Journal of Dermatological Science 74 (2014) 229–235

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Distribution of IL-31 and its receptor expressing cells in skin of atopic dermatitis

Atsuhiko Kato [a,*], Etsuko Fujii [a], Takeshi Watanabe [b], Yoshiaki Takashima [a], Hiroaki Matsushita [a], Takuya Furuhashi [c], Akimichi Morita [c]

[a] Research Division, Chugai Pharmaceutical Co., Ltd., 1-135 Komakado, Gotemba-shi, Shizuoka 412-8513, Japan
[b] Chugai Research Institute for Medical Science Inc., 1-135 Komakado, Gotemba-shi, Shizuoka 412-8513, Japan
[c] Department of Geriatric and Environmental Dermatology, Nagoya City University Graduate School of Medical Sciences, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya-shi, Aichi 467-8602, Japan

ARTICLE INFO

Article history:
Received 21 October 2013
Received in revised form 25 February 2014
Accepted 26 February 2014

Keywords:
Atopic dermatitis
IL-31
IL-31RA
CD11b
Dorsal root ganglia
Nerve-fibers

ABSTRACT

Background: To understand the clinical segments of IL-31 signaling blockade therapy in pruritus of atopic dermatitis (AD), direct detection of the target proteins in the diseased tissues will provide crucial information. There is a lack of direct evidence concerning the cellular origin of IL-31 in AD skins, and data on the expression of IL-31RA in AD are inconsistent. Also, there is no available information regarding IL-31RA protein expression in human dorsal root ganglia (DRG), which mediates the sensation of itch and is the long-suspected source of the protein.
Objective: We sought to obtain direct evidence concerning the distribution of IL-31- and IL-31RA-protein expressing cells and their characteristics in AD skin samples and in human DRG.
Methods: IL-31 was detected immunohistochemically in AD skins, and representative sections were double stained with IL-31 and several immune-markers. IL-31RA was stained immunohistochemically in AD skins and normal human DRG, and representative AD skins were double stained with IL-31RA and PGP9.5 (a nerve marker).
Results: IL-31-positive cells were observed as mononuclear infiltrating cells and as CD11b co-expressing cells in severe AD samples. As for IL-31RA, positive reactions were detected in keratinocytes and nerve fibers in the dermis of AD and in the neurons of normal DRG.
Conclusion: The detection of IL-31 in infiltrating cells of severe AD skin and of IL-31RA in nerve fibers of AD dermis and normal DRG indicates IL-31 signaling may be a contributing factor in the persistence and exacerbation of AD skin lesions.
© 2014 Japanese Society for Investigative Dermatology. Published by Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Pruritus is an essential feature and main symptom of atopic dermatitis (AD), with a high impact on patients' quality of life [1,2]. Although the pathophysiology of pruritus is still not fully understood, it is known that itch, as a cutaneous sensory perception, is excited on neuropeptide-containing unmyelinated nerve fibers in the papillary dermis and epidermis. Several mediators, such as neuropeptides, interleukins, proteases, or cytokines, have been suggested as provoking itch in AD directly or indirectly [3–5].

Interleukin-31 (IL-31) was recently discovered as a new member of the IL-6 family of cytokines, and it signals through a heterodimeric receptor composed of oncostatin M receptor (OSMR) and a IL-31 receptor alpha (IL-31RA) subunit [6,7]. There is emerging evidence that IL-31 signaling may play a role in the pruritus of AD. Transgenic mice that overexpress IL-31 develop severe pruritus and skin lesions similar to AD [8]. In an AD-like murine model (NC/Nga mice), high IL-31 mRNA expression is associated with scratching behavior [9], while an anti-IL-31 antibody reduces scratching behavior [10]. Because of this accumulating knowledge on IL-31 signaling and the pruritus of AD, IL-31 signal blocking is considered a promising therapeutic target for the disease [6,7].

On the basis of this accumulated knowledge, we developed an antibody that specifically binds to IL-31RA and inhibits its signaling as a therapeutic for pruritus of AD. When developing

* Corresponding author. Tel.: +81 550 87 8757; fax: +81 550 87 6383.
E-mail address: katoath@chugai-pharm.co.jp (A. Kato).

http://dx.doi.org/10.1016/j.jdermsci.2014.02.009
0923-1811/© 2014 Japanese Society for Investigative Dermatology. Published by Elsevier Ireland Ltd. All rights reserved.

drugs by molecular targeting, the importance of defining patient segments using a biomarker, such as the expression of target molecules in diseased tissue samples, is widely recognized [11]. To better understand the clinical segments in IL-31 signal blocking therapy for pruritus of AD, information of such systemic or local expression levels or of the distribution of IL-31 and IL-31RA molecules in patients is essential.

In terms of the systemic expression of IL-31, serum levels were higher in AD patients than in the non-atopic healthy individuals and correlated with disease activity [12]. In addition, an IL-31 gene haplotype may be linked with genetic susceptibility to non-atopic eczema in humans [13]. As for local levels in AD skin lesions, an increase of the protein [14] and mRNA [15–17] was detected when biopsy skin samples from AD patients were compared to those of healthy control individuals. The data showing the increase of IL-31 expression levels (both in mRNA and protein levels) in AD skin lesion samples is consistent between studies; however, direct evidence that shows the cellular source of the interleukin in patient skin with immunohistochemistry (IHC) is not available.

