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Immunology Letters 117 (2008) 16–25
www.elsevier.com/locate/

Oral administration of type-II collagen suppresses IL-17-associated RANKL expression of CD4+ T cells in collagen-induced arthritis

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Received 11 January 2007; received in revised form 10 September 2007; accepted 17 September 2007
Available online 22 October 2007

Abstract

The receptor activator of nuclear factor κB ligand (RANKL) is an osteoclastogenic mediator, which is mainly expressed by stromal cells and osteoblast. However, T cells can also be an important provider for RANKL in special condition such as autoimmune arthritis. We examined the RANKL expression of hyporesponsive CD4+ T cells induced by oral feeding with type II collagen in collagen-induced arthritis (CIA) mice. The potential of RANKL expression in CD4+ T cells was downregulated in tolerance, as compared with CIA. One of possible explanations for this phenomenon is that CII-specific T cell activation was intrinsically impaired in oral tolerance, which caused suppression of RANKL expression of CD4+ T cells. We also investigated the extrinsic role of cytokine in this process. IL-17, well-known pro-inflammatory cytokine was upregulated in CIA and downregulated in tolerance. IL-17 had a potential to stimulate T cells to express RANKL in dose-dependent manner. IL-17-associated RANKL expression of CD4+ T cells was downregulated in oral tolerance, suggesting that the induction of tolerance ameliorates IL-17-induced RANKL expression of T cells in murine CIA. We also discovered that CIA – T cells could enhance osteoclastogenesis but not oral tolerance – T cells. Oral tolerance might be promising therapeutic option in viewpoints of modulating autoreactivity of CII which can induce not only IL-17 production but also RANKL expression in CD4+ T cells.

Keywords: Collagen-induced arthritis; Oral tolerance; RANKL; IL-17; CD4+ T cells

1. Introduction

Oral tolerance is a state in which there is an absence of immune responsiveness to protein antigens that have been administered repeatedly by oral feeding [1,2]. Induction of oral tolerance is considered a promising approach to the treatment of chronic autoimmune diseases, including rheumatoid arthritis (RA) [3,4]. We previously showed that tolerization of mice with type II collagen (CII) reduces joint inflammation, increases serum concentrations of IgG1, and decreases serum concentrations of IgG2a, suggesting that tolerized mice have an anti-inflammatory status superior to that of non-tolerized mice [5]. The T cell proliferative response to CII was suppressed in tolerized animals, and production of IL-10 and TGF-β by mononuclear lymphocytes was elevated. We also observed greater production of IL-10-producing immune-

Abbreviations: RA, rheumatoid arthritis; CIA, collagen-induced arthritis; CII, type II collagen; RANKL, Receptor activator of nuclear factor kappa B ligand; CFA, complete Freund's adjuvant; TCR, T cell receptor; OPG, osteoprotegerin.

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0165-2478/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.imlet.2007.09.011

regulatory CD4+ and CD25+ T cells in CII-stimulated splenic T cells from tolerized mice. This subset of T cells is induced by CD11c+ and CD11b+ dendritic cells, which are abundant in Peyer's patches during induction of oral tolerance to CII [6]. Our studies on tolerance showed that Peyer's patches contain CD11c+ and CD11b+, which have tolerogenic properties and play an important role in the induction of oral tolerance by inducing production of regulatory T cells [6].

T cells not only enhance immune regulation in tolerized animals, but also are directly involved in synovial inflammation, cartilage loss, and bone destruction [5,6]. T cells have been reported to enhance osteoclastogenesis through expression of receptor activator of nuclear factor kappa B ligand (RANKL) in response to cytokines, self-antigens, and other immune cells [7–9]. IL-17, a major product of T cells plays important roles in every aspect of synovial inflammation [10]. In addition to its role as an upstream mediator of RA pathogenesis, IL-17 is a potent stimulator of osteoclastogenesis through induction of the crucial mediator, RANKL. Cytokines such as IL-23 and IL-15 stimulate T cells to secrete IL-17, which stimulates osteoblasts and synoviocytes to produce RANKL, initiating RANKL-associated osteoclastogenesis and bone destruction [11,12]. Although the relationship between IL-17 and RANKL expression of stromal cells such as synoviocytes and endothelial cells has been elucidated, studies on the autocrine effects of IL-17 on RANKL expression in T cells are rare.

