Journal of Autoimmunity 80 (2017) 28–38

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Toll-like receptor 4 antagonist TAK-242 inhibits autoinflammatory symptoms in DITRA

Akitaka Shibata, M.D., Ph.D. [a, b], Kazumitsu Sugiura, M.D., Ph.D. [a, c], Yasuhide Furuta, Ph.D. [d], Yoshiko Mukumoto [d, e], Osamu Kaminuma, D.V.M., Ph.D. [f, g], Masashi Akiyama, M.D., Ph.D. [a, *]

[a] Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan
[b] Department of Dermatology, Gifu Prefectural Tajimi Hospital, Tajimi, Japan
[c] Department of Dermatology, Fujita Health University School of Medicine, Toyoake, Japan
[d] Animal Resource Development Unit, RIKEN Center for Life Science Technologies, Kobe, Japan
[e] Genetic Engineering Team, RIKEN Center for Life Science Technologies, Kobe, Japan
[f] Department of Genome Medicine, Allergy and Immunology Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
[g] The Center for Life Science Research, University of Yamanashi, Chuo, Japan

ARTICLE INFO

Article history:
Received 20 November 2016
Received in revised form
23 January 2017
Accepted 31 January 2017
Available online 11 February 2017

Keywords:
Autoinflammation
Deficiency of IL-36 receptor antagonist
Generalized pustular psoriasis
Toll-like receptor 4
LPS
TAK-242

ABSTRACT

Background: *IL36RN* encodes the IL-36 receptor antagonist (IL-36Ra), and loss-of-function mutations in *IL36RN* define a recessively inherited autoinflammatory disease named "deficiency of IL-36Ra" (DITRA). DITRA causes systemic autoinflammatory diseases, including generalized pustular psoriasis (GPP), an occasionally life-threatening disease that is characterized by widespread sterile pustules on the skin, fever and other systemic symptoms. GPP can present at any age, and provocative factors include various infections, medicines and pregnancy.
Objective: We aimed to elucidate the role of toll-like receptor 4 (TLR4) signaling in DITRA and to innovate an efficient treatment for DITRA.
Methods: We generated *Il36rn*$^{-/-}$ mice and treated them with TLR4 agonist to establish DITRA model mice. Furthermore, we administrated TLR4 antagonist TAK-242 to the model mice to inhibit the DITRA symptoms.
Result: *Il36rn*$^{-/-}$ mice treated by TLR4 agonist showed autoinflammatory symptoms in skin, articulation and liver. Thus, we established model mice for DITRA or GPP that show cutaneous, articular, and hepatic autoinflammatory symptoms typical of DITRA or GPP: sterile pustules on the skin, liver abscesses and enthesitis of the hind paws. Additionally, these symptoms were canceled by TAK-242 administration. We demonstrated the inhibitory effects of the TLR4 antagonist TAK-242 on the autoinflammatory symptoms exhibited by the DITRA models.
Conclusion: We suggested that blockage of TLR4 signaling is a promising treatment for DITRA and GPP.

1. Introduction

IL36RN, also known as *IL1F5*, encodes the IL-36 receptor antagonist (IL-36Ra), which is an antagonist of IL-1-family cytokines. Loss-of-function mutations in *IL36RN* define a recessively inherited autoinflammatory disease named "deficiency of IL-36Ra" (DITRA) [1]. DITRA was first described in a subgroup of patients with generalized pustular psoriasis (GPP) [1,2], which is a life-threatening disorder characterized by recurrent episodes of severe skin inflammation, with pustule development associated with fever, malaise, and extracutaneous involvement, including arthritis and neutrophilic cholangitis [2–4]. GPP skin lesions present recurrent sterile pustules with flush on the whole body. Psoriasis vulgaris (PV) exhibits multiple, hyperkeratotic erythematous plaques with scales. PV and GPP are variants of "psoriasis" which is an inflammatory skin disease with accelerated turnover of the epidermal keratinocytes. We reported that the majority of GPP

* Corresponding author. Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan.
E-mail address: makiyama@med.nagoya-u.ac.jp (M. Akiyama).

http://dx.doi.org/10.1016/j.jaut.2017.01.007
0896-8411/

without PV cases in Japanese are caused by mutations in *IL36RN* [2]. *IL36RN* mutations are thought to be a major predisposing factor for GPP, although certain triggering stimuli, such as TLR4 agonist activation, are probably needed for the onset of GPP. IL-36Ra is an IL-1 family cytokine, is an antagonist of the IL-36 receptor (IL-36R; also named IL-1RRP2 or IL-1RL2) and inhibits the activity of IL-36α, IL-36β, and IL-36γ (originally named IL-1F6, IL-1F8, and IL-1F9, respectively), which are also members of the IL-1 family of cytokines and are IL-36R agonists [5–9]. IL-1 is a major mediator of inflammation and exerts effects on the neuro-immuno-endocrine system. The IL-1 system is composed of the two agonist ligands IL-1α and IL-1β [10]. IL-1β is known to be derived from neutrophils and macrophages [11]. These cytokines activate several proinflammatory signaling pathways, such as the nucleolar factor-κB and mitogen-activated protein-kinase pathways, which play important roles in innate immunity [12,13]. Recently, IL-36 cytokines have attracted much attention for their important role in the initiation of psoriasis [1,14].

