Immunology Letters 163 (2015) 143–150

Contents lists available at ScienceDirect

Immunology Letters

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

Treatment of IL-21R-Fc control autoimmune arthritis *via* suppression of STAT3 signal pathway mediated regulation of the Th17/Treg balance and plasma B cells

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ARTICLE INFO

Article history:
Received 21 November 2013
Received in revised form 19 August 2014
Accepted 10 September 2014
Available online 20 November 2014

Keywords:
Autoimmune arthritis
IL-21R-Fc
STAT3
Th17
Plasma cell

ABSTRACT

Interleukin-21 (IL-21) is a T cell-derived cytokine modulating T cell, B cell, and natural killer cell responses. To determine whether IL-21 contributes to pathologic processes, recombinant IL-21 receptor (R) fusion protein (rhIL-21R-Fc) was examined in mice models of autoimmune arthritis (collagen-induced arthritis). DBA/1J mice were immunized with chicken type II collagen and then treated intraperitoneally with rhIL-21R-Fc, which was initiated after the onset of arthritis symptoms in 20% of the cohort. The mice were assessed 3 times per week for signs of arthritis and histologic features as well as serum immunoglobulin. Cytokine messenger RNA levels in the spleen were also examined. STAT3 phosphorylation is dose dependently activated by IL-21 and inhibited by rhIL-21R-Fc *in vitro* using T cells. Treatment of DBA/1J mice with rhIL-21R-Fc reduced the clinical and histologic signs of CIA. The IL-17 and STAT3-expressing $CD4^+$ splenocytes dramatically decreased in the rhIL-21R-Fc treated mice. IL-21R-Fc treated mice also decreased the production of IgG, STAT3 phosphorylation, and plasma cell transcription factor (Blimp1). These findings demonstrate a pathogenic role of IL-21 in animal models of RA, suggesting IL-21 as a promising therapeutic target among human RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by chronic joint inflammation that can culminate in cartilage damage and bone erosion. Though the exact molecular mechanism of RA pathogenesis is yet to be determined, it has become clear that interleukin (IL)-17 producing helper T cell (Th17) plays a central role [1,2].

Signal transducer and activator of transcription (STAT) 3, an essential transcription factor (TF) in Th17 differentiation, is activated by proinflammatory cytokines including IL-6, IL-17, and IL-21 [3,4]. IL-21 belongs to the type I cytokine family, sharing a 4-helix-bundle-type fold structure with IL-2, IL-4, and IL-15 and mainly produced by activated $CD4^+$ T cells. IL-21 is known to affect various kinds of immune cells such as T, B lymphocytes and natural killer cells. IL-21 mediates its function *via* a heterodimeric receptor consisting of IL-21R and a common γ chain (γc) cytokine receptor. Upon binding, the IL-21-IL-21R complex activates Janus kinase (JAK)1 and JAK3, which in turn phosphorylates STAT1 and STAT3. This illustrates the controlling effect from the proliferation of IL-21 on CD4+ T cells and the regulation of differentiating helper T cells, namely the pathogenic Th17 [5]. B cells are associated with RA pathogenesis regarding autoantibody production. IL-21 affects the proliferation and apoptosis of B lymphocytes. This controls the differentiation of B lymphocyte and induces immunoglobulin (Ig) class switching by inducing the B lymphocyte induced maturation protein 1 (Blimp-1) [6,7]. These immune-modulating effects of IL-21

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http://dx.doi.org/10.1016/j.imlet.2014.09.007
0165-2478/

make this a molecule of interest in RA. Noteworthy, the concentration of IL-21 as well as IL-17 in the synovial fluid of patients with RA are significantly higher than those found in osteoarthritis patients. Moreover, the level of IL-17 and IL-21 correlates well with the RA activity [8–10]. Therefore, IL-21 seems to be a promising therapeutic target for RA treatment.

The present study was conducted to investigate the therapeutic effect and underlying molecular mechanism of human IL-21R-Fc using an RA animal model that is collagen-induced arthritis (CIA). The change in differentiation and function of immune cells and arthritis severity with IL-21 blockage was addressed.

2. Material and methods

2.1. Animals

Six-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 examined and approved by the Animal Research Ethics Committee at the Catholic University of Korea.

2.2. Induction of arthritis and injection of hIL-21R

To induce CIA, 100 μg chicken type II collagen (CII) and complete Freund's adjuvant (CFA, Arthrogen-CIA) were injected intradermally into the base of the tail. To examine the effect of human IL-21R on CIA, human IL-21R was injected i.p. 3 times at 2-day intervals after CIA induction. Starting on the next day, three independent observers examined the severity of arthritis 3 times per week. The severity of arthritis was recorded using the mean arthritis index on scale of a 0–4, as reported previously [11]. The final value represented the average index from all four legs recorded by the three independent observers.

