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Blockade of IL-7Rα alleviates collagen-induced arthritis via inhibiting Th1 cell differentiation and CD4+ T cell migration

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ABSTRACT

T cell response is crucial to the pathogenesis and progression of rheumatoid arthritis (RA). IL-7/IL-7R axis has significant effect on CD4+ T cell response, including proliferation, differentiation, survival and migration. However, whether blockade of IL-7/IL-7R axis signaling can relieve RA and what is the potential treatment mechanisms are still remaining unclear. In this paper, we established collagen-induced arthritis (CIA) model and observed the effect of IL-7Rα antibody in the treatment of CIA mice. It is demonstrated that IL-7Rα antibody significantly alleviated clinical symptoms of CIA mice, accompanied with reduced CD4+ T cell number in both spleen and joints. Decreased CII-specific CD4+ T cell proliferation and reduced mRNA expression of inflammatory cytokines in IL-7Rα antibody-treated mice were observed. Subsequently, IL-7Rα antibody treatment in vivo downregulated the percentages of Th1 and Th17 cells and the mRNA expression of T-bet and RORγt gene. Moreover, it was found that IL-7 promoted Th1 cell differentiation in vitro, while having no effect on Th17 cell differentiation. In addition, administration of IL-7Rα antibody reduced the mRNA expression of chemokine receptors (CCR7, CXCR3, CXCR6 and XCR1) on CD4+ T cells and chemokine CXCL2 in joints. The results suggested that IL-7Rα antibody treated CIA mice via the inhibition of CII-specific CD4+ T cell proliferation, the reduction of Th1 cell differentiation and the restrain of CD4+ T cell migration to joint lesion site. This investigation indicates that IL-7Rα is a potential therapeutic target for RA.

1. Introduction

Rheumatoid arthritis (RA) is a systemic chronic autoimmune disease manifested by persistent joint inflammation, leading to progressive cartilage and bone destruction (Smolen et al., 2016). Traditional therapy strategies consist of non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease-modifying anti-rheumatic drugs (DMARDs) and Chinese medicine (O'Shea et al., 2013). However, most drugs not only show poor therapeutic effects but also have side effects (Rainsford, 1999). In the last decades, therapies aimed at pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 are emerging as a major treatment of RA (Calabro et al., 2016), while not all patients respond positively to it (Davignon et al., 2013). Data showed that circulating IL-7 level was significantly reduced in patients who successfully responded to anti-TNF-α, but persisting in non-responders (Pesenacker and Wedderburn, 2013), which indicated that IL-7 might be a novel target anti-inflammatory therapy for RA.

As a member of the IL-2 family (including IL-2, IL-4, IL-9, IL-15 and IL-21), IL-7 is secreted by stromal cells, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells and keratinocytes (Churchman and Ponchel, 2008). IL-7 leads its effect via combining with IL-7Rα chain (CD127) and transferring signal by the common γ chain. It has been reported that IL-7 level was higher in synovial fluid of RA patients than OA (van Roon et al., 2005), and increased IL-7Rα was expressed on CD4+ T cells and CD68+ macrophages in both synovial fluid and synovial tissue of RA patients (Hartgring et al., 2009), indicating that IL-7Rα involves in RA.

IL-7/IL-7Rα signal has been implicated in many autoimmune diseases such as multiple sclerosis (Kreft et al., 2012), Sjogren Syndrome (Jin et al., 2013), inflammation bowl disease (Ji et al., 2015), Ankylosing Spondylitis (Gracey et al., 2016) and RA (Pathak, 2014). Many studies have demonstrated the contribution of IL-7/IL-7R signal on B cells, CD4+ T cells and macrophages/monocytes in the pathogenesis of RA (Bikker et al., 2012a, b). Based on the

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upregulation of IL-7/IL-7R expression in RA patients, the IL-7-driven T cell-dependent autoimmunity, the ability of IL-7-induced inflammatory cytokines secreted by macrophages/monocytes and its tissue destruction afterwards, blockade of IL-7/IL-7R signal may be an effective way to cure RA. To this end, Chen et al. first revealed that therapeutic treatment of IL-7 antibody ameliorated CIA pathology and bone erosion (Chen et al., 2013). As another soluble form of IL-7R (sIL-7R) is produced by synovial fibroblasts and $CD4^+$ T cells, which is higher in RA patients and strongly associated with poor response to TNF-blockade (Badot et al., 2011), the IL-7Rα antibody treatment may be more effective than IL-7 antibody for its blockade of both IL-7R and sIL-7R. Until now, only Sarita A.Y. Hargring reported that prophylactic and therapeutic administration of IL-7Rα antibody decreased arthritis severity, but the therapeutic mechanism is still unclear (Hartgring et al., 2010).

