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Effect of rhTACI-Ig fusion protein on antigen-specific T cell responses from keyhole limpet haemocyanin challenged mice

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ABSTRACT

In addition to modulate B cells function, B cell activating factor belonged to TNF family (BAFF) also regulates T cells response via BAFFR and transmembrane activator and calcium modulator and cyclophilin–ligand interactor (TACI) expressing on activated T cells. This study explored the effect of a recombinant fusion protein containing the extracellular ligand-binding portion of TACI and the Fc portion of human immunoglobulin G (TACI-Ig) on activated T cells that were obtained from antigen-specific T-cell responses mice model induced by keyhole limpet haemocyanin (KLH), the characteristics of KLH-challenged mice were observed simultaneously. KLH immunization leaded to a significant positive relationship between BAFF level in serum and the extent of spleen histopathology. Serum concentration of BAFF, APRIL, IgM and IgG antibodies to KLH, and IL-4 were increased under KLH immunization, but IL-2 synthesis was decreased, resulting in a downregulation of IL-2/IL-4 ratio. Antigen-specific T cells proliferation, IL-5 production, the percentage of Th and activation T cells were significantly upregulated, however, IL-2 secretion and the percentage of naïve T cells were downregulated *in vitro*. RhBAFF co-stimulation further evoked T cells hyperplasy, IL-4 and IFN-γ expression, the subgroups of Th, early antigen activation and activation T cells were also further increased. On the contrary, naïve T cells were further reduced under rhBAFF stimuli. Administration of rhTACI-Ig significantly inhibited T cells proliferation, cytokines production and T cells differentiation, and the inhibitory effects might be associated with its ability to neutralize both the exogenous and endogenetic BAFF and APRIL.

1. Introduction

B cell activation factor from the TNF family (BAFF; also called B lymphocyte stimulator [BLyS]) and a proliferation-inducing ligand (APRIL) are members of the tumor necrosis factor (TNF) family that regulate B-cell maturation, survival and function. BAFF expressed by T cells, monocytes/macrophages, and dendritic cells (DCs) (Batten et al., 2000; Jiang et al., 2011). BAFF transgenic (Tg) mice have an elevated number of B lymphocytes in the periphery, secrete various autoantibodies, and develop a systemic lupus erythematosus (SLE)-like condition leading to severe glomerulonephritis (Wang et al., 2010). BAFF stimulation results in prolonged survival of mature resting B cells (Woodland et al., 2008). The addition of BAFF to cultures of B cells augments class switching of those cells from IgM to IgG (Woo et al., 2011). In humans, high levels of BAFF are detectable in the blood of a percentage of patients with autoimmune diseases, including SLE, rheumatoid arthritis (RA) and Sjögren's syndrome (Bosello et al., 2008; Mandik-Nayak et al., 2008; Marston and Looney, 2010; Varin et al., 2010; Yang et al., 2009). BAFF and APRIL are overexpressed in the synovial fluid as well as in the sera (Tan et al., 2003).

As stated above, inhibition of BAFF signaling is a potential therapeutic option for treatment of autoimmune diseases. BAFF promotes B cells survival and binds to three receptors: B cell maturation antigen (BCMA), transmembrane activator and calcium-modulating and cyclophilin–ligand CAML interactor (TACI) and BAFF-R (Schiemann et al., 2001). All three are expressed on $CD19^+$ B lymphocytes, whereas TACI and BAFFR are expressed by both B cells and activated T cells (Chang et al., 2011). A novel B cell-targeting biological therapeutic drug for the treatment of RA that contains the BAFF-binding extracellular portion of the TACI molecule fused to the Fc portion of human IgG1 (TACI-Ig) is under investigation (Townsend et al., 2010). TACI-Ig can be used to neutralize BAFF or APRIL and prevent them from binding to their receptors on T, B lymphocytes. In human trials for RA and SLE, the effects of TACI-Ig on plasma cells have been demonstrated

Abbreviations: BAFF, B cell activating factor belonged to TNF family; TACI, transmembrane activator and calcium modulator and cyclophilin–ligand interactor; KLH, keyhole limpet haemocyanin; APRIL, a proliferation-inducing ligand; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

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by a rapid drop in serum immunoglobulins and autoantibodies (John Looney et al., 2010). Elevated levels of native BAFF/APRIL heterotrimers, as well as BAFF and APRIL homotrimers in patients with autoimmune diseases are inhibited by TACI-Ig (Dillon et al., 2010). Effects of BAFF and corresponding antagonist TACI-Ig on B cells have been deeply studied; however, less attention has been paid to their potential action on activated T cells. Moreover, T cells also play an important part in the pathology of RA through antigen specific T cells mediated autoimmunity (Raza et al., 2005). Here, we used keyhole limpet haemocyanin (KLH), a copper-containing protein isolated from the mollusc *Megathura crenulata*, to induce an antigen-specific T-cell responses model to further explore the inhibitory effect of TACI-Ig on activated T cells.

2. Materials and methods

2.1. Animals

Adult male C57/BL6 mice weighing 25–30 g (Animal Department of Anhui Medical University, Hefei, Anhui Province, China, Certificate No.: SCXK (Anhui) 2005-0001) were used for all experiments. All 20 mice were acclimatized under standard laboratory conditions. During the experimental period, tap water and commercially available food were given freely. The lighting duration in the breeding room was 12 h (7:00 am to 7:00 pm). The room temperature was 24 °C. All experiments were approved by Ethics Review Committee for Animal Experimentation of Institute of Clinical Pharmacology, Anhui Medical University.

