Therapeutic effects of TACI-Ig on rats with adjuvant-induced arthritis via attenuating inflammatory responses

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Abstract

Objective. To investigate the effects of TACI-Ig, a recombinant fusion protein that modulates B- and T-cell activation by binding and neutralizing B-lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL), in an established adjuvant-induced arthritis (AA) rat model.

Methods. Rats with experimental arthritis were randomly separated into different groups and then treated with TACI-Ig (0.7, 2.1, 6.3 mg/kg), rhTNFR-Fc (2.8 mg/kg), MTX (0.5 mg/kg) or IgG-Fc (6.3 mg/kg), from Day 16 to Day 34 after immunization. Arthritis was evaluated by hind paw swelling, polyarthritis index and histopathological examination. Activities of BLyS, APRIL, IL-1β, IL-2, IL-10, TGF-β1, PGE2, TNF-α, IFN-γ, immunoglobulin (Ig)G1, IgG2a, IgM and IgA were assessed by ELISA. Cluster of differentiation (CD)20 expression was detected by immunohistochemical analysis.

Results. TACI-Ig (2.1, 6.3 mg/kg) treatment significantly reduced the severity of established arthritis using the methods of clinical observation and histopathological examination. TACI-Ig treatment inhibited expression of IgM, decreased the expression of BLyS and APRIL and regulated the balance of pro-inflammatory and anti-inflammatory cytokines in serum of AA rats. Immunohistochemical analysis demonstrated that CD20 production was reduced in spleen.

Conclusions. Data presented here demonstrate that administration of TACI-Ig significantly attenuates progression of experimental arthritis, with reductions in inflammatory response and bone and joint destruction.

Key word: B lymphocyte stimulator, A proliferation-inducing antigen, TACI-Ig, Arthritis, Autoimmune, T cell.

Introduction

RA is a systemic autoimmune disease characterized by synovial membrane hyperplasia and infiltration of inflammatory cells, including activated B cells. Recently, the success of B-cell-targeted therapies, such as rituximab, in the treatment of RA patients has led investigators to reassess the critical role of B cells in RA pathogenesis [1].

Recent reports indicate that B-cell activation, differentiation and survival are determined not only by antigen and T-cell interaction, but also by cytokines, particularly by members of the TNF ligand superfamily [2]. Two closely related TNF family members, B-lymphocyte stimulator (BLyS, also known as BAFF, TALL-1, zTNF-4, THANK and TNSF13B) [3–7] and a proliferation-inducing ligand (APRIL) [8], have been identified that are critical for immune system function [9]. There are three currently identified receptors for BLyS and APRIL: B-cell maturation antigen (BCMA) [10], transmembrane activator and calcium-modulating and cyclophilin ligand interactor (TACI) [11], and BAFF receptor (BAFF-R) [12]. Expression of BCMA and BAFF-R is thought to be restricted to...
B cells [9, 13, 14]. TACI is also expressed by activated T cells [14], which suggests that BLyS and APRIL may also regulate T-cell-mediated immune functions. These ligand–receptor interactions promote survival, proliferation and differentiation of B cells [6, 7, 10, 13, 15, 16]

The individual role of BLyS and APRIL or their receptors is not fully elucidated, but mice treated with TACI-Ig (a soluble TACI fusion protein that neutralizes both BLyS and APRIL) and TACI-Ig transgenic mice have fewer transitional T2 and mature B cells. TACI-Ig overexpression leads to a decrease in circulating immunoglobulin (Ig)G levels, a loss of B1 cells in the peritoneum, and a significant decrease in the number of CD4+ and CD8+ T cells in the spleen and mesenteric lymph nodes [17]. Previous studies showed that administration of TACI-Ig significantly ameliorated disease symptoms in CIA mice, synovium–severe combined immune deficiency (SCID) mouse chimeras, SLE-proneNZB/NZW F1 mice and Hg-treated A.SW mice [17–20].

BLyS is expressed by a few stromal cells, T cells and most myeloid cell lineages, including monocytes, macrophages, dendritic cells and stimulated neutrophils [5, 6], whereas APRIL is expressed at low levels by lymphoid cells and at higher levels by some tumour cells [8]. BLyS can stimulate B cells in vivo [5, 6, 21] and in vitro [7, 22]. Transgenic expression of BLyS in mice leads to enlargement of the spleen and lymph nodes and increased numbers of mature B cells, spontaneously producing antibodies to DNA and other autoantibodies, such as RF [7, 23]. Such mice develop autoimmune-like manifestations reminiscent of human SLE and SS [22].

