Overexpression of CXCL16 in lesional psoriatic skin

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Background: Psoriasis is characterized as an autoimmune disease resulting in an exaggerated innate immune response. The CXC-chemokine ligand 16 (CXCL16) is described to function as an adhesion molecule, a scavenger receptor or as a soluble molecule it acts as a chemoattractant. CXCL16 has been reported to be expressed in a variety of inflammatory diseases. However, no information has been reported in the literature about the expression of CXCL16 in psoriatic skin.

Purpose: The present study was designed to analyze the expression and localization of CXCL16 in human psoriatic skin tissues.

Results: In normal skin, cytoplasmic expression of CXCL16 was increased in keratinocytes of upper epidermal cell layers as compared to the lower epidermal cell layers. In lesional psoriatic skin, CXCL16 immunoreactivity was increased in the cytoplasm of keratinocytes of lower epidermal layer keratinocytes as compared to the normal epidermis. Cytoplastic CXCL16 expression was increased in the capillary endothelial cells of psoriatic dermis as compared to capillary endothelial cells of the normal dermis. Notably, almost all inflammatory cells in the dermis were negative for CXCL16.

Materials and Methods: Ten paraffinized specimens of human lesional psoriatic skin and five paraffinized specimens of normal skin were studied using an immunohistochemical streptavidin-peroxidase technique.

Conclusion: We here report for the first time alterations in the immunohistochemical staining pattern of CXCL16 in lesional psoriatic skin compared to the normal skin. These results suggest that CXCL16 may play a role in the pathogenesis of psoriasis.

Introduction

The expression of the chemokine CXCL16 has been described in a variety of cell types including dendritic cells, macrophages, B-cells, T-cells, smooth muscle cells, endothelial cells, bone marrow stromal cells, neuronal cells, epithelial cells and fibroblasts. Unlike all other members of the CXC chemokine family the chemokine domain of CXCL16 is tethered to the cell surface via a mucine like stalk, which in turn is attached to a transmembrane and a cytoplasmic domain. CXCL16 was independently described as a scavenger receptor for oxidized LDL, suggesting an atheroprotective function. Surface-expressed CXCL16 can also function as an adhesion molecule by binding to its receptor CXCR6, which is expressed on activated T-cells and bone marrow plasma cells. In addition, CXCL16 is also found as a soluble molecule that is constitutively generated by proteolytic cleavage of its transmembrane variant, a process which is mainly regulated by ADAM10 and ADAM17. Soluble CXCL16 is known to induce the chemotaxis of CXCR6-expressing activated T-cells and NK cells. In inflammation and carcinogenesis, CXCL16 has been shown to play an important role in cell activation, adhesion, proliferation and migration.

Recently, Scholz et al. reported that CXCL16 is mainly expressed in keratinocytes throughout the nucleated epidermal layers but not in cells of the dermal layer. Interestingly, after injury of mouse skin soluble CXCL16 was released into the wound fluid and marked expression of CXCR6 expressing T-cells was found in the injured dermis.

Psoriasis is a genetically programmed skin disease of dysregulated inflammation, which results in hyperproliferation of epidermal keratinocytes and consequent abnormal terminal differentiation and impaired barrier function of the epidermis. Using microarray analyses Zhou et al. could demonstrate that a multitude of chemokines including CXCL16 is upregulated in psoriatic skin in comparison to normal skin. CXCL16 protein expression in psoriasis patients has been so far not investigated, therefore we compared with immunohistochemical analysis the expression of CXCL16 in normal skin and in patients with psoriasis, to identify the cell types which express CXCL16 in psoriatic skin.

