MCP-1 mRNA Expression in Basal Keratinocytes of Psoriatic Lesions

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In addition to hyperproliferation of keratinocytes, psoriasis is characterized by pronounced leukocytic infiltration. In contrast to the epidermal localization of neutrophils and T lymphocytes, macrophages are almost exclusively restricted to the dermal compartment. By immunohistologic analysis, these dermal macrophages were mainly encountered in the papillary dermis and arranged along the rete ridges in close proximity to proliferating keratinocytes. Monocyte chemoattractant protein (MCP-1) anti-sense RNA probes yielded abundant signals over the proliferating basal keratinocytes of the tips of the rete ridges, and, to a lesser extent, in cells in the papillae. Thus, the strongest MCP-1 message in psoriatic lesions is found above the dermal-epidermal junction and this may explain the characteristic sub-basal distribution of dermal macrophages. These results suggest that MCP-1 is important in regulating the interaction between proliferating keratinocytes and dermal macrophages in psoriasis pathogenesis. Key words: in situ hybridization, monocyte-macrophage chemotaxis. J Invest Dermatol 101:127–131, 1993

Histopathologically, psoriasis is characterized by an excessive proliferation of keratinocytes as well as by a pronounced inflammatory infiltrate. The juxtaposition of proliferating keratinocytes and immunocompetent cells has been taken to suggest that immunologic processes are involved in the pathogenesis of psoriasis [1]. To better understand the underlying mechanisms, we have investigated the potential factors responsible for the abnormal cutaneous topiobiology of the infiltrating leukocytes.

In psoriasis, the altered spatial distribution of infiltrating leukocytes includes neutrophils that mainly accumulate in the uppermost rete malpighii as singly scattered cells or in Kogoj's pustules and Munroe's microabscesses. We have recently shown by RNA-RNA in situ hybridization [2] that, in psoriatic lesions, transcripts for the chemotactic cytokine NAP-1/IL-8 (neutrophil-activating peptide (synonymous with neutrophil attractant protein-1)/interleukin 8, reviewed in [3]) are mainly, if not exclusively, expressed in the upper layers of the epidermis. This phenomenon may contribute to a) the influx of neutrophils into the epidermis and their further migration to the upper layers, eventually resulting in the formation of subcorneal abscesses, and b) a hyperproliferation of keratinocytes [3]. Likewise, T lymphocytes are not restricted to the dermal compartment in psoriatic lesions, and various cytokines and adhesion molecules have been implicated in regulating T-cell migration into the epidermis [1,4].

Surprisingly, potentially mobile CD 45+, CD 68+ macrophages are almost exclusively restricted to the dermal compartment (therefore termed dermal macrophages) of psoriatic lesions, with only a few cells penetrating the basement membrane (unpublished observation). High densities of dermal macrophages are encountered in the papillary dermis, frequently arranged along the basement membrane of the rete ridges and in close proximity to the extensively proliferating basal keratinocytes ([5] and unpublished observations). At present, neither the specific cytokines/chemotactic proteins nor the identity and location of the corresponding producer cells that contribute to this peculiar distribution of dermal macrophages in psoriatic lesions are known.

Recently, a potent monocyte chemoattractant, the monocyte chemotactic protein-1 (MCP-1), also known as monocyte chemoattractant and activating factor (MCAF) [8] or human JE protein [9] has been isolated and sequenced [6,7]. T lymphocytes [10], fibroblasts [11,12], endothelial cells [12,13], smooth muscle cells [13], and keratinocytes [14] have the potential to secrete MCP-1 in vitro. As all these cell types are integral components of psoriatic lesions, MCP-1 could be one of the factors responsible for monocyte/macrophage chemotaxis in psoriasis. Among the multiple cytokines studied in psoriatic lesions ([16], reviewed in [17]), IL-1 and TNF-α and TNF-β are known to induce MCP-1 production [7,10,11, 16,17]. Moreover, early studies on MCP-1 have shown that platelet-derived growth factor-BB (PDGF-BB) stimulates fibroblasts to produce MCP-1 (JE) [15]. So far, the role of MCP-1 in psoriatic lesions has not been adequately addressed.