Local expression of IL-31RA is less clearly understood than that of its ligand (IL-31) or its heterodimeric partner OSMR. Using skin samples from AD patients, some researchers describe increased IL-31RA mRNA or protein expression than that of healthy control [15], while others could not detect such clear increase [14,16,18]. Furthermore, analysis of the systemic distribution of IL-31 receptor heterodimer revealed that IL-31RA transcripts are most abundantly expressed in the dorsal root ganglia (DRG) of 63 different human tissues [17]. In mice, expression of both IL-31RA mRNA and protein was detected in neurons of DRG and primary afferent fibers projecting into the dermis [19]. Since these data suggest that IL-31 might induce pruritus by directly modulating the function of sensory neurons [7], it is crucial to know the exact distribution of IL-31RA protein within AD patient skin and in human DRG.

Because previous attempts to obtain this necessary information using IHC in formalin-fixed paraffin-embedded skin tissues of AD patients had failed to identify the cellular source of IL-31 [14], we applied IHC to frozen AD skin samples that were expected to have better antigen preservation [20]. Furthermore, we also applied double staining of several immune-cell markers with IL-31 IHC to identify the cellular origin of the protein. As for IL-31RA, because of the above-mentioned conflicting data, we produced a novel mouse antibody that specifically detects human IL-31RA, and stained not only frozen samples of AD skin (some were double-stained with nerve marker), but also normal human DRG tissues.

2. Materials and methods

2.1. Samples

Twenty-four skin biopsy samples were obtained from patients who were clinically diagnosed with AD according to the "Guidelines for Management of Atopic Dermatitis [21]" at the Nagoya City University Hospital between November 2009 and January 2012. After collecting a serum sample, a biopsy was taken of the most severe area of the skin lesions (abdomen or back) in each patient (4 mm in diameter) under local anesthesia (Xylocaine Injection 1% with Epinephrine, AstraZeneca, Osaka, Japan), divided into 4 pieces, and processed into frozen blocks with Tissue-Tek O.C.T. medium (Sakura Finetek, Tokyo, Japan).

All skin and serum samples were obtained from patients who had given their written informed consent before the sample collection. The protocol of the current study was approved by the Ethical Committee for the Study of Human Gene Analysis at Nagoya City University Medical School and the Ethics Committee concerning the Use of Human-Derived Samples at Chugai Pharmaceutical Co., Ltd, which stipulates following the Declaration of Helsinki.

Five fresh, unfixed, completely anonymized, normal human DRG were procured from a tissue supplier (Asterand Inc., Detroit, MI, USA), and at the testing facility (Charles River Laboratories, Pathology Associates, Maryland, Frederick, MD, USA), the tissue samples were processed into frozen blocks with Tissue-Tek O.C.T. medium (Sakura Finetek) and were stored in a tissue bank (at −70 °C). All DRG tissues used in this study were obtained under the guidelines of the tissue supplier, which include provisions for written informed consent and anonymity procedures to protect donor confidentiality. The study protocol was approved by the ethical committee of the Charles River Laboratories, Pathology Associates, Maryland, which stipulates following the Declaration of Helsinki.

2.2. Clinical, serum and pathological scores

As a clinical score for AD, the value of the Severity Scoring of Atopic Dermatitis (SCORAD) and the pruritus visual analog scale (VAS) score were obtained from each patient, and as serum parameters, serum IgE and thymus and activation regulated chemokine (TARC) levels were measured with fluorescence-enzyme immunoassay and enzyme linked immunosorbent assay, respectively (SRL, Inc., Tokyo, Japan).

From the AD patient skin tissue blocks, cryosections of approximately 6 μm were prepared, fixed in acetone for 10 min, and stored below −70 °C prior to staining. One section from each patient was stained with hematoxylin and eosin (HE). Using the HE-stained sections, pathological findings of perivascular inflammation, spongiosis, acanthosis and parakeratosis/hyperkeratosis which are known to characterize the AD lesion [22], were scored as 0–3 along with the severity of the lesions (Fig. S1a).

2.3. IHC

For IL-31 detection, all 24 skin sections were stained by IL-31 IHC that was visualized by diaminobenzidine (DAB; Wako Pure Chemical, Osaka, Japan), and read under a light microscope (OPTIPHOT-2, Nikon, Tokyo, Japan). To identify the nature of IL-31-positive cells observed under light microscopy, we also double stained 2 representative highly IL-31-positive samples with anti-IL-31 antibody and an anti-CD3, -CD11b, or -CD11c antibody, visualized with Alexa Fluor®488 (Life Technologies, Grand Island, NY, USA) and Alexa Fluor®568 (Life Technologies) respectively, and observed them under confocal microscopy (C1si, Nikon). In addition, IL-31 and CD68 double staining was applied to a representative section, visualized with DAB and HistoGreen Substrate kit for peroxidase (AbCys SA, Paris France), and observed under a light microscope (OPTIPHOT-2, Nikon) for further characterization of the IL-31-positive cells.

For IL-31RA detection, all 24 skin sections were stained with IL-31RA IHC and visualized by DAB (Wako Pure Chemical). To identify the nature of the IL-31RA-positive fibrous structures observed in the dermis, we double stained 2 representative positive samples with an anti-IL-31RA and an anti-PGP9.5 antibody, visualized with Alexa Fluor®488 (Life Technologies) and Alexa Fluor®568 (Life Technologies), respectively.

Five normal human DRG cryosections were cut on the day of staining into slices approximately 5 μm thick and were fixed in acetone for 10 min at room temperature. Just prior to staining, the slides were fixed with 10% neutral buffered formalin (NBF) for 10 s. After fixation, the slides were stained with an anti-IL-31RA antibody, and visualized by alkaline phosphatase (Dako EnVision G|2 System/AP Rabbit/Mouse (Permanent Red) kit, Dako, Carpinteria, CA, USA), because the dark brown endogenous lipofuscin

Download English Version:

https://daneshyari.com/en/article/3212840

Download Persian Version:

https://daneshyari.com/article/3212840

Daneshyari.com