Clinical Immunology 180 (2017) 120–127

Contents lists available at ScienceDirect

Clinical Immunology

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

Inhibition of KDM4A activity as a strategy to suppress interleukin-6 production and attenuate colitis induction

Kazuhiro Ishiguro *, Osamu Watanabe, Masanao Nakamura, Takeshi Yamamura, Masanobu Matsushita, Hidemi Goto, Yoshiki Hirooka

Department of Gastroenterology and Hepatology, Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya, Aichi 466-8550, Japan

ARTICLE INFO

Article history:
Received 11 April 2017
Accepted with revision 12 May 2017
Available online 13 May 2017

Keywords:
Fibroblasts
Interleukin-6
Herbal ingredients
Histone H3

ABSTRACT

4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) functions as a hapten and fluoresces upon binding to proteins. Therefore, fluorescence visualization of hapten-proteins is a feature of the colitis induced by NBD-Cl. Using this colitis model, we located activated fibroblasts in the vicinity of hapten-proteins upon colitis induction and observed interleukin (IL)-6 production in the activated fibroblasts. We screened herbal ingredients using primary fibroblasts stimulated with tumor necrosis factor α (TNF-α) and found the suppressive action of Atractylodin on IL-6 production. Under TNF-α stimulation, Atractylodin induced the tri-methylation of histone H3 at lysine residue 9, which impaired the binding between NF-κB and the IL-6 promoter on the genomic DNA. Atractylodin inhibited KDM4A but not KDM6A activity. Atractylodin administration attenuated colitis induction. The KDM4A inhibitor ML324 showed similar actions on IL-6 production and colitis induction. We propose the inhibition of KDM4A activity as a strategy to suppress IL-6 production and attenuate colitis induction.

1. Introduction

Ulcerative colitis and Crohn's disease are chronic inflammatory disorders of the digestive tract that are categorized as inflammatory bowel diseases (IBDs) [1]. The precise etiology of IBDs remains uncertain, and IBDs are sometimes refractory to anti-inflammatory medicine, such as 5-aminosalicylic acid and glucocorticoid [1–3]. Although anti-tumor necrosis factor α (TNF-α) antibody therapy can be effective for the care of patients with refractory IBDs, the efficacy is compromised by the production of antibodies to the anti-TNF-α antibody [4]. Therefore, further analysis of colitis induction and the development of anti-inflammatory therapy are necessary. Experimental colitis models are useful for investigations of the inflammatory reactions in the colon and evaluation of the efficacy of novel anti-inflammatory agents.

4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) induces allergic contact dermatitis as a hapten, which elicits an immune response only when it binds to proteins [5]. Moreover, NBD-Cl fluoresces upon binding to proteins through the substitution reaction of 4-Cl with the amino/thiol groups of amino acid residues (excitation 460 nm, emission 535 nm), whereas NBD-Cl alone does not emit fluorescence [6]. Our previous studies have demonstrated that NBD-Cl-enema treatment induces colitis and that NBD-protein fluorescence is observed in the inflamed mucosa of the colon [7,8]. This NBD-Cl-induced colitis model has shown that macrophages infiltrate in the vicinity of NBD-proteins and that NBD-proteins are endocytosed by macrophages, which activate T cells for colitis induction [7]. Consequently, NBD-Cl-induced colitis is characterised by fluorescence visualization of hapten-proteins, which allows analyses of the inflammatory reactions and assessment of experimental interventions at the site of hapten-protein formation upon colitis induction.

Interleukin (IL)-6 is a pleiotropic cytokine that is involved in the pathogenesis of inflammatory diseases [9]. Blockade of IL-6 signaling with the anti-IL-6 receptor antibody has shown therapeutic benefits in several inflammatory diseases, including Crohn's disease [9,10]. Indeed, IL-6 is the most predominant cytokine produced in NBD-Cl-induced colitis, and colitis induction is reduced by the anti-IL-6 receptor antibody [7]. Therefore, NBD-Cl-induced colitis is an appropriate model to investigate IL-6 expression upon colitis induction and to evaluate the anti-inflammatory effect of agents that suppress IL-6 production. In the present study, we located activated fibroblasts upon colitis induction using this model and showed IL-6 production in the activated fibroblasts.

