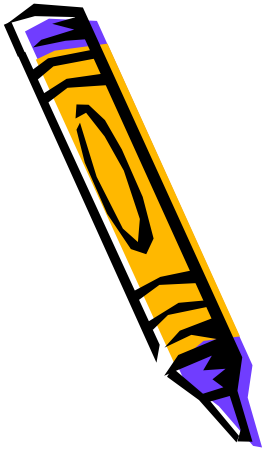
About OMICS Group



- OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

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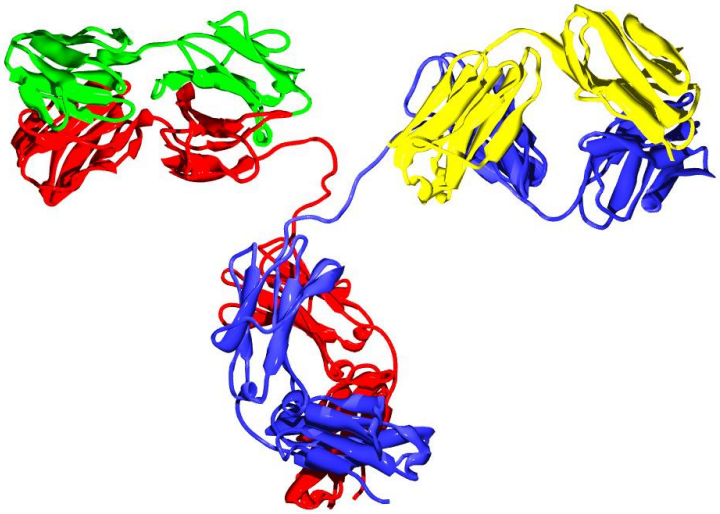
OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Phrama scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

A bispecific antibody against IL-1βand IL-17A is beneficial for rheumatoid arthritis



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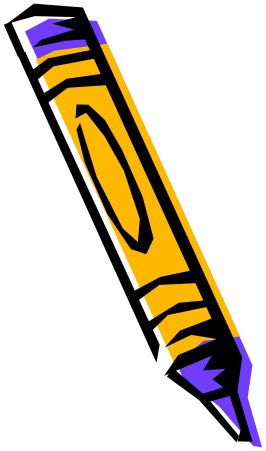
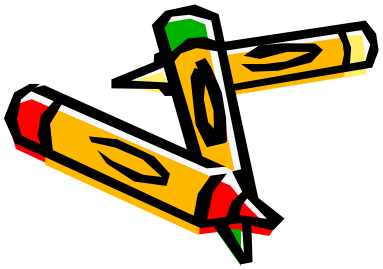
1. Introduction



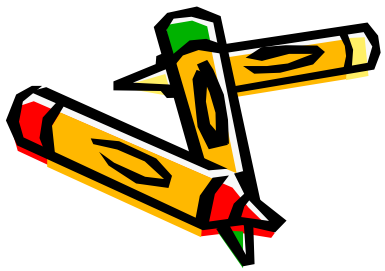
- Rheumatoid arthritis (RA) is one of the most common human auto-immune diseases, characterized by a chronic inflammatory reaction in the synovium of joints. The synovitis involves a massive leukocytic infiltration mainly consisting of macrophages, T lymphocytes and plasma cells. Many proinflammatory cytokines and chemokines expressed in diseased joints are believed to play a pivotal role in recruiting leukocytes to the site of inflammation, and in initiation and progression of the inflammatory process. In particular, tumor necrosis factor a (TNF-a) and interleukin 1 (IL-1) contribute to the main pathology of the disease.



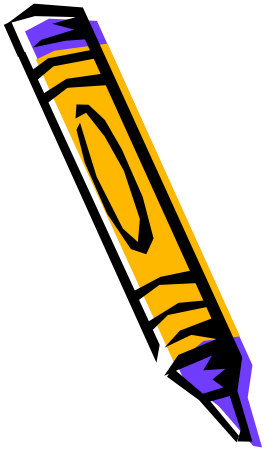
1. Introduction

- Recently, treatments that target TNF-α and IL-1 have begun to be applied in the clinical setting. However, although these therapies can obtain mostly excellent results, they are ineffective in some patients. Therefore, it is tempting to speculate that cytokines or factors

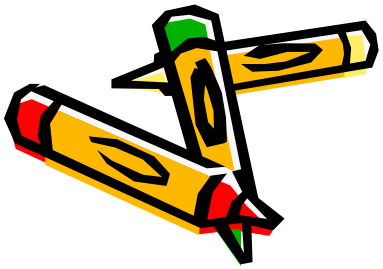
  other than IL-1 and TNF-α also participate in the proinflammatory cytokine cascade.