2.3. 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 IFN-γ, 5′-GAA AAT CCT GCA GAG CCA GA-3′ and 5′-TGA GCT CAT TGA ATG CTT GG-3′, for IL-17, 5′-CCT CAA AGC TCA GCG TGT CC-3′ and 5′-GAG CTC ACT TTT GCG CCA AG-3′, for STAT3, 5′-CCG TCT GGA AAA CTG GAT AAC TTC-3′ and 5′-CCT TGT AGG ACA CTT TCT GCT GC-3′, for RORγt, 5′-TGT CCT GGG CTA CCC TAC TG-3′ and 5′-GTG CAG GAG TAG GCC ACA TT-3′, for Foxp3, 5′-GGC CCT TCT CCA GGA CAG A-3′ and 5′-GCT GAT CAT GGC TGG GTT GT-3′, for IL-10, 5′-AAG TGA TGC CCC AGG CA-3′ and 5′-TCT CAC CCA GGG AAT TCA AA-3′, for Blimp1, 5′-CTG TCA GAA CGG GAT GAA CA-3′ and 5′–TGG GGA CAC TCT TTG GGT AG-3′, and for β–actin, 5′-GAA ATC GTG CGT GAC ATC AAA G-3′ and 5′-TGT AGT TTC ATG GAT GCC ACA G-3′.

2.4. Measurement of immunoglobulin (Ig) concentrations

The serum concentrations of IgG, IgG1, and IgG2a were measured by ELISA, using mouse IgG, IgG1, and IgG2a ELISA quantitation kits (Bethyl Laboratories, Montgomery, TX). To measure the serum levels of type-II-collagen-specific IgG, IgG1 and IgG2a, microtiter plates were coated with type II collagen (4 μg/ml in PBS) at 4 °C overnight and were measured using ELISA with mouse IgG, IgG1, and IgG2a ELISA quantitation kits (Bethyl Laboratories).

2.5. Western blotting

For STAT3 activity analysis, spleen T cells were cultured with IL-21 for 5, 10, 30, and 60 min. To examine the effect of human IL-21R-Fc, isolated T cells were cultured with IL-21 and hIgG1-Fc, anti-IL-21 or hIL-21R-Fc for 10 min. Protein samples were separated by SDS gel electrophoresis and transferred to a nitrocellulose membrane (Amersham Pharmacia Biotech). Membranes were stained with primary antibodies to *p*-STAT3^{Tyr705}, *p*-STAT3^{Ser727}, STAT3 (all

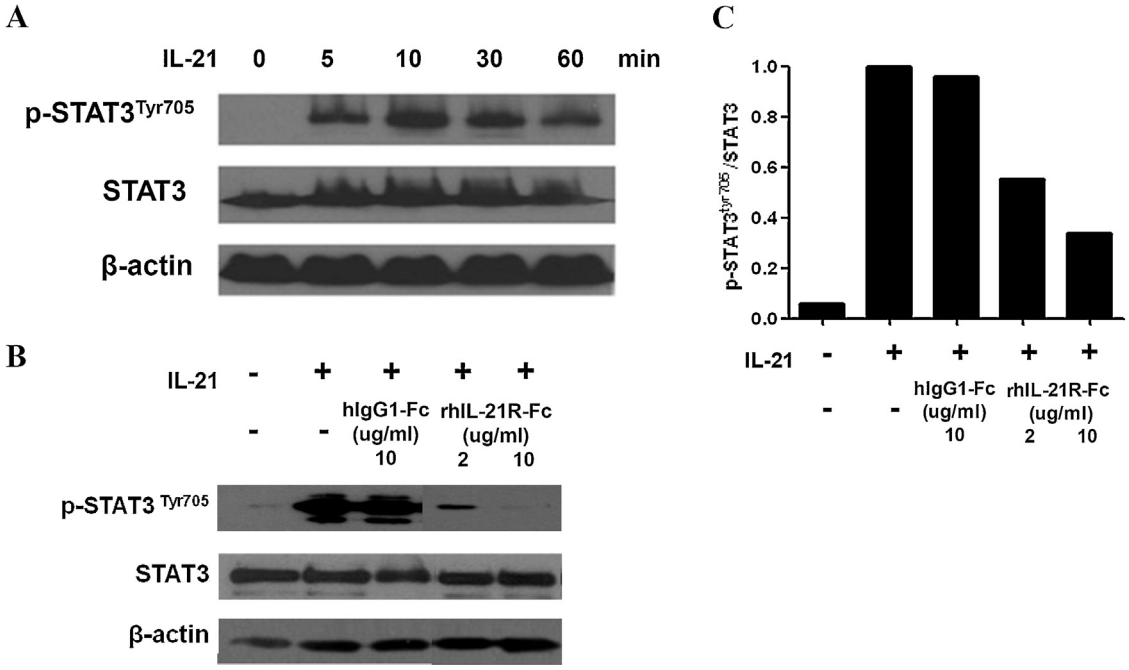

Fig. 1. IL-21-mediated STAT3 activation is inhibited by IL-21 blockade. (A) Mouse splenocytes were stimulated 50 ng/ml recombinant mouse IL-21 for 0–60 min. Cells were lysed and Western blotted with antibodies to pSTAT3. (B and C) Mouse splenocytes were pretreated control Fc (hIgG1-Fc) and recombinant (rh) IL-21R-Fc and then stimulated 50 ng/ml recombinant mouse IL-21 for 10 min. Cells were lysed and Western blotted with antibodies to pSTAT3. Optical densities ratio of p-STAT3^{Tyr705}/STAT3 normalized to β-actin expression are shown in the bar graphs (C). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.