In the present study, we determined the magnitude and location of MCP-1 mRNA expression in psoriatic lesions. In addition, we investigated the spatial relationship of MCP-1 mRNA expression and macrophage localization. To address this issue we used immu-
nohistology to type the inflammatory infiltrate, and in situ hybridization to microanatomically localize these cytokines.

MATERIALS AND METHODS

Selection and Preparation of Skin Sections Fresh incisional biopsy specimens of lesional and non-lesional skin of patients with untreated, long-standing psoriasis were taken under local anesthesia (n = 9). For control purposes, biopsies were taken from five healthy volunteers. The material was snap-frozen and 4-μm cryostat sections were used for immunohistology and in situ hybridization.

Immunohistology For immunohistology the following monoclonal antibodies (MoAbs) were employed: MoAb anti-CD3 (Leu-4, at 1:400, Becton-Dickinson, Sunnyvale, CA) reacting with the T-cell receptor-associated CD3 antigen; MoAb anti-CD45 (HLeu, at 1:100, Becton-Dickinson) the lymphocyte common antigen, specific for a surface protein on all human leukocytes; MoAb anti-human neutrophil elastase (Dako-Elastase, at 1:100, Dakopatts, Copenhagen, Denmark); MoAb S3 (a generous gift from Dr. Pavaresch, Department of Pathology, University of Kiel, Germany) reacting with proliferating cells; MoAbs KiM7 (at 1:2000, Behring Werke, Marburg, Germany); KiM8 (at 1:1000, Behring Werke) anti-CD68 (PGM-1, at 1:1000, Dakopatts) reacting with monocytes/macrophages, applied to detect macrophages of the skin.

For single-color labeling a three-step streptavidin-biotin-peroxidase procedure was used, as described elsewhere [21]. After blocking Fc-receptors with 20% heat-inactivated sheep serum in phosphate-buffered saline (PBS), sections were incubated with specific MoAb overnight at 4°C. Following incubation with biotin-conjugated sheep anti-mouse Ig (Amersham, Amersham, U.K.) and peroxidase-conjugated avidin-biotin (Dako-ABC-peroxidase, Dakopatts) for 1 h at room temperature. Sections were thoroughly rinsed between each incubation step; 3-amino-9-ethylcarbazole (Sigma Co., St. Louis, MO) was used as peroxidase substrate. For control purposes specific MoAbs were replaced by isotype-matched IgG, which consistently yielded negative results.

In Situ Hybridization Preparation of 35S-labeled RNA Probes: MCP-1 cDNA was originally cloned by using the lambdaZap system [7] (Stratagene, La Jolla, CA). For sense and anti-sense reaction products, T3 or T7 polymerase reactions were performed, respectively. Briefly, after linearization of plasmid DNA with appropriate restriction enzymes, 35S-labeled sense and anti-sense probes were obtained by in vitro transcription with T3, T7, or SP6 RNA-polymerase synthesis reaction, respectively. The original linearized template cDNA was eliminated by DNase treatment (Pharmacia LKB Biotechnology). To facilitate penetration of probes into the cells/tissue, alkaline hydrolysis of the RNA probe at 60°C in a carbonate buffer (pH 10.2) was performed according to the formula (min) = [L - Lf)/0.11 X Lf (Lf = initial length in kb, Lf = final size in kb) [22], whereby an average length of 50 - 150 base pairs was generated. The size of 35S-labeled RNA probes was checked after size reduction on a denaturating 6% polyacrylamide gel. Finally, after multiple ethanol precipitation steps, the radioactive riboprobe was adjusted to a specific activity of 2 X 10^6 cpm/μl in 0.1 M Tris-HCl, pH 7.5, containing 1 mM ethylene-diaminetetraacetic acid (EDTA). Generally, 70-95% of UTP-radiounucleotides were incorporated into the RNA probe.