In this study, we investigated the effect of tolerance on T cell expression of IL-17 and IL-10 and on the production of RANKL. The direct effect of IL-17 on RANKL expression of T cells was also investigated. We observed that IL-17 expression was upregulated and IL-10 expression was downregulated in mice with collagen-induced arthritis (CIA), but IL-17 expression was downregulated and IL-10 expression was upregulated in tolerized mice. IL-17 stimulated dose-dependent RANKL expression in T cells. The expression of RANKL by CD4+ T cells was downregulated in tolerized mice compared with CIA mice, and tolerance more specifically suppressed the expression of RANKL when T cells were stimulated with CII. IL-17 and antigen-associated RANKL expression of T cells was downregulated in oral tolerance, suggesting that tolerance ameliorates IL-17 and reactive antigen-induced RANKL expression of T cells in murine CIA.

2. Materials and methods

2.1. Mice

Male DBA/1J mice were purchased from SLC, Inc. (Shizoka, Japan). All mice were used at 6–8 weeks of age and all procedures were approved by the Animal Investigation Committee of Wayne State University.

2.2. Collagens

Bovine CII was kindly provided by Prof. Andrew Kang of the University of Tennessee. The bovine CII was free of other collagen types, proteoglycan, and pepsin.

2.3. Induction of CIA and clinical assessment of arthritis

Type II collagen was dissolved in 0.1N acetic acid (4 mg/ml) by gentle rotation at 4 °C overnight. Mice ($n = 20$) were injected with 100 μg of CII emulsified incomplete Freund's adjuvant (CFA; Arthrogen-CIA, Redmond, WA) (1:1 w/v). Two weeks later, they were boosted i.d. with 200 μg of CII in incomplete Freund's adjuvant (DIFCO, Detroit, Michigan) (1:1 v/v). We used normal mice and mice with adjuvant-induced arthritis as controls. Arthritic indexes [13] were evaluated three times weekly by three independent investigators until 12 weeks after the first immunization. The scale of the arthritis index ranged from 0 to 4. Scores were defined as follows: 0, no evidence of erythema or swelling; 1, erythema and mild swelling confined to the mid-foot (tarsal part) or ankle joint; 2, erythema and moderate swelling extending from the ankle to the mid-foot; 3, erythema and moderate swelling extending from the ankle to the metatarsal joints; 4, erythema and severe swelling encompassing the ankle, foot, and digits.

2.4. Determination of collagen-specific T cell proliferative response

Spleens were removed from mice and washed twice with PBS. Tissues were minced and the cells were filtered through a cell strainer and centrifuged at 1500 rpm at 4 °C for 5 min. The cell pellet was resuspended in RPMI-1640 medium at a concentration of 1×10^5 cells/ml. Cells were then plated in 96-well microtiter plates at a concentration of 2×10^5 cells/well and cultured with 40 μg/well CII for 3 days. Eighteen hours before termination of culture, 1 μCi [^{3}H]thymidine (NEN, Boston, MA) was added to each well. Eighteen hours later, cells were transferred to nitrocellulose filter membranes (Skatron, Lier, Norway), and each membrane was dissolved in Ultima scintillation solution (Packard, Downers Grove, IL). Incorporated radioactivity was counted in a beta counter (Packard Instrument Co., Downers Grove, IL). Data were expressed as stimulation indexes (SIs; the ratio of counts of CII-stimulated cells to counts of unstimulated cells).

2.5. Anti-CII immunoglobulin quantification

Mouse serum samples were collected on the fifth week after the first immunization. Microtiter plates were coated with 4 μg/ml of native CII in 0.05 M sodium carbonate coating buffer (pH 9.6) at 4 °C overnight, followed by 30 min blocking by incubation at room temperature with 1% BSA in TBS (pH 8.0). Serum samples were diluted 1:50,000 in TBS (pH 8.0) containing 1% BSA and 0.5% Tween-20 for anti-CII IgG2a. We used intrinsic serum samples to obtain anti-CII IgG1 antibodies. After 1 h of incubation, microtiter plates were washed five times with TBS (pH 8.0) containing 0.05% Tween-20. Then, each detection antibody/HRP conjugate was diluted 1:50,000 for IgG2a or 1:100,000 for IgG1 and incubated for 1 h at room temperature. The TMB + H_2O_2 system (KPL, Gaithersburg, MD) was used to visualize the reaction. The reaction was stopped by addition of 1N H_2SO_4. Optical density at 450 nm was mea-