The interactions of BLyS and/or APRIL–TACI are important in B-cell-mediated immune responses and in antibody-mediated autoimmune diseases. Although TACI can be expressed on activated T cells, the role of the ligand–TACI pathway in the regulation of T-cell function is unknown. In order to gain further insight into the mechanism of action of TACI-Ig, this study examines the effects of TACI-Ig on disease progression, joint inflammation, bone destruction and the production of inflammatory mediators in a rat model of adjuvant-induced arthritis (AA).

Materials and methods

Regents

The human TACI-Ig fusion protein and IgG-Fc were provided by Yantai Rongchang Pharmaceutical Co, Ltd (Yantai, China). RhTNFR:Fc fusion protein, a soluble human TNF receptor fusion protein, was purchased from Shanghai CP Guojian Pharmaceutical Co., Ltd (Shanghai, China). MTX was purchased from Shanghai Pharmaceutical (Group) Co., Ltd (Shanghai, China). IgG-Fc was used as negative controls. MTX and rhTNFR:Fc were used as positive controls. ELISA kits for BLyS, APRIL, IL-1β, IL-2, IL-10, TGF-β1, PGE2, TNF-α, IFN-γ, IgG1, IgG2a, IgM, and IgA were purchased from Research & Development (R&D) Systems, Inc. (Minneapolis, MN, USA). Anti-cluster of differentiation (CD)20 polyclonal IgG antibody (sc-7735) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Animals

Sprague–Dawley (SD) rats [male, 160 (20) g, Certificate No. 2008-0016] were housed under standard laboratory conditions with free access to food and water. The temperature was kept at 22 (2) °C, and a 12-h light/dark schedule was maintained. The study was approved by the Ethics Review Committee for Animal Experimentation of the Institute of Clinical Pharmacology, Anhui Medical University.

Induction of AA

Complete Freund’s adjuvant (CFA) was prepared by suspending heat-killed *Mycobacterium butyricum* in liquid paraffin at 10 mg/ml. Arthritis was induced in SD rats by intradermal injection of 0.1 ml of CFA emulsion into the left hind metatarsal footpad of rats.

Treatment of AA

Animals were randomly divided into eight groups (n = 10 per group), in which the rats with AA were given TACI-Ig (0.7, 2.1, 6.3 mg/kg, s.c., every other day), rhTNFR:Fc (2.8 mg/kg, s.c., every other day) and MTX (0.5 mg/kg, i.p., every 3 days) from Day 16 to Day 34 after immunization. And for the groups of normal and AA model, rats were subcutaneously given vehicle (NS) at the same time.

Clinical assessment of AA

Evaluation of AA severity was performed by two independent observers with no knowledge of the treatment protocol. From Day 7 after immunization, rats were examined every 3–4 days for two clinical parameters: non-injected hind paw swelling and polyarthritis index. The footpad volume was measured with a water replacement plethysmometer. The arthritis severity in each paw was evaluated by using a macroscopic scoring system ranging from 0 to 4: 0, paws with no swelling and focal redness; 1, paws with swelling of finger joints; 2, paws with mild swelling of ankle or wrist joints; 3, paws with severe inflammation of the entire paws; and 4, paws with deformity or ankylosis. The cumulative score for all paws of each rat was used as the polyarthritis index with a maximum value of 16 [24].