It is known that IL-7 is responsible for T cell development and homeostatic expansion. To better understand the disease of RA, it is important to note the significant role T cells played in its aetiology and pathogenesis. Several widely used animal models for arthritis, such as collagen-induced arthritis or adjuvant arthritis in rodents, are T-cell-dependent, providing a link between pre-clinical and clinical investigation (Firestein, 2003). Either IL-7 deficient or IL-7Rα deficient mice manifested T cell number reduction and weakened proliferation (Giliani et al., 2005). At the same time, IL-7 can prevent the naïve and unstimulated T cells from dying as the homeostatic regulator (Koenen et al., 2013). In RA patients, IL-7 can stimulate Th1 cytokine production in joints, which are drivers of auto-reactive response (van Roon et al., 2003). It can also drive Th1 and Th17 cytokine production in patients with primary SS (Bikker et al., 2012a, b). In EAE model, IL-7 enhanced the differentiation and expansion of Th1 cells and induced the plasticity of Th17 cells by converting them into IFN-γ–producing Th17 cells (Arbelaez et al., 2015). As a biomarker for inflammatory conditions, IL-7 also induced recruitment of monocytes/macrophages to endothelium (Li et al., 2012). Taken together, these implicated the important role of IL-7 in $CD4^+$ T cell proliferation, apoptosis, differentiation and migration in RA. Whether blockade of the IL-7/IL-7R axis would reverse the symptoms of RA is still unknown.

Considering the possible treatment of IL-7Rα antibody in RA and the central role of $CD4^+$ T cell responses in RA pathogenesis, we investigated whether blockade of IL-7Rα had a therapeutic effect on CIA model. Additionally, we further discussed the potential treatment mechanism of this antibody, especially on $CD4^+$ T cell responses, including proliferation, apoptosis, differentiation and migration.

2. Materials and methods

2.1. Mice

DBA/1J mice (eight to ten-week-old, male) were obtained from the Shanghai Laboratory Animal Center, Chinese Academy of Science, Shanghai., and kept under specific pathogen-free conditions at the animal core facility of Shanghai Jiao Tong University School of Medicine. All experiments were conducted according to the Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine.

2.2. Induction, treatment and assessment of CIA

Bovine type II collagen (CII, Chondrex, USA) was dissolved at a concentration of 3 mg/ml in 0.05 M acetic acid and emulsified in equal-volume Freund's complete adjuvant (CFA, Sigma, USA). DBA/1J mice were injected intradermally at the base of the tail with 100 μl emulsion (150 μg CII). On day 21, a booster injection with 50 μl emulsion (75 μg CII) dissolved in equal-volume Freund's incomplete adjuvant (IFA, Sigma, USA) were given. The clinical severity of arthritis was graded on a scale of 1–4 per joint as follows: 0, normal; 1, slight swelling and/or erythema; 2, substantial edematous swelling; 3, substantial edematous swelling plus light joint rigidity; 4, laxity, with a maximum clinical score of 16 per mouse (Mauri et al., 2000). CIA mice were divided into two groups randomly at day 27 (mean clinical score of about 2) and given intraperitoneal injections of 100 μg IL-7Rα antibody (anti-mouse CD127 functional grade purified, eBioscience, USA) or isotype control (rat IgG2a K isotype control functional grade purified, eBioscience, USA) every three days. Clinical scores were evaluated every day after day 21.

2.3. Histopathology and immunohistochemistry

CIA mice were sacrificed and knee joints were fixed with 4% buffered paraformaldehyde and decalcified with 10% ethylene diamine tetraacetic acid (EDTA). Samples were then embedded in paraffin, and sectioned into 5–10 μm slices, and stained with hematoxylin and eosin (H&E), slaframine-fast green staining, immunohistochemistry of CD4 marker and examined by light microscope.

2.4. Micro-CT

The CT images of mice hind legs were acquired on day 37 after 24-h fixation, using a Micro-CT scanner (SkyScan1176, SkyScan, Belgium) at a resolution of 9 μm.

2.5. Cell isolation and tissue preparation

On day 37, spleens and hind paws were collected after the mice were sacrificed. Splenocytes were isolated from spleens through cell strainer. Paws were homogenated in Trizol (Invitrogen, USA) for RNA extraction.

2.6. T cell proliferation assay and cytokine measurement

Splenocytes (0.5×10^6 per well) from mice treated with IL-7Rα antibody and isotype control were cultured in triplicate in complete RPMI-1640 in the presence of CII peptide (40 μg/ml) or pre-coated anti-CD3 (1 μg/ml, BD, USA) and soluble anti-CD28 (1 μg/ml, eBioscience, USA) for up to 72 h. For T cell and APC co-culture assay, MACS-purified $CD4^+$ T cells (Miltenyi Biotec, Germany) were co-cultured with purified naïve $CD11b^+$ cells (Miltenyi Biotec, Germany) at a ratio of 3:1 and were subsequently stimulated with CII peptide (40 μg/ml) for 72 h. $CD11b^+$ APCs purified from both groups were co-cultured with the same CII reactive $CD4^+$ T cells at a ratio of 1:3 in the presence of CII peptide (40 μg/ml) for 72 h. All cultures were maintained at 37 °C in a 5% CO_2. To determine cell proliferation, cells were pulsed with 0.5 μCi [^{3}H]-thymidine (Shanghai Institute of Atomic Nucleus, Chinese Academy of Sciences, China) during the last 18 h before harvest. [^{3}H]-thymidine incorporation was measured as count pulse per minute (cpm) by Microplate counter (PerkinElmer, USA). Culture supernatants of splenocytes and co-culture cells were collected 48 h later and diluted for measurement of IFN-γ, IL-17, TNF-α, IL-6, IL-1β and IL-4 (R&D, USA).

2.7. Th cell differentiation

$CD4^+$ T cells were purified with MACS from naïve DBA/1J mice spleen and incubated with IL-7 at different concentrations, ranging from 0.1 ng/ml to 10 ng/ml overnight. The cells were then washed twice and added Th1 (anti-CD3 1 μg/ml, anti-CD28