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Therapeutic effects of TACI-Ig on collagen-induced arthritis by regulating T and B lymphocytes function in DBA/1 mice

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A B S T R A C T

To investigate the abnormal function of T and B lymphocytes involved in collagen-induced arthritis in DBA/1 mice and the regulation role of TACI-Ig on T and B lymphocytes, collagen-induced arthritis models were established in DBA/1 mice. Mice were divided randomly into eight groups, including normal, collagen-induced arthritis model, TACI-Ig (0.350, 1.105, 3.333, 10, and 30 mg/kg) and IgG-Fc (10 mg/kg) treated groups. The effect of TACI-Ig on collagen-induced arthritis was evaluated by arthritis scores, joints and spleens histopathology, paws radiology, and indices of thymus and spleen. T and B lymphocyte proliferations were assayed by [3H]-TdR method. B lymphocyte stimulator and prostaglandin E2 in serum were assayed by enzyme linked immunosorbent assay. The subsets of T and B lymphocytes were assayed by flow cytometry. Results showed that the onset of paw-swelling was on day 31 after immunization. The peak of inflammation mediators and abnormal proliferation appeared on day 42 and then declined after day 63. Compared with normal mice, collagen-induced arthritis mice have increased arthritis scores, spleen and thymus indices, radiograph scores of joints, and pathology scores of joints and spleens. TACI-Ig could ameliorate these changes and reduce the increased serum level of B lymphocyte stimulator and prostaglandin E2. Further studies showed that TACI-Ig inhibited T and B lymphocyte proliferation response, and inhibited differentiation and activity of T and B lymphocytes in collagen-induced arthritis mice. In conclusion, TACI-Ig has a good therapeutic action on collagen-induced arthritis mice, which might be related to the regulation of TACI-Ig on inflammation mediators and abnormal function of T and B lymphocytes.

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1. Introduction

Rheumatoid arthritis is a systemic autoimmune disease that primarily targets the synovial membrane, cartilage and bone. T and B lymphocytes infiltrate into inflammatory synovial membrane (Seyler et al., 2005). In the last decades, rheumatoid arthritis has been considered to be preferentially mediated by T cells and macrophages. More recently, increasing evidence of an important role of B cells involved in immune dysregulation in rheumatoid arthritis was obtained and fueled by clinical improvements in rheumatoid arthritis patients receiving B cell depleting therapies such as rituximab, an anti-CD20 antibody (Martinez-Gamboa et al., 2007). The tumor necrosis factor family member, B lymphocyte stimulator (BlyS) also known as BAFF, TALL-1, THANX, zTNF4 and TNFSF13B, plays an important role in B lymphocyte maturation and survival (Gao et al., 2007; Kim et al., 2009). BlyS is a type II membrane protein that can function as the membrane-bound form or be proteolytically cleaved into a soluble cytokine. In vitro, soluble BlyS could promote B cell survival and proliferation with anti-IgM (Lu et al., 2009). Overexpression of BlyS is closely involved in the pathogenesis and progression of autoimmune disorders (Gottenberg et al., 2005; Ju et al., 2006; Mandlik-Nayak et al., 2008; Williams et al., 2007). Therefore, BlyS has been considered as an ideal therapeutic target for these conditions (Ding, 2008).

BlyS binds to three receptors: transmembrane activator and calcium modulator cyclophilin ligand interactor (TACI), B cell maturation antigen (BCMA) and BAFF receptor (BAFF-R or BR3). All three receptors are type I single transmembrane receptors and belong to the TNF receptors family. BCMA and BAFF-R are predominantly expressed on B-lymphocytes, and TACI can be found on B cells and activated T cells (Gao et al., 2007; Ju et al., 2009; Kim et al., 2009). TACI-Ig is a soluble glycoprotein comprising a human IgG1-Fc fused with the extracellular domain of the human TACI receptor. TACI-Ig has a major role in B cell function. TACI-Ig not only inhibits B cell proliferation and activation via its three specific cell surface receptors, but also has a therapeutic action on collagen-induced arthritis mice.
Chronic exposure to TACI-Ig is associated with reduced circulating B cells in animals, and a concomitant decrease in circulating immunoglobulin. Because of these activities, TACI-Ig is in clinical evaluation for treatment of various autoimmune diseases and B cell malignancies (Roque et al., 2006).