Histological examination

After ether anaesthesia on Day 35, the secondary hind paws were amputated above the ankle joints and were fixed in 10% neutral-buffered formalin, then decalcified in 5% formic acid and embedded in paraffin. The sections (5 μm) were stained with haematoxylin and eosin (HE), and were examined microscopically. Two blinded observers evaluated cartilage and bone destruction by synovial proliferation, cellular infiltration, pannus formation and cartilage erosion in each preparation on two separate occasions, using the following scoring system [25]: synovial proliferation: Grade 0, proliferation was absent; Grade 1, proliferation was mild with two to four layers of
reactive synoviocytes; Grade 2, proliferation was moderate with four plus layers of reactive synoviocytes, increased mitotic activity and mild or absent synovial cell invasion of adjacent bone and connective tissue; and Grade 3, proliferation was severe and characterized by invasion and effacement of joint space and adjacent cartilage, bone and connective tissue. Cellular infiltration: Grade 0, no changes; Grade 1, few focal infiltrates; Grade 2, extensive focal infiltrates; and Grade 3, extensive infiltrates invading the capsule with aggregate formation. Cartilage erosion: Grade 0, no changes; Grade 1, superficial, localized cartilage degradation in more than one region; Grade 2, localized deep cartilage degradation; and Grade 3, extensive deep cartilage degradation at several locations. Pannus formation: Grade 0, no changes; Grade 1, pannus formation at up to two sites; Grade 2, pannus formation at up to four sites, with infiltration or flat overgrowth of joint surface; and Grade 3, pannus formation at more than four sites or extensive pannus formation at two sites.

We also performed HE staining of tissue specimens of the spleen. Five compartments were evaluated in the spleen: cellularity of periarteriolar lymphoid sheaths (PALS), lymphoid follicles, marginal zone, red pulp and the total number of germinal centres (GCs) in each section. The grading scheme consisted of ordinal categories ranging from 0 (no effect) to 4 (severe effect) [26].

Cytokines and antibodies determination

On Day 35, rats were sacrificed and the serum was collected from peripheral blood and stored at −80°C until use. Concentrations of BLyS, APRIL, IL-1β, TNF-α, PGE2, IL-2, IL-10, IFN-γ, TGF-β1, IgG1, IgG2a, IgM and IgA in serum were measured using ELISA kits. Each serum sample was assayed in duplicate.

Immunostaining

B lymphocyte antigen CD20 was detected by the use of saturating amounts of antibody against CD20 in combination with immunoperoxidase staining according to the manufacturer’s protocol. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections of 4μm that were deparaffinized and endogenous peroxidase activity was blocked with hydrogen peroxide in methanol. The sections were preincubated with goat serum albumin in PBS for 20 min followed by incubation with anti-CD20 polyclonal IgG antibody and for 16 h in a humid chamber at 4°C. After washing with PBS, the sections were incubated with peroxidase anti-goat secondary reagent for 30 min according to the manufacturer’s instructions to evaluate the distribution of CD20. The average optical density of staining with anti-CD20 antibody was analysed with software JEDA 801D Morphology Image Analysis System (version 6.0; JiangSu JEDA Science-Technology Development Co., Ltd, JiangSu, China).

Statistical analysis

Data were expressed as mean (S.D.) The analysis of variance (ANOVA) was used in the program SigmaStat (SPSS Software Products, Chicago, IL, USA) to determine significant differences between means of groups. Spearman’s coefficient of correlation was used to examine the correlation between the severity and histopathology of the animals and the serum BLyS level. P < 0.05 was considered statistically significant.

Results

TACI-Ig inhibits AA

To imitate the clinical scenario of RA, SD rats were subjected to AA. AA developed rapidly and clinical signs like joint swelling, redness and oedema of the feet. The onset of secondary arthritis of the disease appeared on Day 15 after immunization (Fig. 1) (P < 0.01). Rats in the AA and IgG-Fc groups developed typical clinical symptoms of severe arthritis and progressed rapidly. TACI-Ig (2.1, 6.3 mg/kg) administration suppressed the severity of clinical arthritis compared with AA rats, as demonstrated by
both the paw swelling (Fig. 1A) \( (P < 0.01) \) and the polyarthritis index (Fig. 1B) \( (P < 0.01) \). The efficacy was similar to that of MTX and rhTNFR:Fc.

**TACI-Ig prevents ankle joint and spleen histopathology**

Histopathological examination of the ankle joint (Fig. 2A and supplementary figure 1, available as supplementary data at *Rheumatology Online*) and spleen (Fig. 2B and supplementary figure 2, available as supplementary data at *Rheumatology Online*) revealed marked differences between TACI-Ig-treated and AA rats. AA and IgG-Fc-treated rats developed severe arthritis. This was characterized by marked proliferation of synoviocytes, cellular infiltration, pannus formation and joint tissues with resultant erosion of articular cartilage and bone. In contrast, TACI-Ig-treated rats \( (2.1, 6.3 \text{ mg/kg}) \) showed only minimal inflammation or pathological changes. MTX and rhTNFR:Fc had equivalent effects to TACI-Ig.

