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The chemokine receptor CCR3 participates in tissue remodeling during atopic skin inflammation

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ABSTRACT

Background: Recent studies provided insights into the recruitment and activation pathways of leukocytes in atopic dermatitis, however, the underlying mechanisms of tissue remodeling in atopic skin inflammation remain elusive.
Objective: To identify chemokine-mediated communication pathways regulating tissue remodeling during atopic skin inflammation.
Methods: Analysis of the chemokine receptor repertoire of human dermal fibroblasts using flow cytometry and immunofluorescence. Quantitative real-time polymerase chain reaction and immunohistochemical analyses of chemokine expression in atopic *vs.* non-atopic skin inflammation. Investigation of the function of chemokine receptor CCR3 on human dermal fibroblasts through determining intracellular Ca^{2+} mobilization, cell proliferation, migration, and repair capacity.
Results: Analyses on human dermal fibroblasts showed abundant expression of the chemokine receptor CCR3 *in vitro* and *in vivo*. Among its corresponding ligands (CCL5, CCL8, CCL11, CCL24 and CCL26) CCL26 demonstrated a significant and specific up-regulation in atopic when compared to psoriatic skin inflammation. *In vivo*, epidermal keratinocytes showed most abundant CCL26 protein expression in lesional atopic skin. In structural cells of the skin, T_H2-cytokines such as IL-4 and IL-13 were dominant inducers of CCL26 expression. In dermal fibroblasts, CCL26 induced CCR3 signaling resulting in intracellular Ca^{2+} mobilization, as well as enhanced fibroblast migration and repair capacity, but no proliferation.
Conclusion: Taken together, findings of the present study suggest that chemokine-driven communication pathways from the epidermis to the dermis may modulate tissue remodeling in atopic skin inflammation.

Abbreviations: AD, atopic dermatitis; BrdU, 5-bromo-2-deoxyuridine; FSP, fibroblast surface protein; NHDF, normal human dermal fibroblast; qPCR, quantitative real-time polymerase chain reaction.

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1. Introduction

Remodeling of the skin is an important pathophysiologic feature of chronic atopic dermatitis (AD). It is characterized by structural changes of the skin [1–3]. The underlying mechanisms of tissue remodeling in AD, however, are not well understood.

In AD patients proinflammatory cytokines are responsible for the initiation and maintenance of skin inflammation [4]. These cytokines may also contribute to the pathogenesis of skin remodeling. Fibrogenic and fibrosis-associated cytokines (TGF-β1, IL-11 and IL-17) are increased in chronic AD lesions compared with non-lesional AD, or healthy skin [5]. Besides, matrix metallopoteinases and their inhibitors have been shown to contribute to inflammation-induced tissue destruction and remodeling in atopic skin inflammation [3]. Phipps et al. reported the relationship between allergen-induced tissue eosinophilia and markers of remodeling in human atopic skin [6].

Next to cytokines, members of the chemokine superfamily have recently been shown to regulate atopic skin inflammation [7–9]. Chemokines are small cytokine-like proteins that regulate leukocyte trafficking under homeostatic and inflammatory conditions [10,11]. Recently, a defined set of chemokines (CCL1-5, CCL11, CCL13, CCL17–18, CCL20, CCL22, CCL26–27 and CX_3CL1) was identified to initiate and perpetuate atopic skin inflammation [4,12], however, no report demonstrates their contribution to the pathogenesis of cutaneous remodeling during atopic skin inflammation. Notably, serum levels of CCL11, CCL17, CCL22, CCL26, CCL27 and CX_3CL1 are also directly correlated with disease activity and severity suggesting an important role in the immunopathogenesis of AD [7,12].

Beside leukocytes an increasing number of non-hematopoietic cells have been shown to express chemokine receptors and respond to their corresponding ligands. Hence, the aim of the present study was to identify chemokine-mediated communication pathways regulating tissue remodeling during atopic skin inflammation. Here, we systematically analyzed the chemokine receptor repertoire of human dermal fibroblasts and demonstrate expression of functional CCR3 on their cell surface. Moreover, we showed that among all known CCR3 ligands, the chemokine CCL26 is most abundantly expressed in atopic skin inflammation, and that the keratinocytes were the predominant source of CCL26 inducing enhanced migratory responses and repair activity in human fibroblasts. Findings of the present study point to a role for CCL26–CCR3 interactions regulating tissue remodeling in AD.

