Immunology Letters 172 (2016) 56–66

Contents lists available at ScienceDirect

Immunology Letters

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

IL-1 receptor antagonist (IL-1Ra)-Fc ameliorate autoimmune arthritis by regulation of the Th17 cells/Treg balance and arthrogenic cytokine activation

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ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form 15 February 2016
Accepted 17 February 2016
Available online 20 February 2016

Keywords:
IL-1 receptor antagonist
Autoimmune arthritis
Th17 cells
Regulatory T cells
Osteoclastogenesis
Angiogenesis

ABSTRACT

Introduction: IL-1β signalling has a critical role in the pathogenesis of various types of inflammatory arthritis including rheumatoid arthritis (RA). We aimed to investigate the therapeutic effects of human IL-1 receptor antagonist with Fc fragment (hIL-1Ra-Fc) on autoimmune arthritis and to identify the possible mechanisms by which hIL-1RA-Fc exerts anti-arthritic effects in a murine model of RA and patients with rheumatoid arthritis.
Methods: Collagen-induced arthritis (CIA) murine model was established in DBA/1J mice. The levels of various cytokines were determined by using enzyme-linked immunosorbent assay. The mouse joints were assessed for clinical arthritis score and histologic features. Th17 cells and Treg cells were stained by using antibodies specific for CD4, IL-17, CD25, and FoxP3. Osteoclastogenesis was determined by TRAP staining and real-time PCR.
Results: hIL-1RA-Fc reduced the arthritis incidence, histological inflammation and cartilage score in the CIA model. The expression of proinflammatory cytokines, VEGF and RANK, was reduced in the affected joint of hIL-1Ra-Fc-treated mice. hIL-1Ra-Fc-treated mice showed decreased number of Th17 cells with increased number of Treg cells in spleens. hIL-1Ra-Fc reduced Th17 cell differentiation by inactivation of STAT3 signalling, and reciprocally induced Treg cell differentiation through STAT5 signalling. In addition, the expression of IL-17, TNF-α, RANKL, and VEGF was decreased, while Foxp3 gene expression was increased in PBMCs of RA patients after administration of hIL-1Ra-Fc.
Conclusion: The anti-arthritis effects of hIL-1RA-Fc are associated with regulation of balance between Th17 cells and Treg cells and suppression of osteoclastogenesis and angiogenesis in the affected joints.

Abbreviations: ACR, American College of Rheumatology; APC, allophycocyanin; CD, cluster of differentiation; CIA, collagen induced arthritis; CII, type II collagen; CTLA-4, cytotoxic T-lymphocyte antigen 4; DMARD, disease-modifying antirheumatic drug; ELISA, enzyme-linked immunosorbent assay; EULAR, The European League Against Rheumatism; FITC, fluorescein isothiocyanate; FoxP3, fork-head box P3; HIF, hypoxia-inducible factor; hIL-1Ra-Fc, human IL-1 receptor antagonist with Fc fragment; HRP, horseradish peroxidase; i.d., intradermally; IFA, incomplete Freund's adjuvant; Ig, immunoglobulin; IL, interleukin; i.p., intraperitoneally; JIA, juvenile idiopathic arthritis; mAb, monoclonal antibody; MLR, mixed leukocyte reaction; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PE, phycoerythrin; PerCP, Peridinin chlorophyll; RA, rheumatoid arthritis; RANK, receptor activator of nuclear factor kappaB; RANKL, receptor activator of nuclear factor kappaB ligand; RORγt, retinoic acid receptor–related orphan receptor γt; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor β Th17, Type 17 helper T cell; TNF-α, tumor necrosis factor-α TRAP, tartrate-resistant acid phosphatase; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.

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http://dx.doi.org/10.1016/j.imlet.2016.02.011
0165-2478/

1. Introduction

Rheumatoid arthritis (RA) is the most common inflammatory arthritis which is known as systemic autoimmune disorder. RA mainly involves diarthrodial joints and is characterized by hyperplastic synovial membrane and destruction of adjacent bone and cartilage [1]. Excessive synovial proliferation, in other words pannus formation, and bone destruction are the main events which result in functional impairment.

The exact pathogenesis of RA is not completely understood, but it is known that several proinflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-6 have an important role in the development of RA. Since methotrexate was introduced as the first disease-modifying antirheumatic drug (DMARD), several DMARDs have been applied in RA patients and they were found to be effective in pain control and radiologic progression of RA. Since some patients were refractory to various DMARDs, new biologic DMARDs targeting proinflammatory cytokines were introduced and they proved to be superior to conventional DMARDs [4–7]. In addition, depletion of B cells by anti-cluster of differentiation (CD)-20 monoclonal antibody and blocking T cell activation by CTLA4-immunoglobulin (Ig) Fc have been proved to be effective in RA patients who failed to respond to TNF-α blocking agent [8,9]. However, in some RA patients, a therapeutic effect of the aforementioned biologic DMARDs is not observed. Therefore, there is a need for developing new therapeutic agents.

IL-1 family of cytokines has major roles in innate immune reactions, and especially IL-1α and IL-1β are major mediators of several autoinflammatory diseases and proinflammatory activities [10,11]. IL-1β is considered the key cytokine in the pathogenesis of acute gouty arthritis, and blocking IL-1β signalling exerted a therapeutic effect [12,13]. IL-1β has an important role in systemic onset juvenile idiopathic arthritis (JIA) [14]. Blocking the type I IL-1 receptor with anakinra caused improvement in JIA, and most of the patients achieved clinical remission after 1 year of the aforementioned therapy [15]. Also, IL-1β mediates the proinflammatory response in RA by several mechanisms including activation of osteoclasts, synovial fibroblasts, and endothelial cells [16]. Although the previously described IL-1 receptor antagonist (IL-1Ra), anakinra, was not superior to anti-TNF drugs in the field of RA treatment, the efficacy and side effect of hIL-1Ra-Fc needs to be reassessed because it has a longer duration of therapeutic effect than anakinra [17].