Hybridization Procedure: The hybridization protocol described by Mueller et al [23] was used without any modifications. Paraformaldehyde-fixed cryostat sections were treated with protease K (Boehringer, Mannheim, Germany) (50 μg/ml) for 30 min at 37°C to facilitate the accessibility of the radioactive probes to cellular target mRNA. Afterwards, sections were re-fixed in 4% paraformaldehyde, acetylated with acetic anhydride in 0.1 M triethanolamine (pH 8.0, 10 min), dehydrated in graded alcohol, and air dried. Thereafter, sections were overlaid with 20 μl of hybridization solution (50% formamide, 300 mM NaCl, 20 mM Tris-HCl, pH 8.0, 5 mM EDTA pH 8.0, 0.1% BSA, 0.01% Tween 20, 0.1 μg/ml RNase A, 0.02 μg/ml RNase T1 (1 U/ml) (Boehringer) for 30 min at 37°C. For autoradiography, slides were dipped in a NTB-2 Kodak solution (1:2 in 800 mM ammonium acetate) and were exposed for 1 - 5 weeks at 4°C. For the evaluation of developed slides a Zeiss

RESULTS

Immunohistology All sections investigated (n = 9) exhibited the typical histopathologic features of psoriasis (e.g., acanthosis, parakeratinization, Munroe's neutrophil-abscesses, pustules of Kogoj, elongated and distorted papillary vessels). All lesions showed a pronounced leukocytic infiltrate that was mainly localized in the papillary and upper reticular dermis, as shown by staining with MoAb anti-CD45. MoAb KiM7-, MoAb KiM8-, and MoAb anti-CD68-reactive cells (as revealed by sequential sectioning) were found to comprise a major portion of infiltrating cells in the dermal compartment. These cells were clustered in particular high densities in the elongated papillae and were seldomly encountered in the lower epidermal compartment (Fig 1). Frequently, they were aligned along the dermal side of the basement membrane of the elongated and often club-shaped rete ridges like a string of pearls (Fig 1). As opposed to CD68+ dermal macrophages, which were infrequently detected in the epidermis, CD3+ lymphocytes were encountered as singly scattered cells in all epidermal layers of the lesion as previously shown [40]. However, the majority of CD3+ cells resided in the upper dermal compartment, mainly within clusters of infiltrating leukocytes (CD45+ cells > CD3+ cells).

Clusters of neutrophils within the upper stratum malpighii and the parakeratotic layers of long-standing lesions of chronic plaque psoriasis were detected with a MoAb specific for human neutrophil elastase.

The MoAb S3 was used to identify the proliferating keratinocytes in psoriatic lesions. Creactivity with cytokeratin as opposed to analysis with MoAb Ki 67 [24] (J. Gerdes, personal communication) was not observed. In contrast to normal human skin from healthy volunteers, proliferating keratinocytes in psoriatic lesions were not only detected in the basal layer of the rete ridges but were also encountered in the second and third suprabasal layer of the epidermis (Fig 2). The increased proliferation of keratinocytes in these layers as compared to normal skin is in agreement with previous reports [25,26].

In situ Hybridization Using MCP-1-specific 35S-labeled antisense RNA-probes, specific signals were found in all viable layers of the epidermis and in the papillary dermis of psoriatic lesions. Overall labeling in the suprabasal layers was sparse but significantly higher than that of the sense control defining the background of the method. The density of signals appeared higher in the epidermis as compared to the dermal compartment. However, the most conspicuous and consistent finding was the detection of focioculubsilver grain precipitates over the basal epidermal layer of all psoriatic lesions tested (Figs 3A,3B). Interestingly, hybridization signals did not occur along the full length of the basal layer, but were preferentially observed at the tips of the rete ridges. This pattern partly coincided with that seen by anti-S3 immunolabeling with proliferating S3+ keratinocytes, as described above (Fig 2). The specificity of these hybridization signals was confirmed using sense probes or predigestion with RNase, which did not produce cell-associated silver grains above the very low background. In addition, the use of full-length probes (> 800 bp) significantly increased the exposure time, which concomitantly increased the background signals. Sized-reduced riboprobes increased the signal-to-noise ratio and were therefore used in all in situ hybridization procedures.

