International Immunopharmacology 15 (2013) 199–205

Contents lists available at SciVerse ScienceDirect

International Immunopharmacology

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

Preliminary report

Synergistic effects of interleukin-1β and interleukin-17A antibodies on collagen-induced arthritis mouse model

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ARTICLE INFO

Article history:
Received 27 October 2012
Received in revised form 7 December 2012
Accepted 14 December 2012
Available online 29 December 2012

Keywords:
Rheumatoid arthritis
Collagen-induced arthritis
Synergistic effect
IL-1β antibody
IL-17A antibody

ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that mainly causes the synovial joint inflammation and cartilage destruction. Both interleukin-1β (IL-1β) and Interleukin-17 (IL-17) are important proinflammatory cytokines involved in the pathogenesis of RA. We investigated whether combination therapy with IL-1β and IL-17A antibodies would generate the potential for synergistic effects on a collagen-induced arthritis (CIA) mouse model. Mice with CIA were subcutaneously injected with humanized IL-1β antibody, IL-17A antibody, or combination treatment. The effects of treatment were determined by arthritis severity score, histological damage and bone destruction, autoreactive humoral and cellular immune responses and cytokine production. Treatment with IL-1β antibody or IL-17A antibody alone resulted in beneficial effects on clinical and histological parameters of CIA mice. Compared with the single antibody treatments, the combination therapy resulted in a more significant effect in alleviating the severity of arthritis by preventing bone damage and cartilage destruction, reducing humoral and cellular immune responses, and down-regulating the expression of IL-1β, IL-6, IL-17A, IFN-γ, RANKL and MMP-3 in inflammatory tissue. In conclusion, combination treatment with humanized IL-1β and IL-17A antibodies demonstrates synergistic beneficial effects for preventing joint inflammation and cartilage destruction and bone damage in CIA mice model. These studies also provide evidence that combination with IL-1β and IL-17A antibodies may lead to a new combinatorial therapy for RA patients.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that mainly causes the synovial joint inflammation and cartilage destruction [1]. T cells, B cells and inflammatory cytokines such as TNF-α, IL-1β and IL-17 play a pivotal role in the pathogenesis of synovial inflammation and destruction of joint cartilage in RA [2–4].

The type II collagen-induced arthritis (CIA) model shares similar pathologic and physiological properties with human rheumatoid arthritis [5]. In both diseases, immune cells (lymphocytes and macrophages) and synovial tissues produce a series of complex inflammatory cytokines and other soluble mediators in a pathogenic inflammatory cascade. These inflammatory reactions are related with the severity of bone damage and cartilage destruction during the progress of RA [6,7]. CIA has been widely used to illustrate the pathologic mechanisms relevant to human RA and to identify potential therapeutic agents.

IL-1β promotes inflammation and destruction in synovial tissue, bone, cartilage and joint in patients with RA [8,9]. IL-1β is a crucial mediator in the generation of synovial inflammation and pannus [10–12]. It involves in the inflammatory processes in RA by the activation of macrophages, T cells and B cells. IL-1β is also a pivotal cytokine in inducing expression of other inflammatory cytokines, such as TNF-α, IL-6 [13]. Previous study had shown that increased level of IL-1β in synovial tissue [14] was correlated with histomorphological characteristics of arthritis [15,16].

IL-17 is a proinflammatory cytokine produced by activated CD4+ T cells distinct from Th1 or Th2 cells, defined as Th17 cells. IL-17 promotes inflammation by enhancing the production of cytokines such as IL-1β, TNF-α, IL-6 and receptor activator for nuclear factor-κB ligand (RANKL), as well as chemokines such as macrophage inflammatory protein (MIP)-2 and IL-8 [13,17–25]. There is plenty of evidence that IL-17 contributes to the inflammation in the pathogenesis of RA. In collagen-induced arthritis (CIA), an animal model reminiscent in several aspects to RA, IL-17 expression level is elevated in inflamed synovium [26], and synovial membrane cells cultured in vitro could produce IL-17 spontaneously [27,28]. In the process of bone and cartilage destruction, IL-17 could act on osteoblasts and induce the expression of receptor activator for nuclear factor-κ B Ligand (RANKL) which could lead to osteoclastogenesis [29].

Since IL-1 and IL-17 play an important role in the pathogenesis of RA, treatment with IL-1 receptor antagonist (IL-1Ra; anakinra) has been shown to be clinically efficacious in RA patients [30], and anti-IL-17 treatment in experimental arthritis demonstrated therapeutic effects

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http://dx.doi.org/10.1016/j.intimp.2012.12.010

on joint inflammation, cartilage destruction, and bone erosion in a mouse model with collagen-induced arthritis (CIA) [31]. Therefore, several animal studies have focused on the efficacy of IL-1Ra administered alone or in combination with other agents, such as monomeric PEGylated type I TNFR, methotrexate and dexamethasone [32].

Accordingly, the aim of the present study is to investigate the additional therapeutic impacts of combination treatment with IL-1β and IL-17A antibodies on amelioration of arthritis severity, histological damage, humoral and cellular responses and proinflammatory cytokine production in CIA mice.