Previous studies have revealed that TACI-Ig could inhibit T cell and B cell activations in vitro, TACI-Ig administration in mice inhibits antigen-specific activation and priming of T and B cells, further, the possible mechanisms were investigated partially (Gross et al., 2001; Wang et al., 2001). In the current study, collagen-induced arthritis as a most commonly used animal model of rheumatoid arthritis which involves both T and B cell autoimmune reactions to joints (Li et al., 2004) was induced to examine the effects of TACI-Ig on swelling joint, histopathological injuries of joints and spleens. Furthermore, we investigated the mechanism of TACI-Ig in the treatment of collagen-induced arthritis systematically, including the influence on T and B lymphocyte proliferations, the serum level of BLyS, and the effects of TACI-Ig on the proportion of the subsets of T and B lymphocytes.

2. Materials and methods

2.1. Animals

DBA/1 mice (male, 18±2 g) were obtained from Shanghai Institute of Materia Medica, Chinese Academy of Sciences (production license no.: SCXK [HU] 2008-0017). All mice were maintained in specific-pathogen free animal laboratory of Anhui Medical University. All experiments were approved by Ethics Review Committee for Animal Experimentation of Institute of Clinical Pharmacology, Anhui Medical University.

2.2. Materials

Chicken type II collagen (CII), Freund’s complete adjuvant (FCA) and lipopolysaccharides (LPS) from Sigma Chemical Co., USA; concanavalin A (ConA) from Biosharp Co., USA; [3H]-TdR from Aldrich Co., USA; and sodium 1-phenyl-3-methylpyrazol-5-one (PMO) from Shanghai Institute of Applied Physics, Chinese Academy of Sciences; the BLyS enzyme linked immunosorbent assay (ELISA) kit from Eiken Chemical Co., USA; and Biolog’s MTS assay from Promega Co., USA. All cells were cultured in RPMI-1640 medium (Gibco Co., USA) with 10% fetal bovine serum (Gibco Co., USA; the BLyS enzyme linked immunosorbent assay (ELISA) kit from Eiken Chemical Co., USA; and Biolog’s MTS assay from Promega Co., USA. All cells were cultured in RPMI-1640 medium (Gibco Co., USA) with 10% fetal bovine serum (FBS) (Gibco Co., USA) and 1% penicillin/streptomycin (Gibco Co., USA).

2.3. Drugs

TACI-Ig (11.7 mg/ml, Yantai Rongchang Biotechnologies, Ltd.); IgG-Fc (9.64 mg/ml, Yantai Rongchang Biotechnologies, Ltd.).

2.4. Induction of collagen-induced arthritis

CII was dissolved in 0.1 mol/l acetic acid at 2 mg/ml overnight at 4 °C and emulsified with an equal volume of FCA in ice bath under sterile conditions. By immunization with heterologous CII emulsion, emulsion of 0.1 ml was injected intradermally into the back and the base of the tail of DBA/1 mice to induced collagen-induced arthritis on day 0, followed by a booster injection on day 21 and day 28.

2.5. Treatment of collagen-induced arthritis

Animals were divided into 8 groups, containing normal, model, TACI-Ig (0.350, 1.105, 3.333, 10, and 30 mg/kg, intraperitoneal injection, three times per week, treatment of 6 weeks), and negative control IgG-Fc (10 mg/kg, intraperitoneal injection, three times per week, treatment of 6 weeks). After the onset of arthritis, mice were given administration. The vehicle of TACI-Ig and IgG-Fc is normal sodium. Normal mice and model mice were given an equal volume of normal sodium correspondingly.

2.6. Evaluation of arthritis

To quantitatively evaluate the severity of the arthritis, arthritis index was evaluated every week. Inflammation of the four paws was graded from 0 to 4: grade 0, paws with no swelling and focal redness; grade 1, paws with swelling of finger joints; grade 2, paws with mild swelling of ankle or wrist joints; grade 3, paws with severe inflammation of the entire paws; and grade 4, paws with deformity or ankylosis. Each paw was graded and the four scores were totaled so that the maximum possible score per mice was 16 (Xu et al., 2002).

2.7. Radiological examination of paw

On day 70 after immunization, mice were sacrificed by cervical dislocation. Both fore- and hind-paws of mice were removed, X-rayed and bone structures analyzed. The following radiograph criteria were considered: score 0, no bone damage; score 1, tissue swelling and oedema; score 2, joint erosion and disfiguration; and score 3, bone erosion and osteophyte formation (Cuzzocrea et al., 2000). Each paw was graded and the four scores were totaled so that the maximum possible score per mice was 12. The radiographs were evaluated by two independent observers.