To assess the extent of spleen remodelling, histological sections of rat spleen were scored. Significant white and red pulp hyperplasia and GC appearance affected all spleens in the AA rats. In AA rats given TACI-Ig, rhTNFR:Fc and MTX, the degree of spleen damage was significantly reduced: hyperplasia of red pulp and appearance of GCs were extremely reduced, although mild hyperplasia of white pulp was observed to some extent.

**TACI-Ig regulates the concentrations of BlyS, APRIL, IL-1β, TNF-α, PGE2, IL-2, IL-10, IFN-γ, TGF-β1, IgG1, IgG2a, IgM and IgA in serum**

Compared with the normal group, the concentrations of BlyS, APRIL, IL-1β, TNF-α, PGE2, IL-2 and IgM elevated significantly in serum, whereas the concentrations of IFN-γ, IL-10 and TGF-β1 reduced significantly in AA rats (Fig. 3). Treatment with TACI-Ig significantly decreased the concentrations of IL-1β, TNF-α, PGE2, BlyS, APRIL and IL-2 and increased the concentrations of IFN-γ, IL-10 and TGF-β1 compared with AA rats. RhTNFR:Fc and MTX had similar results to TACI-Ig. Of the remaining antibodies, three did not differ significantly between normal and arthritic rats (data not shown).

To examine the correlation between the severity of disease and the serum BlyS level, we measured the coefficient of correlation between the secondary arthritis and histopathology and the BlyS level. Interestingly, the secondary arthritis (Fig. 4) and histopathology significantly correlated with the production of serum BlyS (Table 1).

**TACI-Ig downregulates CD20 expression**

Immunohistochemistry was used to examine the expression and localization of CD20 in AA rats (Fig. 5). We found markedly enhanced expression of CD20 in lymphatic nodule, GCs, marginal zone and mantle zone in spleen of AA and IgG-Fc-treated rats. In TACI-Ig-treated rats \( (2.1, 6.3 \text{ mg/kg}) \), the expression of CD20 was significantly lower than that in AA rats. Similar to TACI-Ig, rhTNFR:Fc reduced the expression of CD20.

**Discussion**

The interaction of BlyS and APRIL with their receptors TACI, BCMA and BAFF-R is critical in the development of the mammalian immune system and the ability of the system to mount an efficient adaptive immune response \[27\]. BlyS and APRIL influence B-cell maturation and survival at several levels. Apart from its role as a B-cell survival factor, BlyS binds to naive and primed/memory CD4+ and CD8+ T cells and costimulates T-cell activation, proliferation and cytokine production *in vitro* \[28\]. BlyS-transgenic mice show increased proportions of T cells with a CD44high, L-selectinlow phenotype suggestive of BlyS-induced expansion of activated effector T cells \[7, 22\]. Both BlyS and APRIL are expressed by activated, but not resting, CD4+ T cells \[29\], and can costimulate T-cell proliferation \[21, 28–30\]. TACI, a high-affinity receptor for BlyS and APRIL, is expressed by B and activated T cells \[12\], and TACI-Ig dampens T-cell proliferation induced *in vitro* by CD3 mAb, as well as CD4-dependent T-cell responses *in vivo* \[31\]. However, the role of BlyS/APRIL receptors in T-cell activation and the therapeutic potential of targeting of the BlyS/APRIL–TACI pathway in RA is unknown, and for this reason, we analysed the effects of TACI-Ig in established AA.

AA, a T-cell-mediated chronic inflammatory stress, is a well-established in vivo model that has been used in numerous studies to investigate the pathogenesis of RA and for identification of potential therapeutic targets \[32\]. The study indicated that blockade of BlyS/APRIL with TACI-Ig regulated cellular immune mechanisms in AA. Analysis of disease progression, assessed by paw swelling and polyarthritis index, showed that treatment with TACI-Ig markedly abrogated the development of arthritis, with these effects observed throughout the course of the study.

Abrogation of disease progression by TACI-Ig was further supported by the histopathological analysis from these animals. TACI-Ig improved articular and splenic status histopathologically in rats with AA. AA and splenic scores were reduced across all disease parameters assessed. Consistent with previous studies \[15, 24, 31\], the current findings support the role of the BlyS/APRIL–receptor pathway in the development of autoimmune diseases and the novel mechanism of action of TACI-Ig in preventing disease progression and attenuating joint inflammation and destruction.