1. Introduction

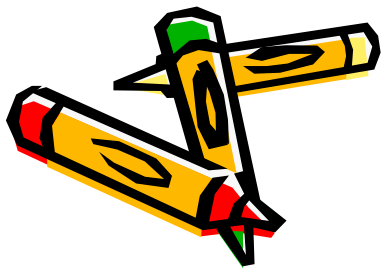


- Interleukin 17 is a recently discovered cytokine that is secreted by a restricted set of cells. IL-17 may play an upstream role in T cell-triggered inflammation by stimulating stromal cells to secrete cytokines and growth factors. It has the capacity to induce IL-6, IL-8, G-CSF, PGE2, and the proinflammatory cytokines TNF-α and IL-1β. IL-17 shares mainly properties with IL-1β and TNF-α. These cytokines activate the common transcription factor NF-κB in a variety of cell types. When IL-1βand IL-17 were combined, an enhancing effect greater than that with one cytokine alone was observed.

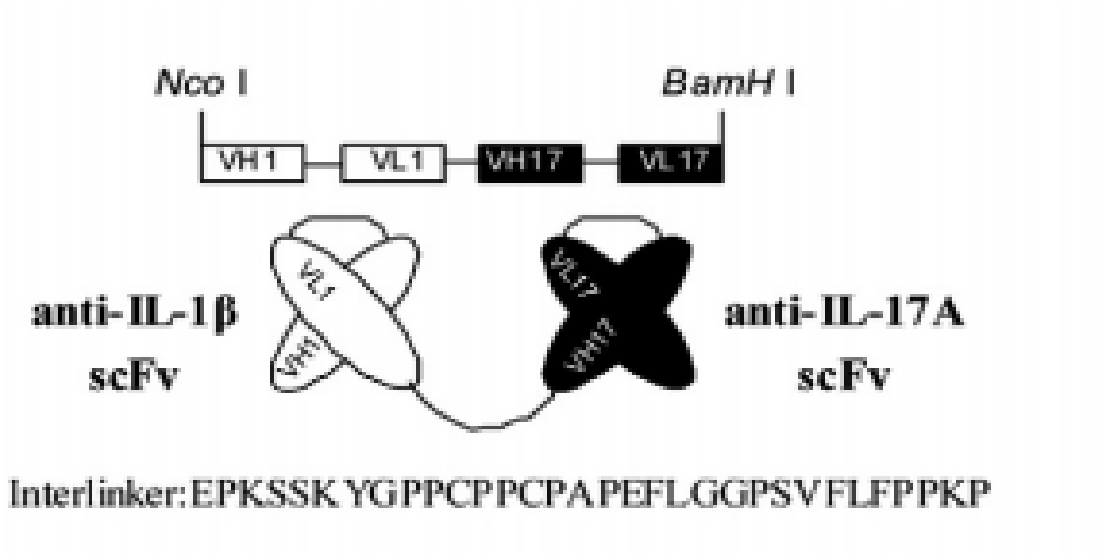


1. Introduction

- In this study, we have analyzed the therapeutic efficacy of scBsAb1/17 on Chick Type II collagen-induced rheumatoid arthritis (CIA) mice. The results showed that scBsAb1/17 has a superior effect than anti-IL-1β antibody or anti-IL-17A antibody alone in CIA mice.