2.8. Indices of thymus and spleen assay

On day 70 after immunization, mice were sacrificed by cervical dislocation. Thymus and spleen were removed and weighed. Thymus index is the ratio of thymus weight to mouse body weight (mg/g), and spleen index is the ratio of spleen weight to mouse body weight (mg/g) (Zhang et al., 2004).

2.9. Histopathology examination of spleen and joint

On day 70 after immunization, mice were sacrificed by cervical dislocation. The spleen of mice was removed and fixed in neutral-buffered 10% formalin routinely processed and embedded in paraffin. Histopathological change of the spleen was graded from 0 to 3: grade 0, normal spleen; grade 1, mild proliferation of white pulp; grade 2, moderate proliferation of white pulp; and grade 3, marked proliferation of white pulp and prominent germinal center. Histopathological examination in spleen was based on the size and cellularity of the periarteriolar lymphoid sheath and marginal zone, and a change in the number of follicles with germinal centers. Histopathological change of the spleen was graded from 0 to 3: grade 0, normal spleen; grade 1, mild proliferation of white pulp; grade 2, moderate proliferation of white pulp; and grade 3, marked proliferation of white pulp and prominent germinal center. The severity of arthritis in joint was graded from 0 to 4 according to the intensity of the lining layer hyperplasia, mononuclear cell infiltration, and pannus formation. Grade 0, normal ankle joint; grade 1, normal synovium with occasional mononuclear cells; grade 2, definite arthritis with a few layers of flat to rounded synovial lining cells and scattered mononuclear cells and dense infiltration with mononuclear cells; grade 3, clear hyperplasia of the synovium with three or more layers of loosely arranged lining cells and dense infiltration with mononuclear cells; and grade 4, severe synovitis with pannus and erosions of articular cartilages and subchondral bones (Tong et al., 2010).
2.1. Assay of BLyS and PGE2 in serum

Triplicates were designed. The cultures were incubated at 37 °C in an cell suspension was purified with a lymphocyte separating medium. Then the cells were washed with PBS buffer three times.

The cell suspension (100 μl) and ConA (100 μl with a final concentration of 3 mg/l) or LPS (100 μl with a final concentration of 4 mg/l) were seeded into a 96-well culture plate simultaneously. Triplicates were designed. The cultures were incubated at 37 °C in an incubator (SHEL LAB Co.) of 5% CO2 for 48 h, 6 h before the end, 3H-TdR 20 μl was added to each well. The radioactivity was measured by LS6500 liquid scintillation counter (Beckman Co.). The results were described as the average of triplicate counts per minute (Xu et al., 2002).

2.11. Assay of BLyS and PGE2 in serum

On day 70 after immunization, the blood of mice was collected. Then serum was separated from the blood in a centrifuge under 2790×10 min. Serum concentrations of BLyS and PGE2 were measured using ELISA.

2.12. Assay of the subsets of T lymphocytes and B lymphocytes

On day 70 after immunization, mice were sacrificed by cervical dislocation. Thymus and spleens were removed under sterile conditions and thymocytes and splenocytes were collected. Then cells were suspended in a DMEM medium at a concentration of 1×107 cells/l. The cell suspension was purified with a lymphocyte separating medium. Then the cells were washed with PBS buffer three times. A 50 μl aliquot of thymic lymphocyte suspension was then transferred into a 12×75 mm polystyrene round-bottom tube and 10 μl of CD3-PE/CD4-FITC, CD25-PE/CD4-FITC, CD154-PE/CD4-FITC, CD69-PE/CD4-FITC, and CD62L-PE/CD4-FITC antibody combinations were added to each tube respectively. A 50 μl aliquot of splenic lymphocyte suspension was then transferred into a 12×75 mm polystyrene round-bottom tube and 10 μl of CD27-FITC/CD19-PE, IgM-FITC/CD19-PE, IgD-FITC/CD19-PE, CD21-FITC/CD19-PE, and CD23-FITC/CD19-PE antibody combinations were added to each tube respectively. The sample was mixed gently, incubated for 20 min at 4 °C, and then analyzed by flow cytometer. Data analysis was performed using a CellQuest™ analysis software.

