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BAFF and its receptors involved in the inflammation progress in adjuvant induced arthritis rats

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ABSTRACT

This study is in order to clear the roles of BAFF and its receptors in the inflammation course of autoimmune arthritis. We used a T cell-mediated experimental autoimmune model adjuvant-induced arthritis (AA) rat to study the profiles of BAFF and its receptors in spleen during the inflammation arthritis induction and the effects of BAFF on DCs functions. *In vivo*, the levels of BAFF and the expression of BAFF-R, TACI were increased in spleen from very early stage of AA. The lesions of spleen were definite correlated with increased levels of BAFF in homogenization. The mature of DCs and increased number in spleen were mainly at early stage of arthritis. In addition, the levels of Interleukin (IL)-12 were found highest and IL-10 were found lowest at this time too. *In vitro* recombinant BAFF promoted maturation of DCs and inhibited the phagocytosis of DCs. Under stimulation of BAFF on DCs, the levels of tumor necrosis factor (TNF)-α, IL-6, IL-12 were increased, IL-10 and transforming growth factor (TGF)-β1 were decreased. Moreover, BAFF-treated DCs induced proliferation of $CD4^+$ T cell. These findings support the crucial pathogenic role of DCs, BAFF, and its receptors in the development of experimental arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease, it is characterized by persistent chronic synovial membrane inflammation, progressive destruction of cartilage and bone [1,2]. In pathogenesis of RA, dendritic cells (DCs), monocytes, macrophages, B cells and T cells all contribute to both systemic inflammation and locality synovitis [3–5].

B cell activating factor (BAFF) is a member of the TNF ligand superfamily, also named B lymphocyte stimulator (BLyS) [6]. The production of BAFF come predominantly from DCs, monocytes, macrophages, astrocytes, fibroblast-like synoviocytes (FLS) and neutrophils [7,8]. Elevated levels of BAFF have been detected in serum and synovial fluid (SF) from RA patients [7,8]. It is worth noting that the increased level of BAFF correlated with disease activity in autoimmune diseases [9–11]. As we all known, BAFF plays an importance role in B cells homeostasis and maturity. BAFF antagonists such as TACI-Ig has been proved to ameliorate disease progression of collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AA) suggests that BAFF affects not only B cells but also T cells [12–14].

BAFF binds to three distinct receptors: B-cell maturation antigen (BCMA), transmembrane activator and calcium-modulator and cytophilin ligand interactor (TACI) and BAFF receptor (BAFF-R/BR3) [15]. BAFF-R is the specific receptor for BAFF, while BCMA and TACI, also bind to a second related TNF family ligand, a proliferation inducing ligand (APRIL). Though many evidences confirmed disturbances expression of both BAFF and its receptors in autoimmune diseases include RA [16–21], but the roles of BAFF and its receptors in the autoimmune diseases course are still unclear.

In this study, we examined the expression profiles of BAFF as well as its three receptors in spleen in AA rat model during the inflammation course. Our findings provide insight into the expression and effects of BAFF in the initiation of the immune response in secondary lymphoid organs, which suggest that BAFF promote progression of arthritis maybe by inducing mature of DCs. Furthermore, co-culture studies of BAFF-treated DCs and $CD4^+$ T cells have revealed a potent role for BAFF-treated DC in enhancing $CD4^+$ T cells proliferation.

2. Materials and methods

2.1. Animals

Lewis rats (male, 150–180 g) were obtained from Bei Jing vital river a charles river company. All the rats were housed under standard laboratory conditions and treated under the Guidelines for Laboratory Animal Research approved by Ethics Review Committee of the Institute of Clinical Pharmacology, Anhui Medical University (Certificate No.

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LLSC2013007). The room condition was kept at temperature 22 °C ± 2 °C, humidity (55 ± 15%) and a 12-hour light/dark schedule.

2.2. Reagents

Alexa Fluor 488 anti-rat CD103 (αE Integrin), Alexa Fluor 488 Mouse IgG1, κ isotype Ctrl (FC) were purchased from BioLegend, Anti-Mouse/Rat CD40 APC, Anti-Rat CD86 (B7-2) PE, Anti-Rat MHC Class II PE, Mouse IgG1 K Isotype Control PE were purchased from eBioscience. FITC-Dextronx were purchased from Sigma. IntegrinαE (CD103) antibody was purchased from BOSTER. Enzyme-linked immunosorbent assay (ELISA) kit for BAFF was purchased from Uscn Life Science Inc. ELISA kit for TNF-α, IL-6 and TGF-β1 was purchased from eBioscience. IL-10, IL-12/P70 was purchased from CUSABIO life science, anti-BAFF-R antibody (sc-28989), anti-BCMA antibody (sc-11746), and anti-TACI antibody (sc-28987), were purchased from Santa Cruz, CA, USA.

2.3. Induction of AA

Rats were immunized by intradermal injection into the right hind metatarsal footpad with 0.1 ml (10 mg/ml) of Complete Freund's adjuvant (CFA was prepared by suspending heat-killed *Mycobacterium butyricum* in liquid paraffin).