It is noteworthy that within the foci of MCP-1 positivity the signal bands were continuous (Figs 1B, 3A,B), suggesting that most of these basal epidermal cells are positive for MCP-1 mRNA. At the margin between non-involved and involved skin, the number of MCP-1 mRNA+ basal cells was significantly decreased (Figs 4A,B). The adjacent non-involved skin of all biopsies studied as well as normal skin from healthy volunteers was devoid of any detectable

Axiophot microscope equipped for polarized incident light (epipolarization) microscopy (Zeiss, Oberkochen, Germany) was used.
MCP-1 mRNA, with the rare exception of single weakly positive basal cells.

In addition, cells with high expression of MCP-1 transcripts were encountered in the upper portion of the papillary dermis of psoriatic lesions (Fig 1B). Single cells in the subpapillary upper dermal compartment also showed high levels of transcripts and these were mainly within clusters of infiltrating leukocytes (Figs 3A,B).

Comparing MCP-1 mRNA expression and the preferential localization of CD68+ dermal macrophages (Figs 1A,B), we frequently (seven of nine lesions tested), but not consistently, observed that dermal macrophages lined the rete ridges in an infrabasal level. Thus, these dermal macrophages were found in the immediate vicinity of basal cells with a high level of MCP-1 mRNA expression (Fig 1B). In addition, CD68+ dermal macrophages also accumulated in the papillae and within clusters of leukocytes in the subpapillary upper compartment, where cells with high MCP-1 mRNA expression have been encountered (Fig 1B). In contrast to the aforementioned correlation between MCP-1 mRNA expression and the localization of dermal macrophages, such observations were not made when CD3+ and neutrophil elastase positive cells were labeled on sequential sections. This suggests that MCP-1 exhibits selective chemotactic properties for macrophages but not for T lymphocytes and neutrophils in vivo.

**DISCUSSION**

The levels of multiple factors/cytokines are known to be altered in psoriasis, as compared to normal skin or other dermatoses, but it is not known how these changes tie into the cascade of events that eventually lead to the morphogenesis of psoriatic lesions.

In the present *in situ* study, we have directed our attention both to dermal macrophages, which, together with neutrophils and T lymphocytes, may have a key role in psoriasis pathogenesis, and to MCP-1 as a potent chemotactic cytokine that could guide these dermal macrophages to sites where MCP-1 is released. Our results show that MCP-1 mRNA is highly expressed in lesional psoriatic skin but not in uninvolved psoriatic or normal skin. Of equal importance is the particularly high expression of MCP-1 in the basal layer of the rete ridges. Furthermore, we detected a correlation between MCP-1 mRNA expression in basal cells and the accumulation of CD68+ dermal macrophages in the direct vicinity of the subepidermal region, suggesting selective chemotactic properties of MCP-1 on macrophages in vivo. The band-like hybridization pattern, with every basal cell in a row expressing MCP-1 mRNA, strongly suggests that basal keratinocytes rather than melanocytes are the major source of MCP-1. This conclusion is substantiated by the finding that freshly cultured melanocytes are devoid of MCP-1 message although they express high levels of NAP-1/IL-8 under the same condition (R. Gillitzer, H. Kock, unpublished observations). Due to the elongation of the papillae and the rete ridges, in particular, the surface of the basal epidermal layer is drastically increased in psoriatic lesions. In parallel, the number of MCP-1 mRNA-expressing keratinocytes is significantly elevated as compared to normal skin.

Thus far, significant post-transcriptional regulation of MCP-1 in cultivated cells has not been reported. Assuming unrestricted translation of MCP-1 transcripts into functional proteins also *in vivo*, which is supported by detected immunoreactivity with MCP-1-specific MoAb in the epidermal compartment as well as on some dermal cells (Gillitzer et al, in preparation), this suggests that high levels of MCP-1 are constantly released in and into the dermal compartment. Therefore, perhaps the most important implication of this study is a chemotactic gradient between the epidermal and dermal compartments in psoriatic lesions. Because the highest levels of MCP-1 transcripts were found in the basal layer of the rete ridges, the steepest MCP-1 gradient supposedly exists between the basal membrane and the sub-basal space where MCP-1 clearance could occur through receptor-bearing target cells, cytokine-binding proteins, and the lymphatic and blood drainage. This would partly explain the fact that dermal macrophages concentrate and line up...
along the dermal/epithelial junction without entering the epidermis. Just recently, detailed ultrastructural analysis revealed that occasionally the basal membrane is interrupted at sites where basal keratinocytes and lining macrophages are in close proximity, supporting our hypothesis that there exist intensive interactions between both cell types. Moreover, MCP-1-producing cells in the dermis, albeit in smaller numbers compared to basal keratinocytes, may influence the location of macrophages.