2.13. Statistical analysis

All data were expressed as mean± standard deviation (S.D.). Differences between groups were evaluated by one-way ANOVA. P values less than 0.05 were considered to be significant.

3. Results

3.1. Effects of TACI-Ig on the arthritis scores of collagen-induced arthritis mice

On day 70 after immunization, mice were sacrificed by cervical dislocation. Thymus and spleens were removed under sterile conditions and thymocytes and splenocytes were collected. Then cells were suspended in a DMEM medium at a concentration of 1×107 cells/l. The cell suspension was purified with a lymphocyte separating medium. Then the cells were washed with PBS buffer three times.

The cell suspension (100 μl) and ConA (100 μl with a final concentration of 3 mg/l) or LPS (100 μl with a final concentration of 4 mg/l) were seeded into a 96-well culture plate simultaneously. Triplicates were designed. The cultures were incubated at 37 °C in an incubator (SHEL LAB Co.) of 5% CO2 for 48 h, 6 h before the end, 3H-TdR 20 μl was added to each well. The radioactivity was measured by LS6500 liquid scintillation counter (Beckman Co.). The results were described as the average of triplicate counts per minute (Xu et al., 2002).

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2.13. Statistical analysis

All data were expressed as mean± standard deviation (S.D.). Differences between groups were evaluated by one-way ANOVA. P values less than 0.05 were considered to be significant.

3. Results

3.1. Effects of TACI-Ig on the arthritis scores of collagen-induced arthritis mice

The onset of paw-swelling was on day 31 after injection of emulsion. The fore-paws of mice swelled before the hind-paws swelled. Medication administration groups were given medication from day 31 to day 70, normal and model groups were given normal sodium simultaneously, and arthritis scores were measured from day 31 every week. Results showed that the swelling peak appeared on day 42, and then declined after day 63. From day 56 to day 70, TACI-Ig (10 and 30 mg/kg) reduced the arthritis scores of model mice significantly. After day 63, TACI-Ig (3.333 mg/kg) was able to reduce the arthritis scores. TACI-Ig (0.350 and 1.105 mg/kg) and IgG-Fc had no obvious effect on the arthritis scores (Fig. 1).

3.2. Effects of TACI-Ig on injuries of joints in collagen-induced arthritis mice

On day 70, both fore- and hind-paws of the animals were removed and X-rayed. Then the hind paws of mice were fixed, decalcified and embedded in paraffin for histological analysis. Radiographs from model mice that showed signs of arthritis with disfiguration, osteolysis and osteophyte production were compared with normal mice. Radiographs from TACI-Ig (3.333, 10, and 30 mg/kg) treated mice showed no disfiguration, and less osteophyte production than model mice. Radiographs from the mice of TACI-Ig (0.350 and 1.105 mg/kg) and IgG-Fc treated groups showed signs of arthritis too. Radiograph scores of model mice were increased compared with normal mice. TACI-Ig (3.333, 10, and 30 mg/kg) reduced the increased radiograph scores of model mice significantly, but TACI-Ig (0.350 and 1.105 mg/kg) and IgG-Fc had no obvious effect on radiograph scores (Fig. 2).

In normal mice, synovioectyes were monolayer and articular cartilages were not infiltrated with inflammation. In model mice, synovioectyes proliferated to multilayers, and articular cartilages were destructed and infiltrated with inflammatory cells. The hyperplastic synovium in model mice formed a large number of fibroblasts and new blood vessels. In mice given TACI-Ig (10 and 30 mg/kg), the synovial hyperplasia and the pannus were inhibited and the destruction of articular cartilages was alleviated. In mice given TACI-Ig (3.333 mg/kg), synovioectyes proliferated to 3 or 4 layers, and articular cartilages were infiltrated with inflammatory cells, but there was a tendency of alleviation. TACI-Ig (0.350 and 1.105 mg/kg) and IgG-Fc had no significant improvement on the histopathology of joints in collagen-induced arthritis mice. Inflammatory scores of joints in model mice were increased in comparison with normal mice. TACI-Ig (3.333, 10, and 30 mg/kg) reduced the increased inflammatory scores of model mice, but TACI-Ig (0.350 and 1.105 mg/kg) and IgG-Fc had no obvious effect (Fig. 3).

