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Therapeutic efficacy of three bispecific antibodies on collagen-induced arthritis mouse model

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ABSTRACT

Interleukin-1β (IL-1β) and interleukin-17A (IL-17A) are inducible factors and important cytokines in the pathogenesis of rheumatoid arthritis (RA). In the present study, three bispecific and neutralizing antibodies (BsAB-1, BsAB-2 and BsAB-3) against both hIL-1β and hIL-17A were constructed, their therapeutic efficacy was compared on collagen induced arthritis (CIA) model mice. In vitro assays demonstrated that the three antibodies could simultaneously bind to target both hIL-1β and hIL-17A. Mice with CIA were subcutaneously administered with one of three antibodies every two days for 29 days, we noticed that, compared with the BsAB-2 and BsAB-3, BsAB-1 antibody therapy resulted in more significant effect on alleviating the severity of arthritis by preventing bone damage and cartilage destruction and substantially decreasing production of CII-specific antibodies. In addition, BsAB-1 antibody was more potent in the inhibition of mRNA expression of IL-2, IL-1β, IL-17A, TNF-α and MMP-3 in the spleen of CIA mice compared to the other two. In summary, BsAB-1 is superior over BsAB-2 and BsAB-3 for the treatment of RA model mice, and may be chosen as an ideal candidate for further development of therapeutic drugs for treatment of RA.

1. Introduction

Rheumatoid arthritis (RA) characterized by synovial inflammation is one of the most common human autoimmune diseases. A large number of inflammatory cytokines express in diseased joints [1,6,7], TNF-α and IL-1β play crucial roles in the recruitment of leukocytes to sites of inflammation, inducement and development RA pathology [2,8,13,14].

Clinical studies have shown that blocking TNF-α and IL-1β was an effective means to mitigate chronic inflammation and damages of articular cartilage [5]. However, the response to these inhibitors made an unsatisfactory effect on a number of patients [3,15]. One of the reasons for these failures may be due to multiple cytokines involved in the disease process. Therefore, it is tempting to speculate that cytokines or factors other than IL-1 and TNF-α also participate in the proinflammatory cytokine cascade [4]. Therefore the development of multiple targets antibody may become an effective means of treatment of RA.

IL-17A is a cytokine secreted by active memory T cells, an increased expression of IL-17A has been detected in the synovial fluid of RA patients [4,9]. IL-17A can stimulate the expression of IL-1β, TNFα, IL-6 and IL-8, suggesting that IL-17A may be an upstream mediator in the RA process [10–12]. IL-17 shares main properties with IL-1β and TNF-α. These cytokines activate the common transcription factor NF-κB in a variety of cell types [16]. Our previous results have shown that when IL-1β and IL-17A were combined, a greater effect than with one cytokine alone was observed [17].

In the present study, three bispecific and neutralizing antibodies with different structures against both hIL-1β and hIL-17A were constructed. The aim is to investigate therapeutic efficacy of the three bispecific antibodies in CIA model mice.

2. Materials and methods

2.1. Materials

Male Kunming mice, 6–8 weeks old, were purchased from Changchun Estar Science And Technology Development Group Co., Ltd. (Jilin, China). They were housed at the animal facility of the

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Laboratory Animals Centre of Northeast Agricultural University. Water and food were provided ad libitum. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Review Board of the Northeast Agricultural University Institute of Biomedicine. Human IL-1β antigen and human IL-17A antigen were purchased from Cell Signaling Technology, Inc. HL-60 and H9C2 cell lines were lab stock.

2.2. Constructions of BsAB-1, BsAB-2 and BsAB-3

The three antibodies were constructed as follows: BsAB-1. A chain was constructed with VH of IL-1β linked with VL of IL-17A by a connecting fragment encoding the first 15 amino acids of CH1, followed by the human CL; B chain was constructed with VL of IL-1β linked with VH of IL-17A by a connecting fragment encoding the first 15 amino acids of CL, followed by the human CH1. The genes of A chain and B chain were cloned into pET-27b vector, respectively, then expressed in *Escherichia coli*. BsAB-2: A chain was formed by VH of IL-1β and VL of IL-17A linked by $(Gly_4Ser)_3$ linker; B chain was formed by VL of IL-1β and VH of IL-17A linked by $(Gly_4Ser)_3$ linker. The vector pExpress used for dicistronic expression of the two chains was constructed from the pET-27b vector. The pelB leader sequence with the ribosome binding site was amplified from pET-27b by primers flanked with *Bam*H I/*Hin*d III. The polymerase chain reaction (PCR) product was digested and cloned into the *Bam*H I/*Hin*d III digested pET-27b vector, containing a pelB signal peptide, to yield the vector pExpress. The genes of A chain and B chain were cloned into the vector pExpress and co-expressed in *E. coli*. BsAB-3: two single-chain Fv fragments, one for hIL-1β, and the other for hIL-17A, were linked by the hinge of human IgG1. The scBsAb1/17 PCR products were cloned into pET-27b vector and expressed in *E. coli*.

All of the three antibodies were purified by the method of inclusion body denaturation and refolding.