Primary fibroblasts are useful for screening to identify IL-6-suppressive agents and for investigations into the molecular mechanism of the suppressive action because they can produce IL-6 and be subcultured over several generations from a stock in liquid nitrogen. Herbal

Abbreviations: ANOVA, analysis of variance; ChIP, chromatin immunoprecipitation; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; 5-EU, 5-ethynyl uridine; FBS, fetal bovine serum; hpf, high-power field; IBDs, inflammatory bowel diseases; IL, interleukin; LPS, lipopolysaccharide; Lys9, lysine residue 9; NBD-Cl, 4-chloro-7-nitro-2,1,3-benzoxadiazole; PBS, phosphate-buffered saline; TNBS, trinitrobenzene sulfonic acid; TNF-α, tumor necrosis factor α.

* Corresponding author.
E-mail address: kio@med.nagoya-u.ac.jp (K. Ishiguro).

http://dx.doi.org/10.1016/j.clim.2017.05.014
1521-6616/

ingredients represent an attractive library from which to find seeds for medical drugs because herbs are traditional remedies for sickness and herbal ingredients have a large variety of chemical structures [11]. In this study, we screened herbal ingredients using primary fibroblasts stimulated with TNF-α to find IL-6-suppressive agents and to investigate the suppressive action at the molecular level. We also evaluated the anti-inflammatory effect of the IL-6-suppressive agents on NBD-Cl-induced colitis.

2. Methods

2.1. Mice

We obtained 8-week-old female BALB/c mice from Japan SLC (Shizuoka, Japan). All mice were kept in a 12-h light/dark cycle with controlled humidity (60–80%) and temperature (22 ± 1 °C) under specific pathogen-free conditions. Food and water were freely available. All animal experiments were performed according to the guidelines of the Institute for Laboratory Animal Research with the approval of the ethics committee of Nagoya University.

2.2. Colitis induction

NBD-Cl (Tokyo Chemical Industry, Tokyo, Japan) was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 200 mg/ml for the stock solution, which was stored at −80 °C. The NBD-Cl stock solution was diluted with ethanol and then with distilled water at a ratio of 1:100:100 to prepare a 1 mg/ml NBD-Cl enema, which induced colitis efficiently with low mortality [7,8]. Trinitrobenzene sulfonic acid (TNBS) was purchased from Wako Chemicals (Osaka, Japan) and dissolved in 50% ethanol to prepare a 10 mg/ml TNBS enema, which induced colitis efficiently with low mortality [12].

On day 0, we lightly anesthetized the mice with isoflurane (Abbott Laboratories, Abbott Park, IL, USA) and inserted a rubber catheter (2 mm outer diameter) fitted onto a 1 ml syringe via the anus. The tip was positioned 2 cm proximal to the anus. Then, 100 μl of the NBD-Cl or TNBS enema was slowly administered to the mice through the catheter. The mice were kept in a head-down position for 30 s and then returned to their cages.

2.3. Fluorescence observation of NBD-proteins, S100A4 and IL-6

The colons were obtained from the mice on day 1 after the NBD-Cl-enema treatment, fixed overnight in 4% paraformaldehyde solution and embedded in paraffin to prepare sections (6 μm). After removal from the paraffin, adjacent sections were used for hematoxylin-eosin staining or fluorescence observation with the BZ-8000 fluorescence microscopy system (Keyence, Osaka, Japan). We used goat anti-S100A4 antibody (Sigma-Aldrich, St. Louis, MO, USA), rabbit anti-goat IgG antibody conjugated with Alexa Fluor 594 (Molecular Probes), rabbit anti-IL-6 antibody conjugated with biotin (Bioss, Woburn, MA USA) and Alexa Fluor 647 conjugated with streptavidin (Molecular Probes, Eugene, OR, USA).

2.4. Cells

Primary fibroblasts were obtained from BALB/c mouse embryos on day 13.5 of gestation as previously described [13]. After three passages, the cells were stored in liquid nitrogen and used within five passages from the stocks. Unless otherwise specified, the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 50 U/ml penicillin G, 50 μg/ml streptomycin, and 125 ng/ml amphotericin B.