2.2. Materials and reagents

RhTACI-Ig and IgG-Fc were kind gifts from Dr. W.X. Wang (Rongchang Pharmaceutical Research Institute, Beijing, China). RhTNFR:Fc fusion protein, a soluble human tumor necrosis factor receptor fusion protein, was purchased from Shanghai CP Guojian Pharmaceutical Co., Ltd. (China). KLH was purchased from Genway Biotech Inc. (San Diego, CA). Bacillus Calmette Guerin (BCG) was the product of Beijing Institute of Biological Products (China). Enzyme-linked immunosorbent assay (ELISA) kits for KLH-immunoglobulins G (IgG), KLH-IgM, BAFF, APRIL, interleukin (IL)-2, and IL-4 were purchased from Research & Development (R&D) Co., Ltd. (US). Anti-mouse FITC-CD4, PE-CD69, PE-CD154 and PE-CD62L antibodies were the products from eBioscience, Inc. (San Diego, CA).

2.3. KLH-challenged mice

For anti-KLH responses, 10 mice were immunized (via the root of tail) with KLH (100 μg) in saline, in a 1:1 emulsion with complete Freund's adjuvant (CFA) that contained mycobacterium tuberculosis strain H37Ra (1 mg/ml, Sigma). Five days after immunization, the same emulsion was injected intradermally again in one hind footpad and saline in the other hind footpad. After 8 days, the spleens were removed and T cell suspensions were prepared as method mentioned as follows (Van Panhuys et al., 2008). Serum levels of anti-KLH-IgG, anti-KLH-IgM, IL-2, IL-4, BAFF and APRIL were detected.

2.4. Histological examination of KLH-challenged spleens

For histopathological analysis, spleens from KLH-challenged mice were fixed in 10% buffered formalin and embedded in paraffin. Spleen sections (3–5 μm) were prepared and stained with hematoxylin and eosin. Microscopic evaluation of KLH-challenged spleen was done by an individual who did not know whether the animals had been challenged. Five compartments were evaluated: cellularity of periarteriolar lymphoid sheaths (PALS), lymphoid follicles, marginal zone (MZ), 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) (Chang et al., 2011).

2.5. Purification of T cells from mice spleen

Nylon fibers column (Wako Chemicals USA, Inc.) was used to isolate spleen T cells. Before use, the column was equilibrated by washing with 20 ml Dulbecco's modified Eagle's medium (DMEM), sealed and incubated for 30 min at 37 °C and 5% CO_2. Spleens were removed, ground through a 70 μm cell strainer, washed with Hanks balanced salt solution and red blood lysed (Sigma RBC lysis buffer). Cells (1.5×10^8) subjected to nylon wool purification were resuspended in 2 ml of warm DMEM, loaded onto the column, and washed with 2 ml warm DMEM. The column was sealed and incubated at 37 °C and 5% CO_2 for 45 min. Cells were then eluted with 10 ml warm DMEM (Lee et al., 2009). Greater than 90% of B cells were removed by this procedure as assessed by flow cytometry.

2.6. Cell proliferation and supernatants cytokines detection

Proliferation was measured by the addition of [^{3}H] thymidine (1 μCi, Academia Sinica, China) for the last 18 h of a 3-day culture; incorporation of radioactivity was analyzed by liquid scintillation counter (LS6500, Beckman, US) (Mosmann, 1983). Purified T cells from immunized mice were cultured (1×10^6/well) in rhBAFF (PeproTech, 0.1 μg/ml) with or without different concentrations of rhTACI-Ig (0.01, 0.1, 1, 10, 100 μg/ml) in DMEM supplemented with 5% fetal calf serum (FCS). Concentrations of IL-2 (in a 24 h culture system), IL-4, IL-5 and IFN-γ (in 72 h culture systems) in cell culture supernatants were determined using ELISA kits obtained from R&D Systems. All assays were performed according to manufacturer's instructions. Optical densities were measured at 450 nm with an ELISA plate reader (ELx808, BioTek, US).

2.7. Flow cytometry analysis

Splenic T cells were bulk cultured (1×10^6/ml) in rhBAFF (0.1 μg/ml) with or without different concentrations of rhTACI-Ig for 3 days. Single-cell suspensions were stained with fluorescence conjugated monoclonal antibodies to CD3, CD4, CD69, CD154 or CD62L (eBioscience) for 30 min at 4 °C. Cell-associated fluorescence was analyzed with a FAC Scan instrument (Epics XL, Beckman Coulter) and associated Cell Quest software (Varfolomeev et al., 2004).

2.8. Statistical analysis

For all experiments, at least triplicate determinations were made for each experimental condition. In indicated cases, the results of representative experiments were shown. All data are expressed as mean and standard deviation (SD). Comparisons between two experimental groups were performed using Student's *t*-test, for multiple group comparisons we used ANOVA with Tukey's post test. Results were considered statistically significant for *P* values less than 0.05.

3. Results

3.1. Inflammatory process and pathological changes in spleens of KLH-challenged mice

To assess whether rhTACI-Ig can abrogate antigen-specific T cell responses *in vitro*, we immunized mice (via 1–2 sites at the base of the tail) with KLH protein suspended in CFA. Five days after immunization, we challenged the animals again with KLH