Results

CXCL16 is expressed in upper epidermal layer of normal skin. In the normal epidermis, CXCL-16 immunoreactivity was restricted to the cytoplasm of keratinocytes of upper epidermal (upper spinous layer and granular) layer (Fig. 1A). In the normal dermis, capillary endothelial cells, venules and arterioles showed no CXCL16 staining (Fig. 1B). Dermal fibroblasts and inflammatory cells in the dermis were not stained with CXCL16. However, CXCL16 immunoreactivity was found in the hair follicle, sebaceous gland and arrector pili-like muscle. Notably, CXCL16 was not expressed in fat cells. All results are summarized in Table 1.
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Inflammatory cells showed increased CXCL16 expression especially in the papillary dermis. All capillary endothelial cells were stained with the CXCL16 antibody (Fig. 2C). Venules and arterioles are positively stained with the CXCL16 antibody. Fibroblasts were also stained with CXCL16 antibody (Fig. 2D). CXCL16 immunoreactivity was found in the hair follicle, sebaceous gland and eccrine sweat glands, and arrector pili-like muscles. However, CXCL16 was not expressed in fat cells. All results are summarized in Table 1.

Table 1  Expression of CXCL 16 in normal and psoriatic skin

| Mean value                             | Normal skin (n = 5) | Psoriatic skin (n = 10) |
|----------------------------------------|---------------------|------------------------|
|                                        | S1†                | PP††                   | S1†    | PP††    |
| Upper epidermal layer keratinocytes    | 1.6*#              | 2.2*#                  | 3.4*#  | 0.9     | 1.2     | 1.6     |
| Lower epidermal layer keratinocytes    | 0.6                | 0.8                    | 0.8    | 2.1***  | 2.8***  | 6.0***  |
| Basal layer keratinocytes              | 0.4                | 0.4                    | 0.4    | 2.2***  | 2.9***  | 6.6***  |
| Dermal inflammatory cells              | 0.2                | 0.2                    | 0.2    | 1.1     | 0.8     | 1.1     |
| Dermal capillary endothelial cells     | 0.2                | 0.4                    | 0.4    | 1.7***  | 2.6***  | 4.4***  |
| Dermal fibroblasts                     | 0.2                | 0.2                    | 0.2    | 1.1     | 0.9     | 1.3     |
| Sebaceous glands                       | 1.2                | 2.0                    | 2.2    | 1.7     | 2.1     | 2.8     |
| Hair follicle                          | 1.6                | 1.4                    | 2.4    | 1.7     | 1.9     | 3.3     |
| Arrector pili muscle                   | 1.0                | 2.0                    | 2.0    | 1.7     | 2.2     | 3.4     |
| Eccrine glands                         | 1.6                | 2.2                    | 3.8    | 1.8     | 2.3     | 4.2     |
| Adipose tissue                         | 0.2                | 0.2                    | 0.2    | 0.4     | 0.5     | 0.5     |

p value; *p < 0.05, **p < 0.01, ***p < 0.001 (increased as compared to the corresponding compartment in normal skin, #: increased as compared to the lower epidermal cell layer in normal skin). †: Immunoreactivity score (IRS): IRS = SI x PP. Percentage of positively stained cells (PP): 0 = no stained cells, 1 = >0–25% stained cells, 2 = >25%–50% stained cells, 3 = >50%–75% stained cells, 4 = >75%–100% stained cells. ††: Immunoreactivity score (IRS): IRS = SI x PP.

Figure 1. CXCL16 immunoreactivity in normal human skin. Note that CXCL16 is expressed mainly in keratinocytes of upper epidermal cell layers (A; arrow). CXCL16 expression was detected arrector pili-like muscle in dermis (B; arrow). Original magnification x200 (A), x200 (B).
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Recently, it has been shown that CXCL16 plays an important role in the initiation of host defenses against bacterial and viral infections in normal skin. Soluble CXCL16 can be released by ADAM10 in keratinocytes. We reported recently that ADAM10 is overexpressed in the upper epidermal keratinocytes in normal skin. Interestingly, in this study, CXCL16 expression was also increased in normal skin in keratinocytes of the upper epidermal cell layers. These findings suggest that upper epidermal layer keratinocytes may play a role in host defenses in normal skin, not only as a physical barrier but also via production of CXCL16.

