidermal gradient of GRO-α mRNA is generated. The secreted peptides could, however, be redistributed from the upper epidermal layers to the papillary dermis in the extracellular compartment, binding to components of the extracellular matrix [31].

A detailed understanding of psoriatic tissue infiltration therefore requires the analysis of both lesional chemokine mRNA and protein distribution. Tettelbach *et al* [16] examined psoriatic and other inflammatory or neoplastic skin lesions with monoclonal antibodies specific for MGSA/GRO. They showed that GRO-α immunoreactivity was mainly associated with keratinocytes of the stratum spinosum and stratum germinativum. In contrast to GRO-α mRNA, however, the protein was also detectable in some normal skin specimens. Performing similar experiments with two monoclonal antibodies directed against IL-8, Sticherling *et al* [32] described intracellular IL-8 immunoreactivity in normal human epidermis. Depending upon the antibody, the staining pattern was either basal or suprabasal. The suprabasal IL-8 immunoreactivity is maximal in normal skin and appears substantially reduced in psoriatic lesions. It is suggested that increased release of preformed intracellular IL-8 could play a role in the pathogenesis of psoriatic tissue infiltration, in addition to newly synthesized material. With our *in situ* hybridization experiments, we were not able to detect GRO-α or IL-8 mRNA in the basal or suprabasal layers, nor were we able to demonstrate mRNA expression of these chemokines in normal or uninvolved skin. We cannot exclude that expression is too low to be detectable by our analysis, despite the proven sensitivity of our radioactive *in situ* hybridization technique. A combined immunohistochemical and *in situ* hybridization analysis with serial cryostat sections is currently in preparation to rule out differences due to variations in clinical disease patterns. This analysis could reveal further correlations between protein and mRNA expression and help to clarify the molecular mechanisms underlying psoriatic tissue infiltration.

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