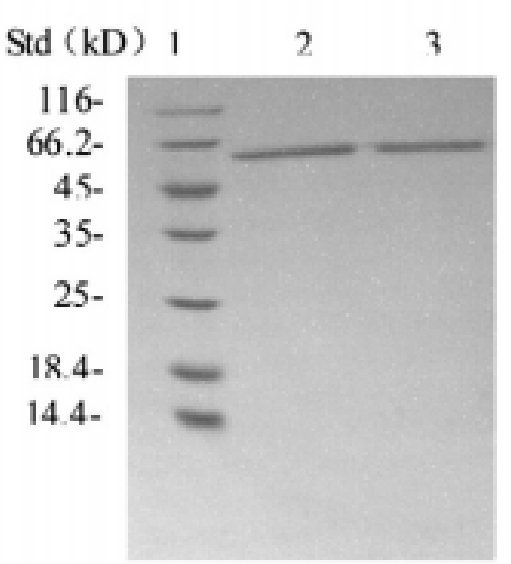


2. Construction of scBsAb1/17

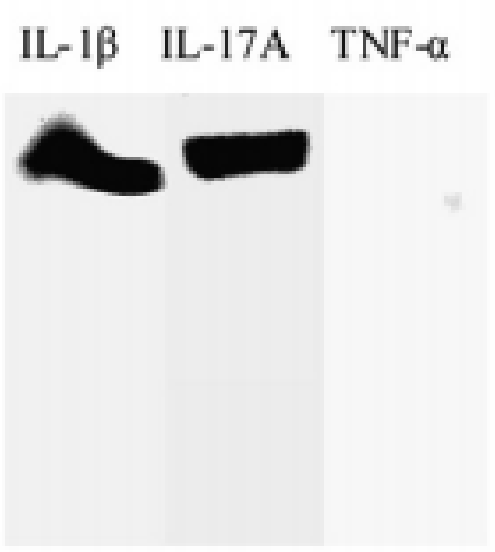


Two single-chain Fv fragments, one for hIL-1β, and the other for hIL-17A, are joined by a 32-amino-acid interlinker from the hinge of the human IgG.

3. SDS–PAGE and western blot analysis of scBsAb1/17

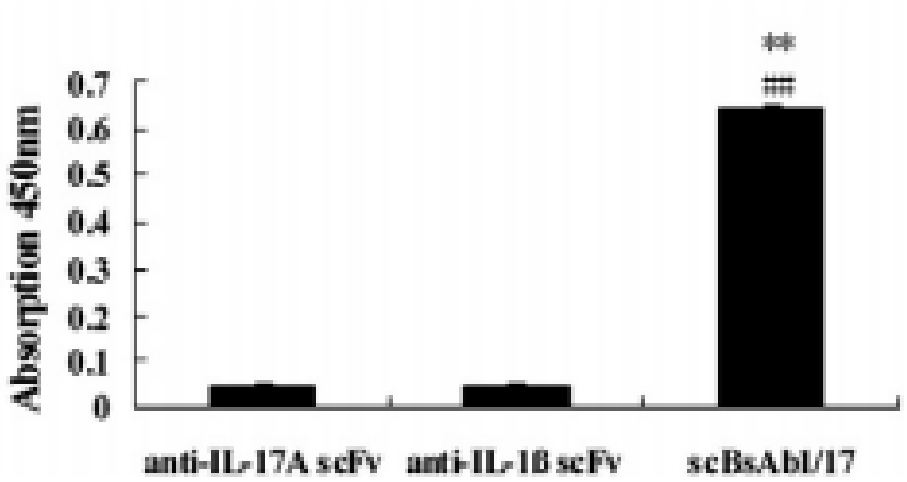


SDS–PAGE analysis of the purified antibodies under non-reducing and reducing conditions. Yield was approximately 50 mg/L. ~57 kDa