In the past few years, a murine model of PV was generated by activation of TLR7, which recognizes viral single-stranded RNA [15,16]. *Il36rn*$^{-/-}$ mice that were administered with a TLR7 agonist displayed more severe phenotypes associated with psoriasis vulgaris [17]. Although these models successfully reproduce the simple, acute skin inflammation of PV, they only partially reproduce the symptoms of DITRA. Concerning other TLRs, recent findings have indicated the importance of TLR4 and persistent inflammation in the development of obesity [18]. Moreover, Ballak et al. [19] reported that in obese mice, TLR4-signaling activation was attenuated by IL-37 which is an antagonist of IL-1-family cytokines. Therefore, we focused on the role of TLR4 instead of TLR7 in the onset of autoinflammatory reactions associated with DITRA. In this study, we established a model of autoinflammatory syndromes associated with DITRA via TLR4 activation in *Il36rn*$^{-/-}$ mice and successfully inhibited the onset of DITRA-related symptoms by using a selective TLR4 antagonist.

2. Methods

2.1. Mice

Il36rn$^{+/-}$ mice (Accession No. CDB1242K: http://www2.clst.riken.jp/arg/mutant%20mice%20list.html) were generated using a targeting vector designed to eliminate a part of exon 1 through the entire exon 3 by homologous recombination (Fig. 1a). A C57BL/6N embryonic stem cell line, HK3i [20], was transfected with the targeting vector by electroporation. We obtained F1 heterozygous mice by mating them to wild-type mice. Then, we intercrossed *Il36rn*$^{+/-}$ mice to generate *Il36rn*$^{-/-}$ mice. Males at 8–12 weeks were included in the experiment, and no randomization or blinding was used. Genotypes of the mice were determined by PCR analysis of the tail tissue DNA using the following primers: P1 (5′-ATGCATCCAAAGGCAGGTAA), P2 (5′- GCTTGGCTGGACGTAAACTC), P3 (5′- TGGAGCTCATGATGGTTCTG) and P4 (5′- AGGATCCTGCTCAGTTCTTCC). Primer sets P1-2 and P3-4 correspond to knockout allele 293bp and WT allele 205bp (Fig. 1b).

2.2. Antibodies, receptor agonists, and antagonists

Antibodies, receptor agonists, and antagonists used in this study for *in vivo* treatment were as follows: anti-mouse IL-17a antibody (catalog number: 16-7173-85; eBioscience, San Diego, CA, USA), mouse IgG1 K-isotype control functional grade purified antibody

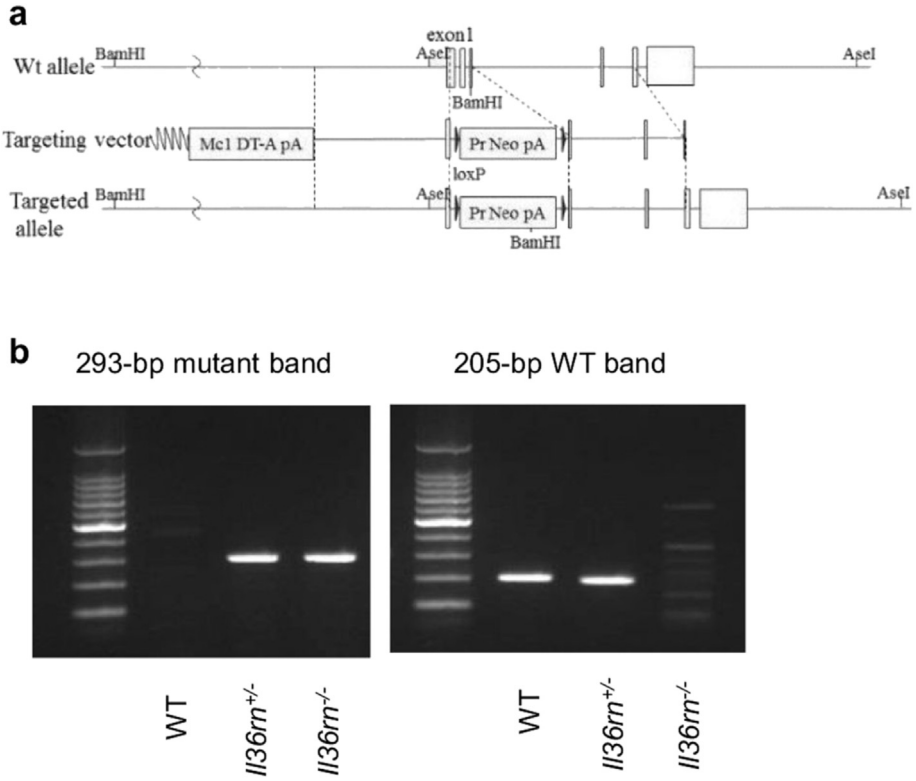

Fig. 1. Establishment of the IL-36Ra deficient mice. (**a**) The targeting vector was constructed by disrupting exon 1 through the entire exon 3. (**b**) PCR reaction amplified a 293-bp mutant band, and a 205-bp fragment corresponding to the wild-type (WT) genomic *Il36rn* DNA.