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International Immunopharmacology 7 (2007) 1360–1368

International Immunopharmacology

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T cells are involved in the development of arthritis induced by anti-type II collagen antibody

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Received 21 March 2007; received in revised form 30 May 2007; accepted 31 May 2007

Abstract

T cells play an important role in initiating autoimmune responses and maintaining synovial inflammation in rheumatoid arthritis. Although, anti-type II collagen antibody-induced arthritis (CAIA) is generally believed to be a T cell- and B cell-independent model, the detailed pathogenesis of CAIA remains unclear. In the present study, to elucidate the contribution of T cells to the pathogenesis of CAIA, we evaluated the effects of CTLA4 Ig and cyclosporin (CsA). Arthritis was induced in mice by intravenous injection of anti-type II collagen antibody followed by intraperitoneal injection of lipopolysaccharide. CTLA4 Ig was intraperitoneally administered and CsA was subcutaneously administered; then the severity of arthritis was evaluated by scoring the edema and erythema of paws and by measuring hind paw thickness. Paw samples were collected 12 days after the antibody injection, and the mRNA expression levels were analyzed by real-time quantitative polymerase chain reaction. Administration of CTLA4 Ig ameliorated the increases in arthritic score and paw thickness in the later phase, but not in the early phase of arthritis. CsA suppressed the increases in arthritic score and paw thickness in both the early and later phases of arthritis. CTLA4 Ig and CsA suppressed mRNA up-regulation of T-cell markers, CD3 and CD25, and immune response-related mediators, IFN-γ and IL-12. They also suppressed the up-regulation of macrophage marker, F4/80, and proinflammatory cytokines, TNF-α, IL-1β and IL-6. The results provide direct evidence that arthritis in this model is T-cell activation dependent.

Keywords: Anti-type II collagen antibody; CTLA4; Cyclosporin; Rheumatoid arthritis

1. Introduction

Rheumatoid arthritis (RA) is a common chronic autoimmune and inflammatory disease characterized by the destruction of the synovial joints, leading to progressive disability with loss of joint function. RA has a prevalence of 1% worldwide and results in substantial morbidity and premature mortality [1–3].

Collagen-induced arthritis (CIA) is a widely used animal model for human RA. CIA is induced by immunization with type II collagen in adjuvant, which causes the rapid onset of acute arthritis with severe paw swelling and erythema. Although the exact pathogenic sequence of events remains unknown, it is generally believed that after T-cell activation, both cell-mediated and humoral immunity to collagen are involved in triggering an inflammatory cascade with the production of proinflammatory cytokines and growth factors, which

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doi:10.1016/j.intimp.2007.05.021

contribute to perpetuating the disease process. A variant of CIA, anti-type II collagen antibody-induced arthritis (CAIA), is evoked by administering a combination of monoclonal antibodies to type II collagen together with LPS [4,5]. Compared with the classical CIA model, the CAIA model provides advantages including: rapid induction within a week, MHC haplotype independence, and high reproducibility [5]. The CAIA model is used to screen compounds for anti-arthritic activity and to study the mechanisms involved in the development of arthritis. Although the development of CAIA is different from that of CIA because it skips the induction phase of CIA in which T-cell activation and antibody production by B cells occur, the resulting clinical arthritis shares many common characteristics.

T cells play an important role in initiating autoimmune responses and maintaining synovial inflammation by activating synovial macrophages and fibroblasts to produce inflammatory mediators. The critical role of T cells in the pathogenesis of RA has led to the development of T-cell-directed suppressive therapies. The importance of T-cell activation as a starter and motor for RA is reviewed by Skapenko et al. [6]. T-cell activation requires two distinct signals. The first signal is an antigen-specific interaction between the T-cell receptor and nominal antigen presented on the surface of an antigen-presenting cell. The second signal is provided through co-stimulatory molecules, of which CD28 is considered to be the most important. CD28, which is constitutively expressed on the T-cell surface, binds to both CD80 (B7-1) and CD86 (B7-2), which are present on antigen-presenting cells. The CD28–B7 interaction results in enhanced T-cell proliferation, cytokine production, and resistance to apoptosis [7]. Several lines of evidence have demonstrated that the CD28–B7 interactions play an important role in some immune diseases, including multiple sclerosis [8], inflammatory bowel disease [9], and RA [10]. Cytotoxic T lymphocyte-associated antigen-4 (CTLA4), a second receptor for B7, is expressed on the surface of activated T cells and is also important in immune regulation. CTLA4 competes with CD28 to bind B7 and functions as a counter-regulatory receptor that attenuates T-cell responses by down-regulating T-cell activation [11–13], facilitating antigen-specific apoptosis [14], and suppressing secretion of both Th1 and Th2 cytokines [11,13,15]. In RA patients, CTLA4 Ig (CTLA4 IgG_1, abatacept) demonstrated favorable therapeutic effects [16–18].

Cyclosporin (CsA) is a potent inhibitor of T-cell activation, with documented efficacy in the treatment of severe RA, both as monotherapy and as combination therapy in conjunction with methotrexate [19,20]. CsA interferes with T-cell activation by inhibiting the intracellular phosphatase calcineurin in complex with an intracytoplasmic protein, cyclophilin A [19]. Inhibition of calcineurin causes a blockade of T cell-derived cytokines such as TNF-α, IL-2 and IFN-γ [21].

T-cell proliferation and activation are considered critical in the development of CIA, and the administration of CTLA4 Ig and CsA suppress the severity of arthritis [22,23]. However, the role of T cells in the development of CAIA is not well understood. In the present study, to elucidate the contribution of T cells to the pathogenesis of CAIA, we evaluated the effects of CTLA4 Ig and CsA on the arthritis induced by anti-type II collagen antibody. We found that both CTLA4 Ig and CsA had significant anti-arthritic effects, which indicates that T cells are involved in the development of CAIA.

2. Materials and methods

2.1. Animals

Six-week old, female BALB/c mice (Charles River Laboratories Japan Inc., Japan) were used. Each group consisted of 5 animals for observation of clinical arthritis markers and analysis of gene expression. Animals were housed in plastic cages (5 mice per cage) with wood tip bedding and maintained in a temperature- and humidity-controlled room (23±2 °C and 55±10%, respectively) with a fixed 12-hour light/dark cycle (7:00 a.m. to 7:00 *p*.m.). Animals were housed under controlled environmental conditions with free access to standard laboratory food and water.

2.2. Induction of arthritis

Arthritis was induced using the methods of Terato et al. [5]. On day 0, mice were intravenously injected with 2 mg of an arthritogenic monoclonal antibody cocktail (Chondrex, LLC, Seattle, USA). On day 3, mice were intraperitoneally injected with 25 μg of lipopolysaccharide (LPS).

2.3. Experimental design

Murine CTLA4 Ig (0.1 mg) or control Ig (an isotype-matched non-specific antibody, 0.1 mg) was intraperitoneally administered every other day. The CTLA4 Ig protein consisted of the extracellular domain of murine CTLA4 fused to the Fc region of murine IgG2a. Cyclosporin (Sandimmune, Novartis Pharma AG, Basel, Switzerland, 25 mg/kg) or vehicle (saline) was subcutaneously administered twice a day. Mice in the intact group were not treated with either antibody/LPS or compounds. On day 12, mice were anesthetized by intraperitoneal administration of a mixture of ketamine (50 mg/kg, Sankyo, Japan) and xylazine (10 mg/kg, Bayer, Japan). Anesthetized mice were bled from the abdominal artery, and