3.3. Effects of TACI-Ig on histopathology of spleens in collagen-induced arthritis mice

The normal spleen architecture included two major functional zones, the hematogenous red pulp and the lymphoid white pulp. White pulp was considered to be composed of lymphoid follicles and periarteriolar lymphoid sheaths. In model mice, white pulp proliferated, germinal centers emerged, and spleens were infiltrated with inflammatory cells. TACI-Ig (3.333, 10, and 30 mg/kg) alleviated these abnormalities on varying degrees. TACI-Ig (0.350 and 1.105 mg/kg) and IgG-Fc had no significant improvement on the histopathology of spleens in collagen-induced arthritis mice. Pathology scores of spleens
in model mice were increased compared with normal mice. TACI-Ig (3.333, 10, and 30 mg/kg) reduced the increased pathology scores of model mice, but TACI-Ig (0.350 and 1.105 mg/kg) and IgG-Fc had no obvious effect (Fig. 4).

3.4. Effects of TACI-Ig on indices of thymus and spleen in collagen-induced arthritis mice

The indices of thymus and spleen were assayed on day 70. Results showed that thymus index and spleen index of model mice were increased compared with normal mice. TACI-Ig (3.333, 10, and 30 mg/kg) moderated the increase of thymus and spleen indices of collagen-induced arthritis mice. TACI-Ig (0.350 and 1.105 mg/kg) and IgG-Fc had no obvious effect on indices of thymus and spleen (Fig. 5).

3.5. Effects of TACI-Ig on T lymphocyte and B lymphocyte proliferations in collagen-induced arthritis mice

Purified thymocytes are T cells, and purified splenocytes are B cells in major, which also contain a small amount of T cells. Results showed that T lymphocyte and B lymphocyte proliferations in model mice were increased compared with normal mice. TACI-Ig (3.333, 10, and 30 mg/kg) could reduce ConA-induced T lymphocyte proliferation, and reduce LPS-induced B lymphocyte proliferation in collagen-
induced arthritis mice. TACI-Ig (0.350 and 1.105 mg/kg) and IgG-Fc had no obvious effect on T lymphocyte and B lymphocyte proliferations (Fig. 6).

### 3.6. Effects of TACI-Ig on serum BLyS and PGE2 in collagen-induced arthritis mice

The level of serum BLyS and PGE2 was assayed by ELISA. Results showed that the concentration of serum BLyS and PGE2 was increased in model mice. TACI-Ig (3.333, 10, and 30 mg/kg) could reduce the concentration of serum BLyS and PGE2 significantly. The concentration of serum BLyS in TACI-Ig (1.105 mg/kg) treated mice was decreased also. TACI-Ig (0.350 mg/kg) and IgG-Fc had no obvious effect on the level of serum BLyS and PGE2 in collagen-induced arthritis mice (Fig. 7).

### 3.7. Effects of TACI-Ig on the subsets of T lymphocytes and B lymphocytes in collagen-induced arthritis mice

The subsets of T lymphocytes and B lymphocytes were assayed by flow cytometer. Results showed that the percentages and numbers of activated T helper cells (Th cells) (CD4^+CD25^+ and CD4^+CD69^+) and Th cells expressing costimulatory molecules CD154 (CD4^+CD154^+) in thymus of model mice were significantly higher compared with normal mice. Unactivated Th cell (CD4^+CD62L^+) counts were reduced in model mice. TACI-Ig (1.105, 3.333, 10, and 30 mg/kg) could reduce the percentages of CD4^+CD25^+ and CD4^+CD154^+ cells. TACI-Ig (3.333, 10, and 30 mg/kg) could reduce the percentage of CD4^+CD69^+ cells, and increase the percentage of CD4^+CD62L^+ cells. But these various dosages of TACI-Ig had no obvious effect on the percentage of CD3^+CD4^+ cells. We choose TACI-Ig 10 mg/kg as an optimal dosage, and show its effects on the subsets of T lymphocytes in a figure (Fig. 8).
In addition, the percentages of immature B cells (CD19+IgM+), activated B cells (CD19+CD23+ and CD19+CD21+), memory B cells (CD19+CD27+) and mature B cells (CD19+IgD+) were increased in model mice. TACI-Ig (1.105, 3.333, 10, and 30 mg/kg) could reduce the percentage of CD19+IgD+ cells. TACI-Ig (3.333, 10, and 30 mg/kg) could reduce the percentages of CD19+IgM+, CD19+CD23+ and CD19+CD21+ cells. But these various dosages of TACI-Ig had no obvious effect on the percentage of CD19+CD27+ cells. Similarly, we choose TACI-Ig 10 mg/kg as an optimal dosage, and show its effects on the subsets of B lymphocytes in a figure (Fig. 9).