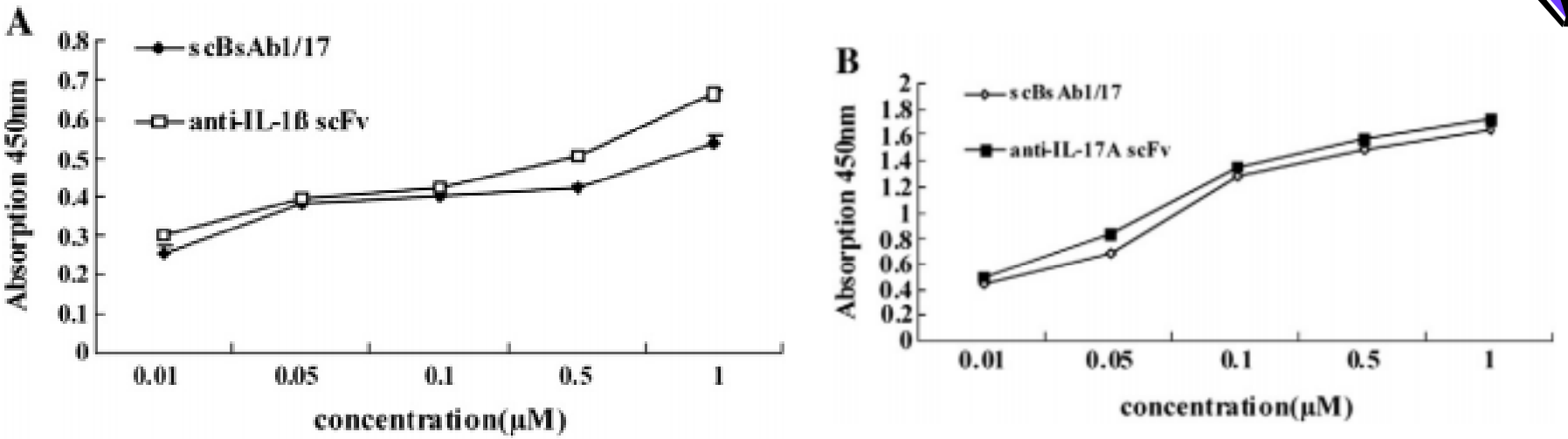
Specificity of scBsAb1/17 was analyzed by Western blot. scBsAb1/17 could bind to hIL-1β and hIL-17A specifically, but could not bind to TNF-α.

4. Bispecific reaction of scBsAb1/17 with both hIL-1β and hIL-17A



scBsAb1/17 or the mono-specific antibodies (50 nM) for hIL-1β and hIL-17A was first incubated with hIL-1β (2 μg/mL) in solution and then transferred to a microtiter plate coated with hIL-17A (100 ng/well), followed by mouse anti-IL-1β monoclonal antibody and goat anti-mouse IgG-HRP. One-way ANOVA reveals a significant difference, **P<0.01 vs anti-IL-17A scFv, ##P<0.01 vs anti-IL-1β scFv. **scBsAb1/17 binds to both its targets simultaneously.**

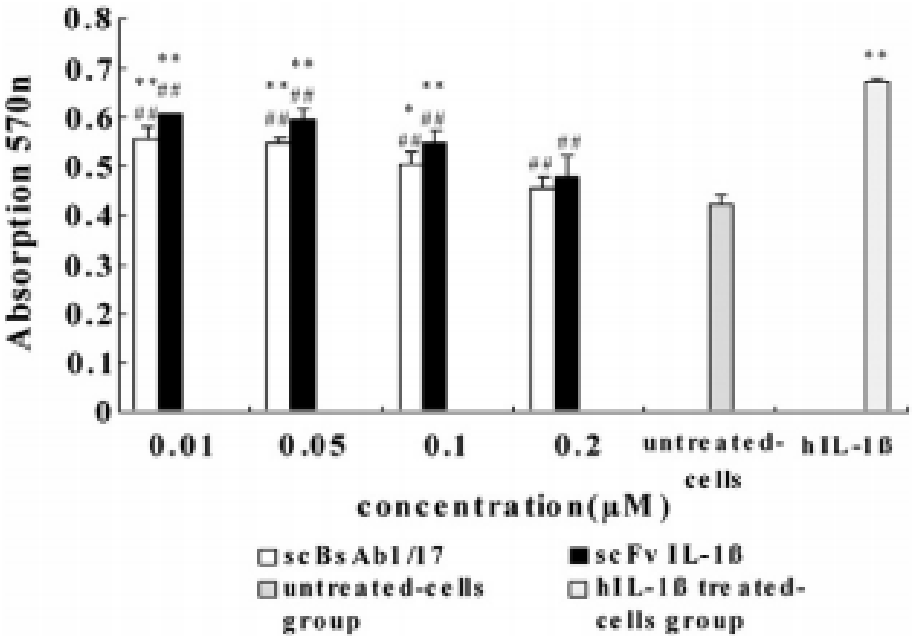
5. Affinity analysis of scBsAb1/17



ELISA plates were coated with various amounts of scBsAb1/17 or the mono-specific antibodies for hIL-1β and hIL-17A. 100 μL antigens (0.1 μg/mL) hIL-1β or hIL-17A was added to the wells respectively, followed by mouse anti-IL-1β monoclonal antibody or mouse anti-IL-17A monoclonal antibody and goat anti-mouse IgG-HRP. (A) Binding affinity of scBsAb1/17 to hIL-1β antigen. (B) Binding affinity of scBsAb1/17 to hIL-17A antigen. Results are showed as the mean±S.D. of triplicate samples.

scBsAb1/17 bound both hIL-1β and hIL-17A, and had similar antigen-binding efficiency to the respective monovalent single-chain antibody molecules.

