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Regulatory immune responses induced by IL-1 receptor antagonist in rheumatoid arthritis

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ABSTRACT

Anakinra, a human recombinant IL-1 receptor antagonist, is approved for the treatment of RA. In this study, 12 patients received the placebo plus MTX treatment, 38 patients received Anakinra combined with MTX treatment. Compared with the placebo plus MTX group, serum levels of IL-17, IFN-γ, IL-21 and IL-1β significantly decreased, the percentages of Th17 cells and Th1 cells were lower and the percentage of Treg cells was higher after receiving Anakinra combined with MTX treatment. The observed regulatory immune responses collectively correlated with clinical improvement in treated patients. A substantial response, ACR 20 at 24 w were consistent with those at 12 w, 16 w and 20 w, and was accompanied by a marked improvement in RA related laboratory parameters. The study reveals that the combination of Anakinra and MTX is safe and well tolerated, which induces regulatory immune responses and significantly provides greater clinical benefit than the placebo plus MTX group.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder of unknown etiology characterized by symmetric, erosive and disabling polyarthritis and a wide array of extraarticular complications (Scott et al., 2010). The inflammatory process leads to cartilage and bone destruction. Long-term studies have demonstrated that significant disability occurs in 50–70% of patients after 10–15 years of disease (Schuna, 1998). Although the etiology and pathogenesis of RA remain unknown, it is generally considered an autoimmune pathology in which autoreactive T cells of pathogenic potential, such as Th1 and Th17 cells, are thought to play an important role (Park et al., 2005; Chen et al., 2007; Afzali et al., 2007; Gaston, 2008). There is evidence that these T cells are activated during the disease process and accumulate in the inflamed synovium, leading to perpetuation of the joint inflammation and tissue destruction (Gaston, 2008; Sato, 2008; Lubberts, 2008; Singh et al., 2007). On the other hand, regulatory T cells (Treg) are deficient in patients with RA, further unbalancing the immune system toward a pro-inflammatory state (Gaston, 2008; Sato, 2008; Lubberts, 2008; Singh et al., 2007; Boissier et al., 2008). Importantly, the cytokine milieu built progressively in inflamed synovium over the course of RA is particularly critical to maintain inflammatory process in the joint. The cytokine milieu carries the inflammatory signature of RA and is crucial in the differentiation and maintenance of pathogenic T cells, and in the dysfunction of regulatory T cells (Boissier et al., 2008; Lipsky et al., 2000; Wang et al., 2008).

IL-1 is produced by a variety of cells that are part of the innate immune system. There is increasing evidence that constant activation of the innate immune system occurs in several chronic inflammatory processes, including RA (Schiff, 2000). IL-1 has effects on cartilage degradation leading to damage as well as inhibiting repair and is a potent stimulus to osteoclasts, leading to bone erosion. The IL-1β-NF-κB axis is a key pathway in the pathogenesis of RA and is central in the production of proinflammatory mediators in the inflamed synovium. NF-κB activation in fibroblast-like synoviocytes contributes to the pathogenesis of RA by activating the transcription of a family of MMPs (Vincenti et al., 1998; Lee et al., 2009). These MMPs are major products of cytokine-stimulated FLS and efficiently degrade the collagenous components of cartilage and bone, which leads to joint deformity and a great deal of pain in RA patients. Thus, reducing the synthesis of IL-1 or blocking the effects of an overabundance of IL-1 may offer a

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therapeutic option for RA. IL-1 receptor antagonist (IL-1ra) is an endogenous blocker of the cytokine (Kay and Calabrese, 2004). Evidence supporting an anti-inflammatory role of IL-1ra in vivo is demonstrated by the observation that IL-1ra-deficient mice spontaneously develop autoimmune diseases similar to RA as well as vasculitis (Nicklin et al., 2000; Horai et al., 2000, 2004). Anakinra, a human recombinant IL-1ra, is approved for the treatment of RA. Anakinra differs from native IL-1ra by the addition of an N-terminal methionine. Anakinra blocks the biologic activity of IL-1 by binding to IL-1R type I with the same affinity as IL-1β. A randomized double-blind placebo controlled trial showed long-term safety and maintenance of clinical improvement following treatment with Anakinra in patients with RA (Nuki et al., 2002; Chen, 2010).

Increased levels of Th17 cells can be detected in IL-1Ra−/− mice even preceding the onset of arthritis. In addition, the results of cytokine-blocking studies demonstrated that IL-17 contributes to the inflammation and bone erosion in this model (Koenders et al., 2008). Gabay et al. suggested that myeloid cell-derived IL-1Ra plays a critical role in the control of the development and the severity of CIA by modulating Th1 and Th17 responses in lymphoid organs (Lamacchia et al., 2010). Some studies have found that proinflammatory cytokines, such as IL-1beta and IL-6, were all essential for human Th17 differentiation (Chung et al., 2009; Volpe et al., 2008; Acostoa-Rodriguez et al., 2007). But the developmental regulation of Anakinra on T cell subsets balance on RA patient is unclear.

Methotrexate (MTX) is the most commonly used conventional disease-modifying antirheumatic drug. Combination regimens that employ MTX plus other biological agents, such as etanercept or infliximab have been used successfully to combat disease that remains active or progressive despite treatment with MTX alone. In order to further support the use of combination therapy with MTX and a biological agent, we observed the immunological changes and evaluated the efficacy of Anakinra in combination with methorexate in patients with active rheumatoid arthritis. In this study, we evaluated the regulatory effect of IL-1 receptor Antagonist on restoring the Treg/Th17, Th1 balance. The finding described here has important implication in the understanding of the role of IL-1 receptor Antagonist in treatment of RA and suggests IL-1 as a good target for treating this autoimmune disease.