4. Discussion

Rheumatoid arthritis is a disabling systemic autoimmune disease of indefinite pathogenesis characterised by a chronic inflammatory process that leads to the destruction of the synovial membrane, cartilage, and bone. It is associated with a worsening quality of life and reduced life expectancy because of joint swelling, arthrodynia and
impaired joint function. BlyS-transgenic mice have elevated serum immunoglobulin levels, including rheumatoid factor, as well as circulating immune complexes (Khare et al., 2009). The elevated serum BlyS level and synovial fluid BlyS level have been detected in rheumatoid arthritis patients, and the concentration of BlyS in rheumatoid arthritis synovial fluid has been reported to be higher than the serum concentration of BlyS (Cheema et al., 2001). Therefore, BlyS has been considered as an ideal therapeutic target for rheumatoid arthritis. Presently, there are mainly three drugs (Belimumab, BR3-Fc, and TACI-Ig) in clinical trials being used to treat rheumatoid arthritis through inhibiting BlyS (Vital and Emery, 2008).

In animal studies TACI-Ig resulted in a reduction in mature B cells in peripheral blood and lymphoid organs, as well as a reduction in circulating immunoglobulin (Bilsborough et al., 2008; Carbonatto et al., 2008). In a phase Ib trial in human rheumatoid arthritis there was a reduction in rheumatoid factor (RF) and anti-CCP antibodies as well as serum immunoglobulin. The drug penetrated inflamed joints and there was a trend to improved disease activity and good safety profile (Tak et al., 2008).

In the present study, we have found that TACI-Ig could inhibit secondary inflammatory reactions and histopathological injuries of joint and spleen, moderate the increase of spleen indices and thymus indices, and modulate radiological changes of paw in collagen-induced arthritis mice. These findings prove that TACI-Ig attenuates the severity of arthritis, and reverses structural changes in collagen-induced arthritis mice. It is known to all, rheumatoid arthritis is a disabling disease because of the destruction of cartilage and bone. The direct effect of TACI-Ig on disfigurement, osteolysis and osteophyte production has a high potential therapeutic value for clinical treatment of rheumatoid arthritis.

Further studies showed that TACI-Ig modulates the level of BlyS and PGE2 in serum. High concentrations of PGE2 have been detected in the synovial fluid of patients with rheumatoid arthritis, and cytokine-activated synovial cells are a primary source of PGE2 in affected joints. Cyclooxygenase 2 (COX-2) and microsomal PGE synthase 1 (mPGES-1), both of them being enzymes involved in PGE2 generation (Kapoor et al., 2006; Kojima et al., 2002, 2005; Murakami et al., 2000; Stichtenoth et al., 2001), are highly expressed in the synovium of patients with rheumatoid arthritis and in models of arthritis (Kojima et al., 2005; Murakami et al., 2003; Westman et al., 2004). Furthermore, PGE2 is specifically implicated in the symptoms of arthritis because neutralizing antibody against PGE2 is able to inhibit acute and chronic inflammation in the rat adjuvant arthritis model (Kojima et al., 2009). Our findings that TACI-Ig inhibits inflammation in collagen-induced arthritis mice accompanying the decreased serum level of BlyS and PGE2 suggest that TACI-Ig plays its anti-inflammation role by reducing the level of inflammation mediator PGE2 in serum.

Moreover, TACI-Ig could inhibit T and B cells’ proliferative response, and inhibit differentiation and activity of T cells and B cells in collagen-induced arthritis mice. ConA is regarded as a mitogen for mouse T cells. ConA-induced T lymphocyte proliferative response reflects the reproductive activity of T cells. LPS can stimulate polyclonal mouse B-cell proliferation and is regarded as a mitogen for mouse B cells. LPS-induced B lymphocyte proliferative response is used for evaluating the reproductive activity of B cells. But it has been demonstrated that LPS-activated B cells acquire differential modulatory effects on T-cell polarization, and such modulatory effects of B cells on T cells are dependent on the stimulation with LPS in a dose-dependent manner (Xu et al., 2008). So, LPS-induced splenic lymphocyte activation may be T cells proliferation. The study presented here demonstrates that TACI-Ig could inhibit T and B cells’ proliferative response in collagen-induced arthritis mice without excluding the effects of LPS-activated B cells on T cells.