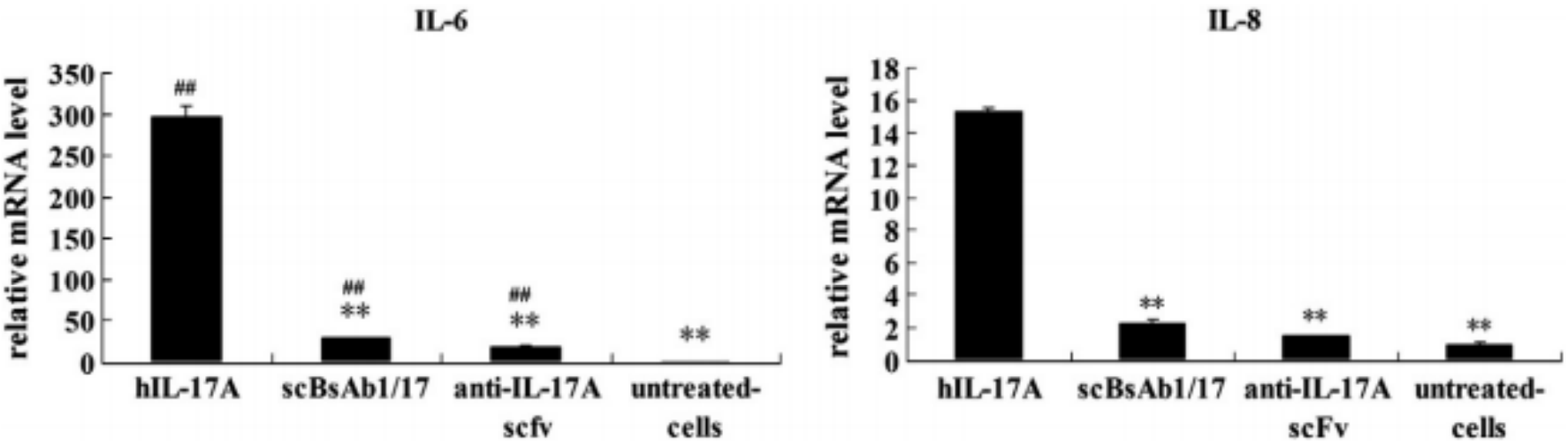
6. scBsAb1/17 inhibits proliferation of L929 cells stimulated by hIL-1β



L929 cells in growth medium were seeded in 96-well plates and cultured overnight. Fixed amount of hIL-1β (10 ng/mL, 50 μL/well) and various amounts of antibodies (50 μL/well) were added into the culture and incubated with the cells for 48 h, MTT method was used to detect the cell density at 570 nm. The data are the means±S.D. of quadruplex samples. *p<0.05 vs untreated-cells group, **p<0.01 vs untreated-cells group, ##p<0.01vs hIL-1β Ag group, (Tukey Kramer test).

Both scBsAb1/17 and anti-IL-1β scFv could block hIL-1β-induced cell proliferation in a dose-dependent manner. scBsAb1/17 had similar inhibition capacity to that of anti-IL-1β scFv.

7. scBsAb1/17 blocks gene expression of IL-6 and IL-8 stimulated by IL-17A



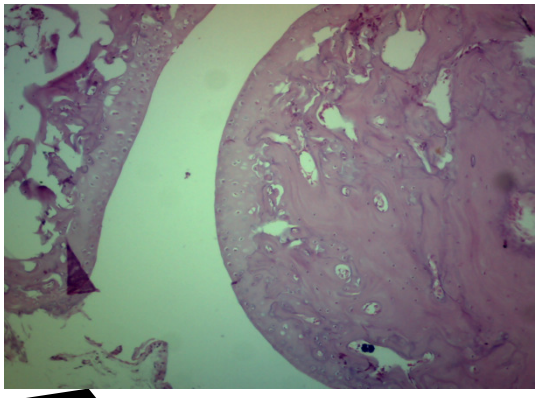
Hela cells were seeded in 6-well plates. When cells grew to 90% confluence. Cells were treated with both hIL-17A (50 ng/mL) and scBsAb1/17 (20 μg/mL) for 12 h. The cells were collected and total RNA was extracted. Gene expression of IL-6 and IL-8 was measured by real-time PCR. The untreated cells were used as negative control. The Cells stimulated with hIL-17A antigen were used as positive control. Data represent mean±S.D. of triplicate samples. **p<0.01 vs hIL-17A antigen group, ##p<0.01vs untreated-cell group (Tukey Kramer test).