2. Materials and methods

2.1. Animals

Male DBA/1 mice 7–8 weeks old with a mean weight of 25–30 g were purchased from Harbin Veterinary Research Institute (Harbin, China). They were treated and maintained at the animal facility of Pharmaceutical Biotechnology Laboratory of Northeast Agricultural University. The animals were fed with standard rodent chow and provided water ad libitum. The health status of the animal colony was monitored in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

2.2. Induction of CIA and assessment of arthritis

Each mouse was immunized with 100 μl of a 1:1 (v/v) emulsion 0.1 mol/l acetic acid containing 2 mg/ml of chicken type II collagen (SIGMA) and Freund's complete adjuvant (SIGMA) at the base of the tail. Mice were immunized a second time 7 days later.

The severity of the arthritis in each limb was scored on an established scoring system of 0–4: 0 = no visible evidence of arthritis, 1 = mild swelling confined to the ankle or wrists, 2 = erythema and moderate swelling, 3 = edema erythema and moderate swelling of more than 2 joints, 4 = severe joint swelling of the entire ankle, foot and digits, or dysfunction of the limb. A total arthritis score per mouse was determined by summarizing the scores of all four limbs. Assessment of the arthritis score was carried out by three observers independently.

2.3. Antibody preparation

IL-1β and IL-17A humanized antibody genes were cloned into the pEE eukaryotic expression plasmid (LONZA, Switzerland). Then the expression vectors were transfected into Chinese hamster ovary (CHO) cells by Lipofectamine 2000 (Invitrogen) for stable expression. After the bulk selection, the positive clones were detected by ELISA and cultured in serum-free medium for antibody purification. The supernatant of the serum-free medium was purified on Protein-A agarose column (GE, USA) with AKTA purifier (GE, USA). The purified IL-1β and IL-17A humanized antibodies had shown high specificity and neutralization activity in vitro (data not shown).

2.4. Antibody treatment

Mice were divided into 4 CIA groups and 1 healthy control group (n = 9 mice per group). The CIA groups were given one of the following treatments 3 times per week: phosphate buffered saline (PBS), IL-1β antibody (10 mg/kg subcutaneously), IL-17A antibody (10 mg/kg subcutaneously), combination with the two antibodies (10 mg/kg in total subcutaneously). The treatment was started on day 14 after the initial immunization with chicken type II collagen, and the mice were sacrificed at the day 70.

2.5. Measurement of anti-collagen IgG in the sera

Sera were collected at the day of sacrifice (day 70) by retro orbital blood drawing. All the sera samples were centrifuged and were stored at −80 °C for later use.

Levels of anti-collagen IgG in the sera were determined by ELISA using mouse anti-type II collagen IgG assay kit (Cayman chemical company, USA) for capture and detection. Absorbance (450 nm) was measured with an ELISA plate reader (BioTek, USA). The measurements were carried out in triplicate. The concentration of IgG antibody was calculated by a standard curve prepared in the anti-type II collagen IgG assay kit.

2.6. Analysis of splenocyte proliferation and cytokine production

At 10 weeks after the first CII immunization, spleens were removed under aseptic condition and single cell suspensions were prepared. Splenocytes were suspended to 5×10^6 cells/ml and cultured in RPMI 1640 (GIBCO) containing 10% FBS with PHA (20 μg/ml) and CII (100 μg/ml) for 72 h in 96-well plates. The cell proliferation was determined by MTT assay.

After 72 h of the splenocytes cultured, the splenocyte supernatants were collected and analyzed for cytokine (IL-1β, IL-17A) production by ELISA according to the supplier's instructions (R&D Systems).

2.7. RNA isolation and real-time quantitative PCR

RNA from the cartilage tissue was isolated with Trizol (Invitrogen) according to the manufacturer's protocol. RNA was reverse transcribed into cDNA using the reverse-transcription kit (Promega, USA).

The cDNA was used for real-time quantitative PCR (ABI 7500, Applied Biosystems, Carlsbad, CA, USA) to analyze IL-1β, IL-6, IL-10, IL-17A, IFN-γ, TNF-α, MMP-3, and RANKL. β-actin mRNA was used as an endogenous control to allow the relative quantification of IL-1β, IL-6, IL-10, IL-17A, IFN-γ, TNF-α, MMP-3, and RANKL. The amplified PCR products were quantified by measuring the calculated cycle thresholds (C_t) of samples mRNA and β-actin mRNA. Relative multiples of change in mRNA expression were calculated by $2^{-\Delta\Delta Ct}$. The mean value of normal group target levels became the calibrator (one per sample) and the results are expressed as the n-fold difference relative to normal controls (relative expression levels).

2.8. Histopathologic analysis

After the mice were sacrificed at the end of the experiment, the ankle joints were removed and fixed in 10% formalin, then decalcified in decalcifier solution for 2–3 days. The joints were trimmed and embedded in paraffin, and 4 μm sections of whole ankle joints were cut with a microtome and stained with hematoxylin and eosin (H&E). The severity of arthritis in the joints was scored on a scale of 0–5 (0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe), for 3 different parameters: joint inflammation, cartilage destruction, and bone destruction. Scoring was performed in a blind manner by 3 independent observers.

2.9. Statistical analysis

All data are expressed as mean ± S.D. Unless otherwise noted, combined experimental data were analyzed by one-way analysis of variance (ANOVA), followed by Student two-tailed *t* test. All statistical analyses were performed using SPSS13.0 software. $P<0.05$ was considered statistically significance.