IL-17 producing type 17 helper T cells (Th17) have emerged as major helper T cells in the pathogenesis of RA [16,18]. On the contrary, fork-head box P3 (FoxP3)-expressing regulatory T cells (Treg) displayed immunologic self-tolerance and suppressive effect in inflammatory arthritis [19,20]. Recently, several experiments revealed novel candidates for RA treatment by displaying the regulation between Th17 cells and FoxP3-expressing Treg cells [21–24]. IL-1 signalling is known to enhance the development of Th17 cells by downregulation of Foxp3 expression [25].

Therefore, we hypothesized that hIL-1Ra-Fc would suppress arthritis in an animal model of collagen-induced arthritis by blocking the IL-1β signalling pathway. With the aim of revealing the pathophysiologic mechanism of IL-1β signalling and the therapeutic effect of hIL-1Ra-Fc in an animal model of RA, we investigated whether hIL-1Ra-Fc affects the balance between Th17 cells and Foxp3-expressing Treg cells.

2. Materials and methods

2.1. Animals

Seven-week-old male DBA1/J mice (SLC, Inc., Shizuoka, Japan) were maintained in groups of five in polycarbonate cages in a specific pathogen-free environment and were fed standard mouse chow (Ralston Purina, Gray Summit, MO) and water ad libitum. All experimental procedures were assessed and approved by the Animal Research Ethics Committee at the Catholic University of Korea.

2.2. Patients with RA

To investigate in vitro effects of hIL-Ra-Fc in peripheral blood mononuclear cells (PBMCs) of RA patients, we obtained peripheral blood samples from two RA patients. All of the patients fulfilled the 2010 American College of Rheumatology (ACR)/The European League Against Rheumatism (EULAR) classification criteria for RA and presented high titer of Anti-citrullinated protein antibody. Patient 1 was a 62-year-old male with a disease duration of 6 months, and patient 2 was a 51-year-old female with a disease duration of 77 months. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital. All patients gave written informed consent prior to participation in the study.

2.3. Induction of arthritis and injection of hIL-1Ra-Fc

To induce CIA, 100 μg of bovine type II collagen (CII) and complete Freund's adjuvant (Chondrex, Inc., Redmond, WA) were injected intradermally (i.d.) into the base of the tail. Two weeks later, they were boosted i.d. with 100 μg of CII and incomplete Freund's adjuvant (IFA). To examine the effect of administering hIL-1Ra-Fc, hIL-1Ra-Fc (50 mg/kg or 100 mg/kg) was injected intraperitoneally (i.p.) at 2-day intervals after CIA induction. After CIA induction, three independent observers examined the severity of arthritis three times a week. The severity of arthritis was recorded using the mean arthritis index on a scale of 0–4, as previously described [26].

2.4. Mixed leukocyte reaction (MLR)

For T cell specific proliferation response, normal DBA1/J spleen cells or healthy PBMCs were cultured with anti-mouse CD3 (0.5 μg/ml) or anti-human CD3 (0.5 μg/ml) and hIL-1Ra-Fc (0.1, 1, 10, 100 ng/ml) for 72 h. Spleen single cells of CIA mice and hIL-1Ra-Fc injected CIA mice were cultured with anti-mouse CD3 (0.5 μg/ml) for 72 h. Before the final 16 h of the total culture time, cells were treated with 25 μCi/ml of [^{3}H]-thymidine (GE Healthcare, Piscataway, NJ). Then the radioactivity was measured with a Micro Beta (Pharmacia Biotech, Piscataway, NJ).

2.5. Real-time polymerase chain reaction (PCR)

Relative expression of specific mRNAs was quantified by real-time PCR using SYBR Green I (Roche Diagnostics). The following sense and antisense primers were used: for mouse IL-17, 5′-CCT CAA AGC TCA GCG TGT CC-3′ (sense) and 5′-GAG CTC ACT TTT GCG CCA AG-3′ (anti-sense); for mouse RANKL, 5′-TGT ACT TTC GAG CGC AGA TG-3′ (sense) and 5′-CCA CAA TGT GTT GCA GTT CC-3′ (anti-sense); for mouse VEGF, 5′-TCT TCA AGC CGT CCT GTG TG-3′ (sense) and 5′-AGG ACC ATT TAC ACG TCT GC-3′ (anti-sense); for mouse IFN-γ, 5′-GAA AAT CCT GCA GAG CCA GA-3′ (sense) and 5′-TGA GCT CAT TGA ATG CTT GG-3′ (anti-sense); for mouse TGF-β, 5′-GTA ACG CCA GGA ATT GTT GC-3′ (sense) and 5′-ACC GCA ACA ACG CCA TCT AT-3′(anti-sense); for mouse IL-10, 5′-AAG TGA TGC CCC AGG CA-3′ (sense) and 5′-TCT CAC CCA GGG AAT TCA AA-3′(anti-sense); for mouse Foxp3, GGC CCT TCT CCA GGA CAG A-3′ (sense) and 5′-GCT GAT CAT GGC TGG GTT GT-3′ (anti-sense); for mouse β-actin, 5′-GAA ATC GTG CGT GAC ATC AAA G-3′ (sense) and 5′-TGT AGT TTC ATG GAT GCC ACA G-3′ (anti-sense); for human RANKL, 5′-ACC AGC ATC AAA ATC CCA AG-3′ (sense) and 5′-CCC CAA AGT ATG