In light of the impressive therapeutic effect of TACI-Ig in AA, we investigated whether TACI-Ig reduces the expression of BlyS and APRIL in serum. Mounting evidence from animal models and human studies supports important roles for BlyS and APRIL in the development of autoimmune diseases \[22, 33–35\]. Several studies also have revealed the presence of increased BlyS levels in serum from patients with autoimmune diseases, such as SLE, RA and SS \[33, 36, 37\]. In human RA, the concentration of BlyS in the SF is greater than in the blood.
Consistent with these findings, we also observed that the level of BLyS in serum remarkably increased and significantly correlated with the clinical manifestation and histopathological parameters. TACI-Ig decreased the elevated serum BLyS and APRIL levels of AA rats. The current results support the role of the T-cell-expressed BLyS/APRIL-receptors pathway in vivo that contributes to the development of AA.

The effect of TACI-Ig on inflammatory mediators was determined by analysis of cytokines and Igs. TACI-Ig treatment decreased the increased IgM level in serum. The results are consistent with previous findings that mice either transgenic for BLyS or injected with BLyS showed significantly increased levels of serum IgM [6, 28]. In particular, treatment with TACI-Ig significantly decreased the levels of the Th1-cell cytokine IL-2 and...
FIG. 3 Effects of TACI-Ig on serum cytokine levels in rat AA model. On Day 35, rats were sacrificed and the serum was collected from peripheral blood. Levels of BLyS and APRIL (A), IL-1β and TNF-α (B), IL-2 and IFN-γ (C), IL-10 and TGF-β1 (D), PGE2 (E) and IgM (F) in serum were measured by ELISA as described in the ‘Materials and methods’ section. *P < 0.05, **P < 0.01 compared with normal group; #P < 0.05, ##P < 0.01 compared with model group [n = 6–8, mean (S.D.)].
increased Th2-cell cytokine IL-10 and Th3-cell cytokine TGF-β1. The role of these cytokines in the development of autoimmune disorders is well established [38]. In addition, the production of pro-inflammatory cytokines IL-1, TNF-α and PGE2 were released from activated T cells and macrophage in RA patients, which are considered to be the important participants in the pathophysiology of RA [39-42], were reduced after treatment with TACI-Ig. Together, these findings suggest that via modulation of the BLyS/APRIL-receptors pathway, TACI-Ig regulates Th1, Th2 and Th3 cell function and results in the down-regulation of pro-inflammatory cytokines and up-regulation of anti-inflammatory cytokines, and their associated inflammatory pathways.

How do BLyS and APRIL influence T-cell activity and function? There may be several possible explanations, first, interaction of BLyS/APRIL with TACI may modulate T-cell function in an autocrine manner because BLyS and APRIL are expressed by activated T cells, and second, BLyS and APRIL may modulate T-cell functions in a paracrine manner. T cells present BLyS/APRIL to B cells and/or antigen-presenting cells (APCs), which lead to activation in paracrine manner through TACI, BCMA and BAFF-R, in turn, the activated B cells and/or APCs may provide co-stimulatory signals for activation and maturation of T cells. Therefore, TACI-Ig exerts its therapeutic effects on AA as a decoy receptor might through either neutralizing BLyS or APRIL, thus preventing them from binding to their receptors, TACI, BCMA and BAFF-R, but whether TACI is the key or only receptor involved has not been determined.

Taken together, the findings of this study demonstrate that TACI-Ig has profound therapeutic effects on AA and is as effective as rhTNFR-Fc and MTX, two drugs that have been confirmed effective in the therapy of human RA. This study provides the first evidence of the ability of TACI-Ig to reduce BLyS and APRIL levels and regulate T-cell function in AA rats. BLyS/APRIL–TACI interactions play an important role in the pathogenesis of RA and could be manipulated therapeutically to regulate immune response. TACI-Ig, therefore represents a potentially new immunotherapeutic agent for the management of arthritis as well as other autoimmune diseases that involve both B and T cells.

**Rheumatology key messages**

- BLyS/APRIL may play a pathogenic role in autoimmune diseases that involve T cells.
- TACI-Ig is a potential therapeutic approach for RA.

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Yan Chang et al.