2. Materials and methods

2.1. Patients

Total of 50 RA patients with rheumatoid arthritis according to the criteria of the American College of Rheumatology were included in this study. The study protocol was approved by the Institutional Medical Ethics Review Board of GuangHua Rheumatology Hospital. Informed consent was obtained from all participating patients before sample collection. All the patients had active disease (defined as the presence of 4 swollen joints, ≥6 tender joints and at least 2 of the following: morning stiffness of ≥45 min in duration, or reythrocyte sedimentation rate (ESR) ≥28 mm/h, or a plasma C-reactive protein (CRP) concentration ≥2.0 mg/dl). All the patients were receiving methotrexate (MTX) with stable dose of 7.5–15 mg per week, and had taken no other disease-modifying antirheumatic drugs (DMARDs) within 4 w. In this study, in the MTX group, 12 patients received a stable weekly dose of MTX (7.5–15 mg/wk orally), and placebo was administered as subcutaneous injection per day. And in the combined-therapy group, 38 patients received Anakinra plus MTX treatment, and Anakinra was given as subcutaneous injection of 80 mg per day with stable dose of MTX (7.5–15 mg/w) orally. The course of treatment in both groups lasted for 24 w.

2.2. Analysis of cytokine production

Serum was obtained after centrifugation and stored at −80 °C for the measurement of cytokines. Serum cytokines were measured quantitatively by IL-17 ELISA (R&D Systems, Minneapolis, MN), IFN-γ ELISA (R&D Systems, Minneapolis, MN), IL-21 ELISA (eBioscience, San Jose, CA) and IL-1β ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Briefly, 96-well microtiter plates were pre-coated overnight at 4 °C with 100 μl/well of capture antibody in coating buffer. Wells were then blocked at 37 °C for 1 h and washed five times with cold washing solution. Each sample was added and incubated for 2 h with a biotinylated detecting antibody. Plates were washed and incubated for 30 min with streptavidin-conjugated horseradish peroxidase prior to color development. The sensitivity for IL-17 was 15 pg/ml, for IFN-γ was 8 pg/ml, for IL-21 was 31 pg/ml and for IL-1β was 0.057 pg/ml.

2.3. Cell perparation

Blood samples were taken into collection tubes containing sodium heparin. Peripheral blood mononuclear cells (PBMC) were separated by Ficoll-Hypaque centrifugation (Amersham Biosciences, Piscataway, NJ) from RA patients.

2.4. Surface and intracellular staining

For intracellular cytokine staining, PBMC were stimulated with phorbol 12-myristate 13-acetate (PMA) (50 ng/ml; Sigma, Saint Louis, MO), ionomycin (500 ng/ml; Sigma, Saint Louis, MO) for 5 h. After incubation with a protein transport inhibitor (BD PharMingen, San Diego, CA) to prevent cytokine secretion, cells were stained with anti-CD4-Percp-Cy5.5 (BD PharMingen, San Diego, CA). After washing, intracellular IL-17 and IFN-γ were stained with anti-IL-17-PE (eBioscience, San Diego, CA) and anti-IFN-γ-APC (BD PharMingen, San Diego, CA). To analyze Treg, anti-CD4-Percp-Cy5.5 (BD PharMingen, San Diego, CA) and anti-CD25-PE (Miltenyi Biotec, Sunnyvale, CA) were used for surface staining. Cytofix/Cytoperm buffer set (eBioscience, San Diego, CA) was used for intracellular staining. Cells were fixed and made permeable for 60 min at 4 °C and were stained with anti-Foxp3-FITC (eBioscience, San Diego, CA) for 60 min at 4 °C in permeabilization buffer. A FACSCalibur (BD Biosciences, San Jose, CA) and FlowJo software (Tree Star, San Carlos, CA) were used for flow cytometry.

2.5. Real-time quantitative RT-PCR

Total RNA was isolated from PBMC pellets using the RNeasy Mini Kit (Qiagen, Chartsworth, CA). Genomic DNA was then removed from total RNA using RNase-free DNase Set (Qiagen, Chartsworth, CA). First strand cDNA was subsequently synthesized using Sensiscript RT Kit and random hexamers (Qiagen, Chartsworth, CA) according to the manufacturer's instructions. Primer Express software (Applied Biosystems, Foster city, CA) was used to design primers from published cDNA sequences. BLAST searches were conducted on the primer nucleotide sequences to ensure gene specificity. The primer sequence genes were as follows: GAPDH, sense 5′-GTGAAGGTCGGAGTCAACG-3′, antisense 5′-TGAGGTCAATGAAGGGGTC-3′; RORC, sense 5′-CGGGCCTACAATGCTGACA-3′, antisense 5′-GCCACCGTATTTGCCTTCAA-3′; T-bet, sense 5′-GCCTACCAGAATGCCGAGATTA-3′, antisense 5′-TCAAAGTTCTCCCGGAATCCT-3′; Foxp3, sense 5′-CGGACCATCTTCTGGATGAG-3′, antisense 5′-TTGTCGGATGATGCCACAG-3′. The mRNA expression was determined by real-time PCR using SYBR Green Master Mix (Applied Biosystems, Foster city, CA). Thermocycler conditions comprised of an initial holding at 50 °C for 2 min and