The MCP-1 transcripts in the few scattered cells at the tips of the papillae could be responsible for the accumulation of dermal macrophages in this region. Whether an additional barrier (e.g., chemotactic stimuli and adhesion molecules within the dermal compartment) to macrophage exocytosis exists is still unknown. In contrast to dermal macrophages, neutrophils face the highest concentration of chemotactic molecules in the upper stratum malpighii, which in psoriasis contains focal clusters of keratinocytes expressing high levels of NAP-1/IL-8 mRNA message [2], eicosanoids, and C5a [24]. As a result, neutrophils migrate through the epidermis and characteristically cluster in the upper malpighian layer and/or the parakeratotic stratum corneum.

The role of dermal macrophages in the subbasal spaces is not known and equally unknown is their capacity to secrete cytokines that could, in turn, stimulate keratinocytes. Whereas we were not able to detect TNF-α message in dermal cells by in situ hybridization [2], others have shown by immunohistologic methods that dermal macrophages express TNF-α reactivity [34]. This, however, does not prove a de novo synthesis of TNF-α but could also reflect binding to TNF-α–specific surface receptors, which are known to be abundant on monocytes. On the other hand, the proximity of dermal macrophages to the highly proliferative basal keratinocytes is strongly suggestive of a dialogue between both cell types. MCP-1 may be considered to be one of the signals of communication and further functional studies are needed to elucidate whether MCP-1 is not only chemotactic but also functions as an activating signal, as suggested by Matsushima et al [8]. As the in situ technique used in the present study does not allow the simultaneous localization of cytokine mRNA and leukocyte subtype-specific antigens, the few scattered cells responsible for the MCP-1 mRNA transcripts in the dermis remain unknown. Fibroblasts [11,12], endothelial cells [12,13], lymphocytes [10], and even monocytes themselves [6] could account for dermal MCP-1 message.

In tumor cell lines, MCP-1 secretion in vitro does not require the presence of known stimuli (summarized in [36]), but for other cells, multiple cytokines are known to induce and modulate MCP-1 expression. Among them are TNF-α [12,17,37], IL-1 [7,10,11], and IFN-γ [14,18], which may exhibit synergistic effects [14]. Conflicting results exist concerning the level of IL-1 (IL-1α and IL-1β) and TNF-α in psoriatic skin. Some investigators found elevated levels

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for IL-1 [28,29], whereas others reported decreased IL-1 concentrations in psoriatic lesions [16,30,31]. With respect to TNF-α, neither we [2] nor others [16] have been able to detect elevated concentrations in the in situ mRNA level and the protein level, respectively.

PDGF-B could also be considered a triggering signal for MCP-1 expression because it has been shown to be produced by keratinocytes [38] and other cells that constitute psoriatic lesions (fibroblasts, endothelial cells). Indeed, we have found elevated levels of PDGF-B expression in dermal cells of psoriatic lesions (Gillitzer et al., in preparation). Because keratinocytes in vivo do not express detectable levels of the appropriate receptor subtype for PDGF-B (PDGF-β-type receptor) [34] it may be assumed that dermal rather than epidermal cells are the appropriate targets. Interestingly, PDGF-B has been reported to exhibit direct chemotactic properties for macrophages [39] and, therefore, could directly stimulate monocyte chemotaxis.

Although the role of MCP-1 in the initiation of psoriatic lesions remains to be elucidated, its high expression in continuous, though focally localized, populations of basal epidermal cells suggests an important role of this molecule in the pathogenesis of psoriatic lesions. Our microanatomic analysis of MCP-1 mRNA expression may help to explain one of the pathologic features typical for psoriasis, such as the almost exclusive accumulation of dermal macrophages in the subepidermal space.

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REFERENCES