Th cells are a sub-group of lymphocytes that play an important role in establishing and maximizing the capabilities of the immune system. Th cells are involved in activating and directing other immune cells, and are particularly important in the immune system. They are essential in determining B cell antibody class switching, in the activation and growth of cytotoxic T cells, and in maximizing bactericidal activity of phagocytes such as macrophages. Although macrophages and neutrophils are the major sources of mediators of inflammation, CD4+ T cells play a key role in the initiation and perpetuation of collagen-induced arthritis by producing IFNγ, a potent inducer of the inflammatory response (Brand et al., 2003). CD4+CD25+ T cells have been reported to play a critical role in the regulation of autoimmune diseases, including rheumatoid arthritis and collagen-induced arthritis (Luys et al., 2002; Morgan et al., 2003, 2005; Van Amelsfort et al., 2004; Wraith et al., 2004; Yudoh et al., 2000).

Results showed that TACI-Ig down-regulated CD4+CD25+ T cells, the early T cell activation marker CD69 and costimulatory molecules CD154 expressed on CD4+ cells in thymuses of collagen-induced arthritis mice, and up-regulated T-cell unactivation marker CD62L expressed on CD4+ cells in thymuses of collagen-induced arthritis mice, but had no obvious effect on the percentage of CD3+CD4+ cells. This finding demonstrates that TACI-Ig accommodates T cell immune response through inhibiting the differentiation and activation of CD4+ T cells, instead of reducing the counts of CD4+ T cells. It is often assumed.
Fig. 8. Effects of TACI-Ig on the subsets of T cells in collagen-induced arthritis mice. The expression of molecules on T cells was detected by flow cytometer. Percentage of cells in each upper right quadrant in dot plot is indicated. TACI-Ig (10 mg/kg) could reduce the percentages of CD4+CD25+, CD4+CD154+, and CD4+CD69+ cells, and increase the percentage of CD4+CD62L+ cells.
Fig. 9. Effects of TACI-Ig on the subsets of B cells in collagen-induced arthritis mice. The expression of molecules on B cells was detected by flow cytometer. Percentage of cells in each upper right quadrant in dot plot is indicated. TACI-Ig (10 mg/kg) could reduce the percentage of CD19⁺IgM⁺, CD19⁺CD23⁺, CD19⁺CD21⁺ and CD19⁺IgD⁺ cells.
that immunological memory depends on populations of memory CD4 T cells that are long-lived. This assumption was questioned by adoptive transfer experiments, reported several decades ago, that observed a rapid loss of memory in recipients that were not challenged immediately with specific antigen (Gray and Matzinger, 1991). Our finding is likely demonstrating that TACI-Ig has no effect on the immunological memory of T cell.

CD19 is a B cell-specific surface antigen that is expressed by early pre-B cells from the time of heavy chain rearrangement until the molecule is eventually down-regulated during terminal differentiation into plasma cells (Nadler et al., 1983). CD19 belongs to the immunoglobulin domain-containing superfamily of transmembrane receptors. As a component of the B-cell receptor complex, CD19 regulates the threshold for B-cell activation (Engel et al., 1995). Relatively small changes in CD19 surface expression can lead to loss of tolerance and autoantibody production (Sato et al., 2004). Recently, it was reported that the surface expression of CD21 and CD23 is regulated by BLYS, and the coordinated expression of the CD21 and CD23 genes is coincident with the survival effects of BLYS (Debnath et al., 2007; Gorelik et al., 2004). Results showed that TACI-Ig down-regulated CD19+CD21+ cells, CD19+CD23+ cells, and autoantibody-producing B cells (CD19+IgM+ and CD19+IgD+) in spleens of collagen-induced arthritis mice, but had no obvious effect on the percentage of CD19+CD27+ memory B cells. This finding demonstrates that TACI-Ig accommodates B cell immune response through inhibiting the differentiation and activation of B cells, which has no effect on the immunological memory of B cells. Taken together, these present findings suggest that TACI-Ig has a good therapeutic action on collagen-induced arthritis, which might be related to the modification of inflammation-potentiating – the abnormal expression of the subsets of T lymphocytes and B lymphocytes in collagen-induced arthritis mice.

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