2.3. CD spectra

The CD spectra of three proteins were assayed by Spectrometer (ASCO,J-815 CD Spectrometer) and the spectra were in the wavelength range 200–300 nm. The samples of three antibodies dissolved in Tris-HCl buffer (20 nM) at the concentration of 0.2 mg/mL.

2.4. ELISA binding assay

In this assay, three antibodies was tested for their capability in simultaneously binding two target antigens: three antibodies (50 nM) were first incubated with 2 μg/mL hIL-1β (Cell Signaling Technology) in solution at 37 °C for 2 h and then transferred to a microtiter plate coated with 100 ng/well hIL-17A (Cell Signaling Technology) at 37 °C for 1 h, the plates were washed three times with PBST(0.05% Tween-20 in PBS), 100 μL mouse anti-hIL-1β monoclonal antibody (eBioscience) was added and incubated at 37 °C for 1 h, followed by incubation with goat anti-mouse IgG-HRP (R&D) to measure the plate-bound activity. A total of 100 μL TMB solution (1 mg/mL TMB, sodium acetate buffer, pH 6.0, 0.006% H_2O_2) was added into the plates after washing the plates five times with PBST. The reaction was stopped with 50 μL of 1 M H_2SO_4. Absorbance was measured at 450 nm in an ELISA reader.

2.5. Assessment of NO and ROS

HL-60 cells were cultured for 2 h in the presence of IL-17A, which combined with three antibodies at 37 °C for 1 h, respectively. Cells only stimulated with IL-17A as positive control, absence of stimulus as negative control. Put ROS probe (Beyotime) into medium in dark area at 37 °C for 30 min, then wash the cells with PBS three times. In the end cells were suspended with 500 μL PBS and assayed by the fluorescence intensity through FCM (BD). Use IL-1β, which combined with three antibodies at 37 °C for 1 h, respectively, to stimulate the H9C2 cells for 2 h. Put NO probe (Beyotime) into medium in dark area at 37 °C for 30 min, wash the cells with PBS three times. Last suspend the cells with 500 μL PBS and the fluorescence intensity were assayed through FCS (BD).

2.6. Induction of CIA

Chicken type II collagen (CII) was dissolved in 0.1 M acetic acid to a concentration of 2 mg/mL. This was emulsified in equal volumes of Freund's complete adjuvant (CFA, Sigma). The mice were immunized in the right hind paw with 100 μg of chicken CII. On day 8, the mice received an intraperitoneal (IP) booster injection of 100 μg of CII once more. After arthritis occurred, on day 21, the mice were randomly divided into four groups (n = 10), including BsAB-1 group (8 nmol/kg), BsAB-2 group (8 nmol/kg), BsAB-3 group (8 nmol/kg), CIA control group (normal saline), and selecting another normal 10 mice as normal group. Each experimental group was injected corresponding antibodies every two days, the CIA control and normal group were injected the same volume saline as before. The treatment was performed once every two days from day 21 to 49 after the first immunization.

2.7. Assessment of arthritis

Mice were considered as arthritic when significant changes in redness and/or swelling were noted in the digits or in other parts of the paws. Joint swelling was observed and scored every four days, using a scale of 0–4; where 0 = no swelling; 1 = little toe joint swelling; 2 = toe joints and toe swelling; 3 = ankle swollen feet below; 4 = including ankle, including all the paw edema. Scores for the four limbs were summed and each animal was given the resulting score (between 0 and 16). Scoring was done by 3 independent observers, then we got the mean value.

2.8. Measurement of serum for anti-CII antibodies

On the 49th day after the first immunization, blood samples were taken from each mouse and anti-CII IgG antibodies in sera were detected by ELISA. Dilute 2 mg/ mL CII (Sigma) with PBS, then the concentration of CII was 5 μg/mL. The sample was added into the appropriate wells on the plate and incubated over night at 4 °C. Block enzyme-

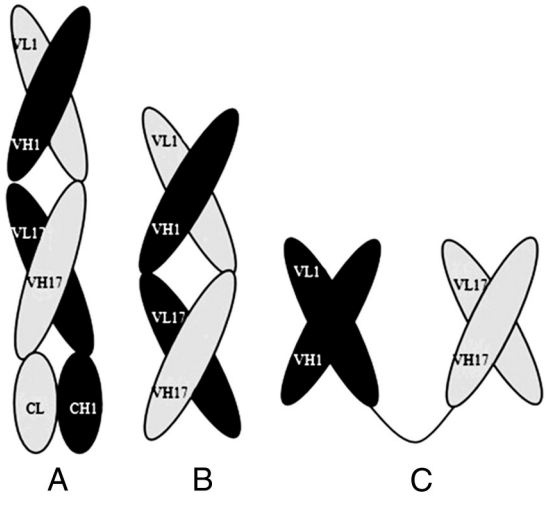

Fig. 1. The hypothetic structure of three antibodies. (A) Linear and hypothetic structure of BsAB-1. (B) Linear and hypothetic structure of the BsAB-2. The two chains are joined together through Inter-chain disulfide. (C) Linear and hypothetic structure of the BsAB-3. The two scFvs are joined together by an interlinker.