2.4. Assessment of AA

Rats were inspected daily by two independent observers. From day 7 after immunization, non-injected hind paw swelling and polyarthritis index were detected and detailed recorded. The non-injected hind paw swelling was measured with a volume meter (YLS-7B, Ji Nan, China). The arthritic severity in each paw was evaluated through 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 four paws of each rat was used as the polyarthritis index with a maximum value of 16 [12].

2.5. Histological examination

Rats were sacrificed after ether anesthesia, synovium and spleen were fixed in 4% paraformaldehyde. The serial paraffin sections were stained with hematoxylin and eosin (HE) and histopathological changes were evaluated under blinded conditions. The changes of spleen were assessment as before [12,22]. The grading scheme is from 0 (no change) to 4 (severe lesion), the cumulative score of each rat with a maximum value of 20.

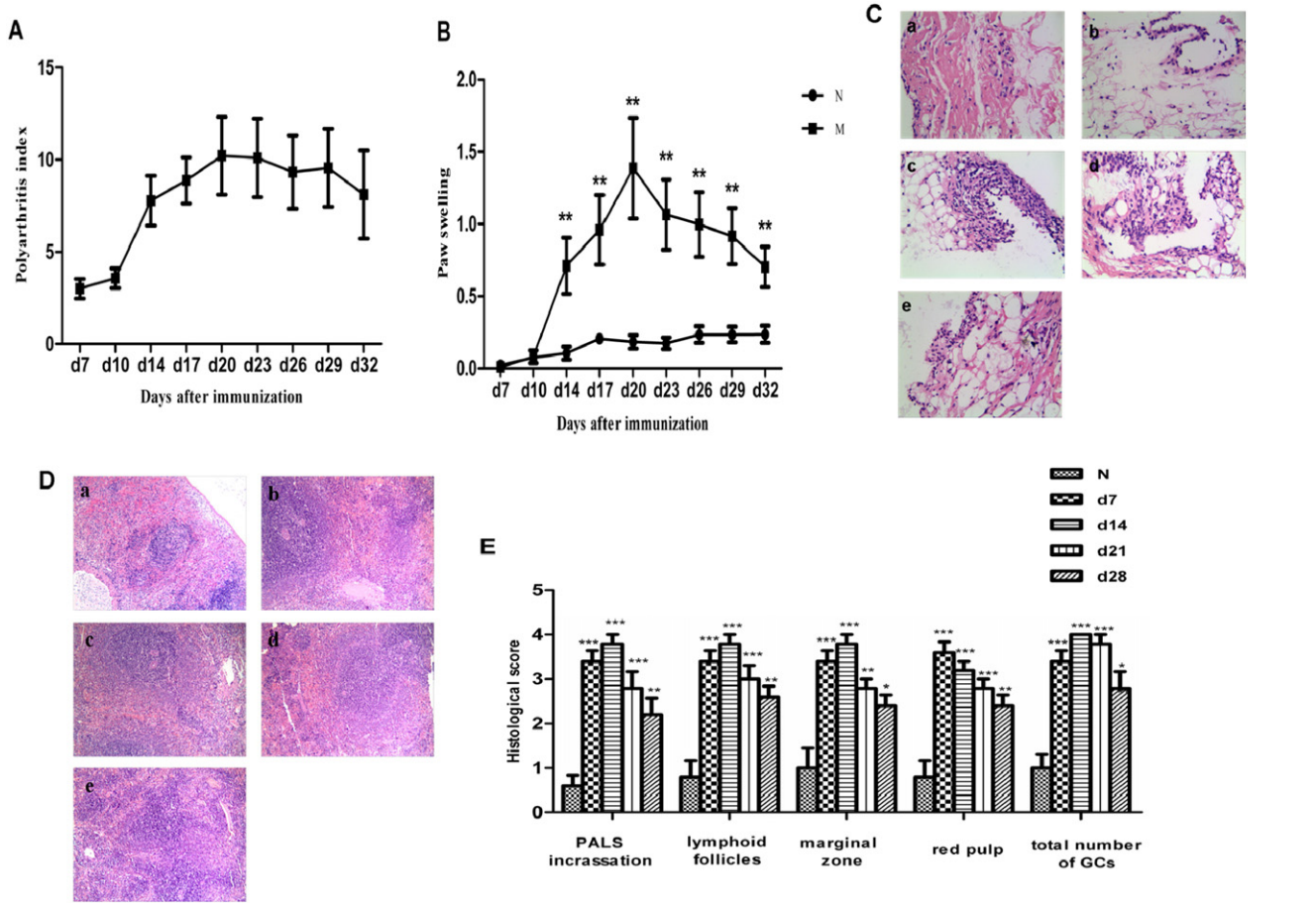

Fig. 1. The changes of clinical parameters and histological pathology in the inflammation progress of AA rats. A: Polyarthritis index for all paws, B: paw swelling of non-injected hind (n = 12, mean ± S.D.). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* normal group. Lewis rats were immunized with CFA on day 0, and were assessed from day 7 every three to four days, C: histological pathology of synovium (HE, 100×). a: Normal rat; b: 7 days after immunized with CFA; c: 14 days after immunized with CFA; d: 21 days after immunized with CFA; e: 28 days after immunized with CFA, D:histological pathology of spleen(HE, 100×) the groups are the same as "C", E: histopathological score of spleen (n = 5, mean ± S.D.). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* normal group.