2. Materials and methods

2.1. Patients

Six-millimeter punch biopsies were taken from healthy individuals ($n = 29$), non-lesional ($n = 9$) and lesional ($n = 37$) psoriasis, non-lesional ($n = 29$) and lesional ($n = 65$) chronic AD, and prurigo nodularis ($n = 46$) patients. AD was identified according to the criteria defined by Hanifin and Rajka [13]. All clinical diagnoses were confirmed by histological evaluation. Atopy patch tests were performed with house dust mite preparations containing a mixture of *Dermatophagoides farinae* (10%) and *Dermatophagoides pteronyssimus* (10%) antigens in petrolatum (Chemotechnique Diagnostics) as described previously [9]. A total of 17 patients with atopic dermatitis and positive house dust mite prick tests were selected for the study. Tests were performed with Finn Chambers (Epitest) on healthy appearing dorsal skin. Of those tested, seven patients reacted positively and were chosen for the study. Patients were studied as follow: after 2, 6, and 48 h of application, the patches were removed and test results were determined. Patch sites and untreated normal skin were biopsied, snap frozen in liquid nitrogen and stored at −70 °C. All 7 patients included showed positive reactions to repeated atopy patch tests and patch testing with petrolatum alone was uniformly negative in all patients. The studies were approved by the local ethics committee.

2.2. Quantitative real-time RT-PCR (TaqMan) analysis

Quantitative real-time RT-PCR analyses were performed, as previously described [7]. Briefly skin biopsies specimens were homogenized in liquid nitrogen using Mikro-DismembratorU (Braun Biotech, San Diego, CA) and RNA was extracted using TRIzol (Invitrogen Life Technologies). Total RNA of 4 μg was treated with DNase I (Boehringer Mannheim) and reverse transcribed. The cDNA was subjected to qPCR analyses (ABI PRISM 7000 Sequence Detection Systems (Applied Biosystems) continuously during 40 cycles) of chemokine and receptor expression. Target gene expression was normalized to 18S RNA expression. Primers and probes specific for chemokines and their receptors were obtained from Applied Biosystems.

2.3. Cell cultures, hemopoietic factors and reagents

Human primary epidermal keratinocytes, and normal human dermal fibroblasts (NHDF) were cultured in keratinocyte (KGM-2), or fibroblast (FGM-2) growth medium (all Clonetics, San Diego, CA) as described previously [14]. Cultured cells were either left untreated or stimulated for different period of time with either TNF-α (100 ng/ml) plus IL-1β (5 ng/ml), or IFN-γ (50 ng/ml), or IL-13 (10 ng/ml), or IL-4 (50 ng/ml), or hGM-CSF (50 ng/ml, Schering-Plough Research Institute, Kenilworth, NJ). All other reagents were purchased from R&D Systems Inc. (Minneapolis, MN).

2.4. Histology and immunofluorescence

To determine tissue remodeling and changes within the dermal compartment, skin specimens from healthy volunteers ($n = 10$) or AD patients ($n = 37$) with chronic lesions were fixed in 4% formaldehyde in 0.075 M phosphate buffer, dehydrated in ethanol, and infiltrated with paraffin at 60 °C. Sections of 5 μm thickness were cut. Slides were stained by elastic van Giesson, and Masson's trichrome. Immunofluorescence analyses of CCL26 protein expression in normal skin of healthy volunteers ($n = 9$) and lesional skin of AD patients ($n = 10$) was performed using acetone fixed cryo-sections. Sections were blocked with PBS ± 2% donkey serum and stained overnight with a goat anti-human CCL26 or isotype controls antibodies, respectively (goat IgG; Santa Cruz Biotechnology, Heidelberg, Germany; Jackson Immunoresearch, West Grove, PA). Subsequently, sections were stained with an AlexaFluor 555-labeled donkey anti-goat antibody (R&D Systems, Mineapolis, MN) and DAPI (Invitrogen, Karlsruhe, Germany). Images were captured using a Zeiss Cell Observer System (Zeiss, Oberkochen, Germany). CCL26 protein expression was quantified using the measurement module of the ImageJ-Tool (NIH, Bethesda, MD, USA).

2.5. Flow cytometric analyses

NHDFs (10^6 cells) were washed in PBS, then incubated and stained with phycoerythrin-conjugated rat anti-human anti-CCR3 (IgG2a) monoclonal antibody (BD Pharmingen, San Diego, CA), or appropriate isotype control. Finally, samples were fixed in 1% paraformaldehyde and subsequently washed in PBS. Fluorescence was quantified by FACScan and CellQuest software (Becton Dickinson Biosciences, San Jose, CA).