hIL-17A-stimulated cells showed an increase of IL-6 and IL-8 gene expression. scBsAb1/17 at 20 μg/mL was sufficient to inhibit the hIL-17A-stimulated the production of IL-6 and IL-8.

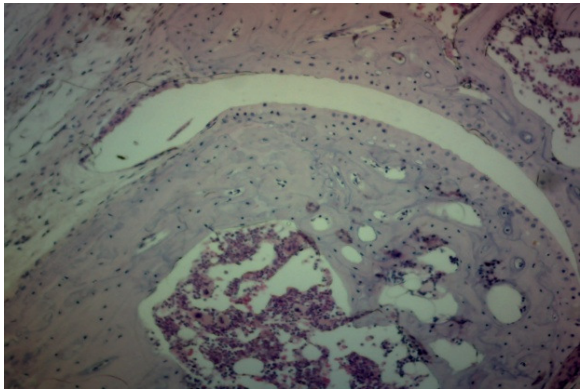
8. CIA Model

Healthy mouse hind foot

Model mouse hind foot

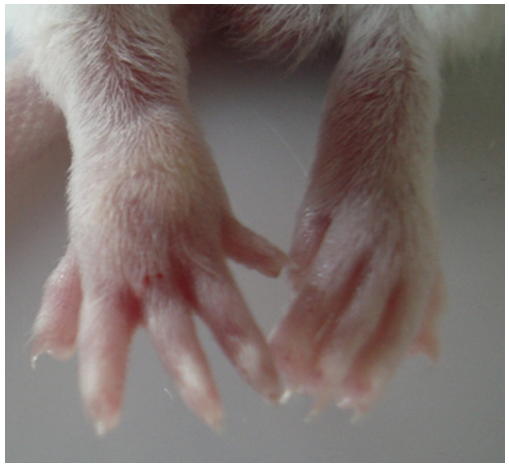


Healthy mouse articular cavity, Histopathology

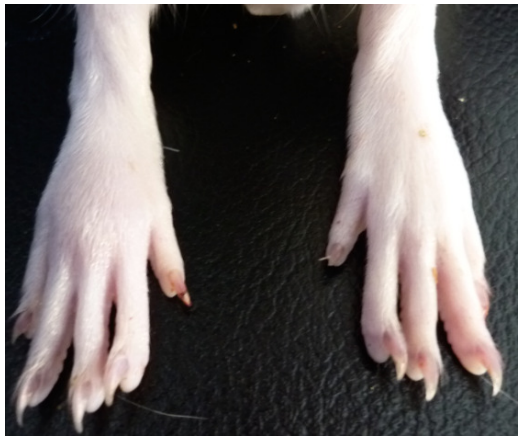


Model mouse articular cavity, narrower, synovial cell proliferation

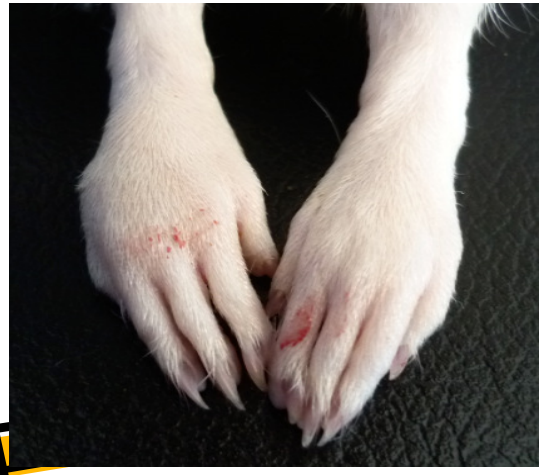
9. scBsAb1/17 treatment



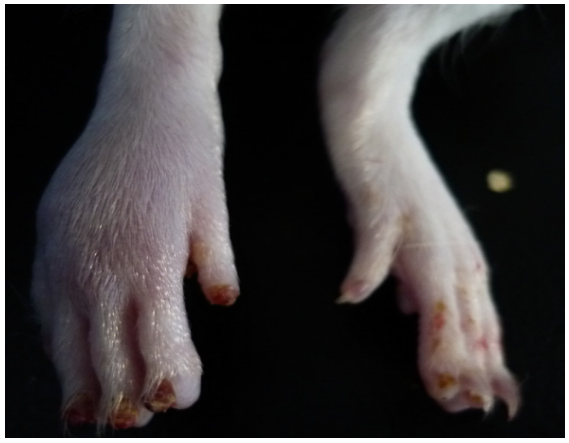