Psoriasis is a genetically programmed skin disease of dysregulated inflammation, which results in hyperproliferation of epidermal keratinocytes and consequential abnormal terminal differentiation. It has been shown that toll-like receptors (TLR) 2,3 leads to production of AMPs, cytokines and chemokines including CXCL16 in keratinocytes. In addition, TLR 2 was reported to be increased in psoriatic skin by infectious or endogen ligands. Recently, it was postulated that CXCL16 could be released from normal keratinocytes into the wound fluid following injury. Therefore, it is possible to suggest that trauma of unaffected psoriatic skin may induce upregulation of CXCL16 and could thereby trigger the formation of psoriatic plaques.

Epidermal keratinocytes do not only form a passive physical barrier, but also initiate and regulate cutaneous immune responses. CXCL16 plays an important role in the uptake of various pathogens and chemotaxis of CXCR6 expressing T- and NKT-cells. Recently, it has been shown that CXCL16 plays an important role in the initiation of host defenses against bacterial and viral infections in normal skin. Soluble CXCL16 can be released by ADAM10 in keratinocytes. We reported recently that ADAM10 is overexpressed in the upper epidermal keratinocytes in normal skin. Interestingly, in this study, CXCL16 expression was also increased in normal skin in keratinocytes of the upper epidermal cell layers. These findings suggest that upper epidermal layer keratinocytes may play a role in host defenses in normal skin, not only as a physical barrier but also via production of CXCL16.

Discussion

To our knowledge, this is the first report to show that protein expression of CXCL16 is increased in lesional psoriatic skin. Keratinocytes have been identified as important producers of chemokines in the skin. Cutaneous injury, the trigger for the Koebner phenomenon (formation of a psoriatic plaque after trauma of unaffected skin), is known to induce upregulation of antimicrobial peptides (AMPs) such as β-defensin (HBD). It has been shown that toll-like receptors (TLR) 2,3 leads to production of AMPs, cytokines and chemokines including CXCL16 in keratinocytes. In addition, TLR 2 was reported to be increased in psoriatic skin by infectious or endogen ligands. Recently, it was postulated that CXCL16 could be released from normal keratinocytes into the wound fluid following injury. Therefore, it is possible to suggest that trauma of unaffected psoriatic skin may induce upregulation of CXCL16 and could thereby trigger the formation of psoriatic plaques.

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Psoriasis is a genetically programmed skin disease of dysregulated inflammation, which results in hyperproliferation of epidermal keratinocytes and consequential abnormal terminal differentiation. In this study, we found overexpression of CXCL16 in the lower epidermal keratinocytes of lesional psoriatic skin in contrast to normal epidermis. This result may be due to the changes of the epidermal differentiation pattern. Recently, we reported that ADAM10 is also overexpressed in the lower epidermal layer keratinocytes in lesional psoriatic epidermis. In the normal skin and in vascular inflammation soluble CXCL16 acts as a chemoattractant for CXCR6 expressing Th1 cells. This suggests that upregulated soluble CXCL16 released by ADAM10 in the lower

Figure 2. CXCL16 immunoreactivity in psoriasis vulgaris. Note that expression of CXCL16 is increased in the lower epidermal cell layers (B; arrow). In papillary dermis, increased immunoreactivity of CXCL16 is detected in the almost all capillary endothelial cells (C; arrow). In deep reticular dermis, CXCL16 expression was increased in arteriole and venule (D; arrow) and in a proportion of fibroblasts. Original magnification x100 (A), x200 (B), x400 (C), x400 (D).
portion of psoriatic epidermis, may facilitate leukocyte recruitment through interaction with CXCR6 of Th1 cells in psoriatic dermis. In this study, we also found CXCL16 overexpression in the capillary endothelial cells of psoriatic dermis. Given that ADAM10 is a main sheddase of CXCL16 in endothelial cells, it is possible that ADAM10 may play an important role in the leukocyte diapedesis during psoriatic inflammation. In addition, it is conceivable that CXCL16 in the capillary endothelial cells of the psoriatic dermis initially act as an adhesion molecule. Later, soluble CXCL16 released by ADAM10 may act as a T-cell chemoattractant in psoriatic inflammation. Until the early 1980s, psoriasis was believed to be a disease primarily resulting from dysregulated epidermal keratinocyte proliferation, while the cutaneous inflammatory infiltrate was believed to be a secondary event. It has been shown that proliferation of psoriatic keratinocytes starts after sensitization with IFN-γ. In addition, CXCL16 was known to play an important role in the proliferation of endothelial and/or smooth muscle after the treatment with IFN-γ. Recently, CXCL16 has been shown to play an important role in mesangial cell proliferation, prostate cancer and pancreatic ductal carcinoma. Therefore, it is possible that CXCL16 also influences the proliferation of psoriatic keratinocytes (epidermal hyperplasia), psoriatic capillary endothelial cells and/or vascular smooth muscle cells (psoriatic vascular angiogenesis).