Primary macrophages were isolated as previously described [14]. Briefly, 2 ml of 3% thioglycollate medium was injected into the peritoneal cavity of each mouse. Three days later, peritoneal exudative cells were collected by peritoneal lavage with cold phosphate-buffered saline (PBS). The cells were resuspended in culture media and seeded into plates. Twenty-four hours later, the non-adherent cells were removed by washing twice with PBS. Flow cytometry showed that >90% of the adherent cells expressed the macrophage marker F4/80 [15]. Therefore, the adherent cells were used as primary macrophages.

2.5. Herbal ingredients

We purchased Atractylodin, Barbaloin, Catalpol, Eleutheroside B, Gentiopicroside, Liquiritin and Nodakenin from Wako Pure Chemical (Osaka, Japan); Dulcoside A and Samin from Nagara Science (Gifu, Japan) (purity of each ≥98%). The herbal ingredients were dissolved in DMSO to generate stock solutions. The stock solutions were diluted at a ratio of 1:200 for the experiments. DMSO alone was used as a vehicle control.

2.6. IL-6 production

The cells were seeded into 24-well plates (4×10^4/well). One day later, the fibroblasts were stimulated for 24 h with 0–100 ng/ml TNF-α (Sigma-Aldrich) in DMEM containing 0.1% FBS in the presence of 0–20 μM herbal ingredients or 0–10 μM ML324 (BioVision, Milpitas, CA, USA). Alternatively, the macrophages were stimulated with 100 ng/ml lipopolysaccharide (LPS) (*Escherichia coli* serotype O111:B4) (Sigma-Aldrich). The IL-6 concentrations in the media were determined with an ELISA kit (R&D Systems, Minneapolis, MN, USA). IL-6 production (%)

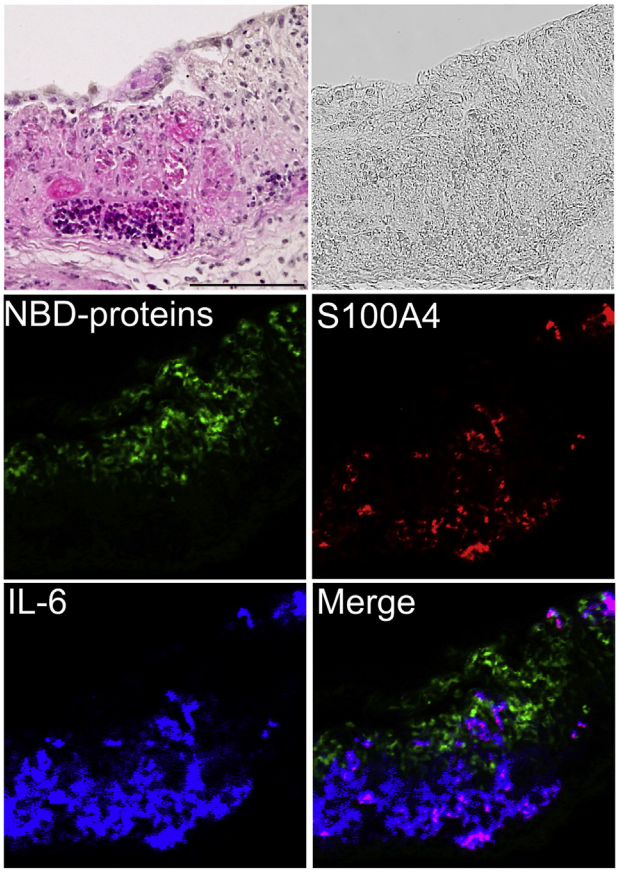

Fig. 1. NBD-proteins and S100A8/IL-6-expressing cells upon colitis induction. On day 1 following NBD-Cl-enema treatment, the colon was obtained from mice to prepare paraffin sections. After removal from paraffin, adjacent sections were used for hematoxylin-eosin staining or fluorescence observation of NBD-proteins, S100A4 and IL-6. Three independent experiments showed similar results. Bar, 100 μm.