CIA Model



scBsAb1/17 treatment

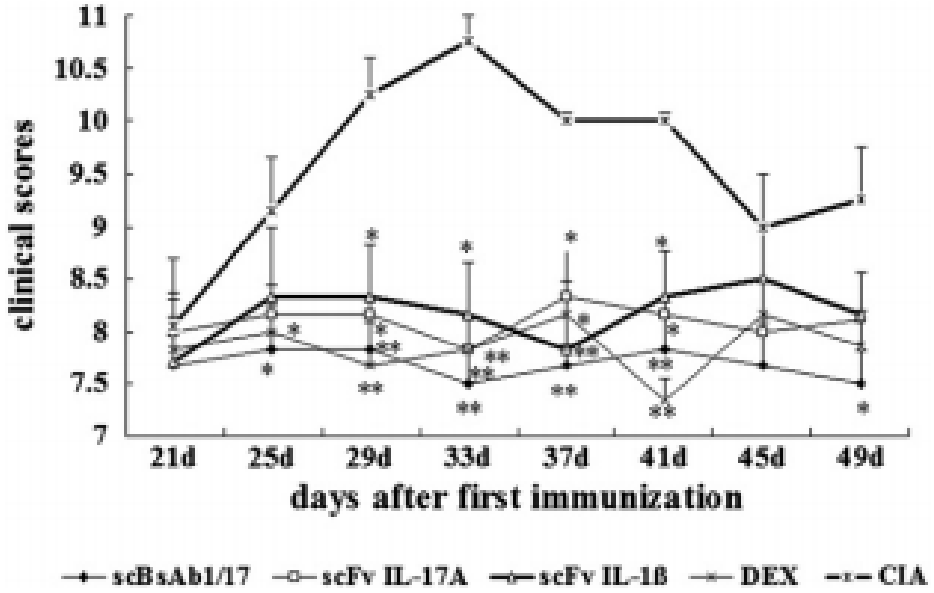


anti-IL-1β scFv



anti-IL-17A scFv treatment

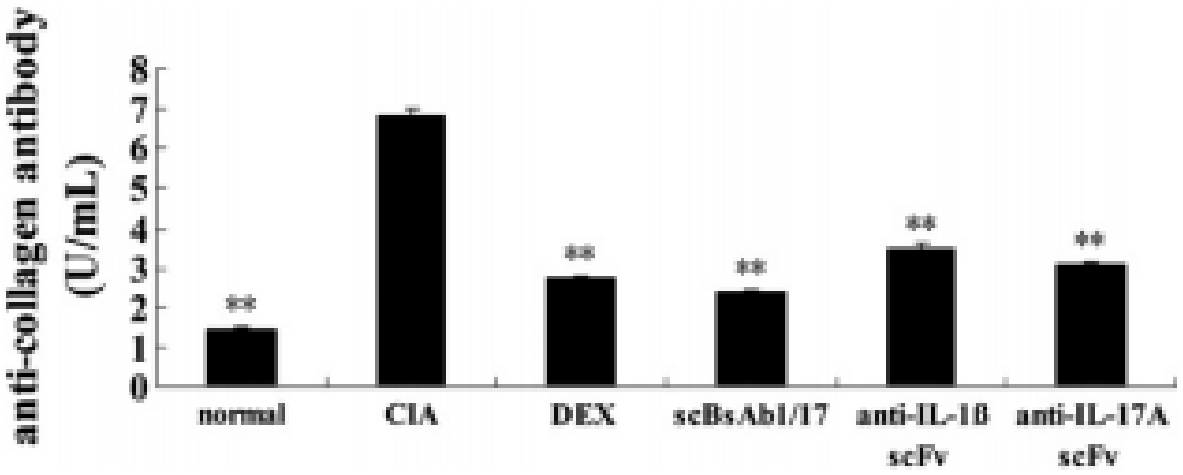
10. scBsAb1/17 alleviates clinical symptoms of CIA mice



Mice were randomly divided into six groups at 21d after the first immunization, and then antibodies and dexamethasone were administered every 2 days from day 21 to 49. The degree of arthritis was observed every 4 days from day 21 to 49. Results are expressed as mean±S.D. (n=6). Two-way ANOVA reveals a significant difference, **p<0.01 vs CIA group, *p<0.05 vs CIA group.