It has been described, that autoimmune diseases could be treated by interfering with leukocyte endothelium interaction. Recently, it was demonstrated that rheumatoid arthritis patients, responding to anti-TNFα therapy, showed a strongly decreased CXCL16 expression, and it was concluded that CXCL16 may represent one of the crucial chemokines regulated by infliximab treatment. Our findings indicate that CXCL16 may be involved in the development of psoriasis. Given that CXCL16 is not only an interferon-γ but also a TNFα regulated chemokine, it is possible to suggest that CXCL16 may be a useful therapeutic target in psoriasis.

In conclusion, the major findings of the present study were as follows: (1) expression of CXCL16 is abundant in lower psoriatic epidermal keratinocytes. (2) expression of CXCL16 is increased in all capillary endothelial cells in the psoriatic dermis. These results indicate that CXCL16 may be of importance for the pathogenesis of psoriasis, involving leukocyte recruitment and migration, and alterations in keratinocytes proliferation/differentiation. Therefore, further studies should be performed to investigate if CXCL16 could be a potentially useful therapeutic target for the treatment of psoriasis patients.

Materials and Methods

Skin samples. For immunohistochemistry, paraffin specimens of lesional psoriatic skin (n = 10) and paraffin specimens of normal human skin (n = 5) from the dermatology clinic of the Saarland University Hospital were used. Diagnosis was confirmed by a certified pathologist.

Antibodies. Immunohistochemical analysis was performed using specific polyclonal antibodies and a sensitive streptavidin-peroxidase technique as established in our laboratory. The polyclonal rabbit antibody to human CXCL16 was from Acris Antibodies (Acris Antibodies, Hiddenhaven, Germany).

Immunohistochemical staining procedure. For antigen retrieval, paraffin sections (5 μm) were microwave-treated according to standard procedures. Incubation steps were performed in a moist chamber at room temperature (RT). The slides were incubated overnight at 4°C with primary antibody of CXCL16 in 1:100 dilutions. After intermediate washing steps (PBS, 2 x 5 min), the sections were incubated with biotin-labeled goat anti-rabbit IgG (1:400, 30 min, RT, DAKO, Hamburg, Germany), and then with streptavidin-peroxidase complexes (1:400, 30 min, RT, DAKO). After rinsing, the sections were incubated with 3-amin-9-ethylcarbazole (AEC, Sigma Immuno Chemicals, Munich, Germany) as a substrate for the peroxidase reaction, transferred into tap water, and mounted with AquaTex (Merck, Darmstadt, Germany). In control sections, primary antibody was replaced with rabbit IgG1 (DAKO). No immunoreactivity was observed in these control sections.

Evaluation of immunohistochemical staining patterns. Microscopic analysis were performed by two independent observers (SO, JR), as described previously. CXCL16 staining intensity (SI) was assessed using a 4-point immunoreactivity scale (0 = no staining, 1 = weak staining intensity, 2 = moderate staining intensity, 3 = strong staining intensity). Percentage of CXCL16-positively stained cells (PP) was assessed using a 5-point scale from, no positive cells to almost every cell positive (0 = no stained cells, 1 = >0–25% stained cells, 2 = >25%–50% stained cells, 3 = >50%–75% stained cells, 4 = >75%–100% stained cells). Overall CXCL16 immunoreactivity was evaluated using a resulting immunoreactivity score (IRS) (IRS = SI x PP).

Statistical analysis. The statistical significance for the immunohistochemical staining was analyzed by the paired t-test.

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