Similar to the Dexamethasone (DEX)-treated control, scBsAb1/17 as well as anti-IL-1β scFv and anti-IL-17A scFv ameliorated both the clinical symptoms and hind paw swelling with reduced arthritic score compared to that of the CIA mice.

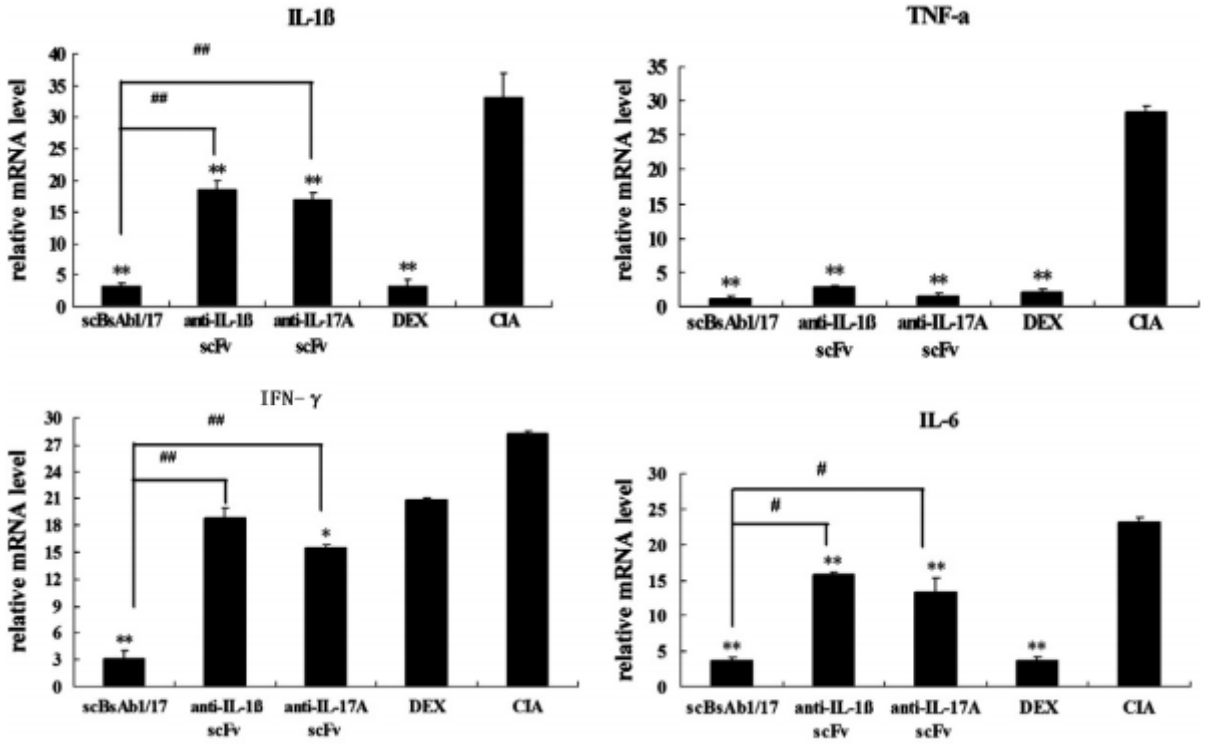
11. Reducing Effect of scBsAb1/17 on serum anti-CII antibodies



The antibody against type II collagen of the CIA mice was markedly higher than normal mice. Treatment with various antibodies resulted in a remarkable decrease of the antibodies against type II collagen. **scBsAb1/17** was even more potent in **reducing the type II antibody** although the difference was not statistically significant compared to other antibody treatment.

The data are expressed as means±S.D. **p<0.01, vs CIA group.

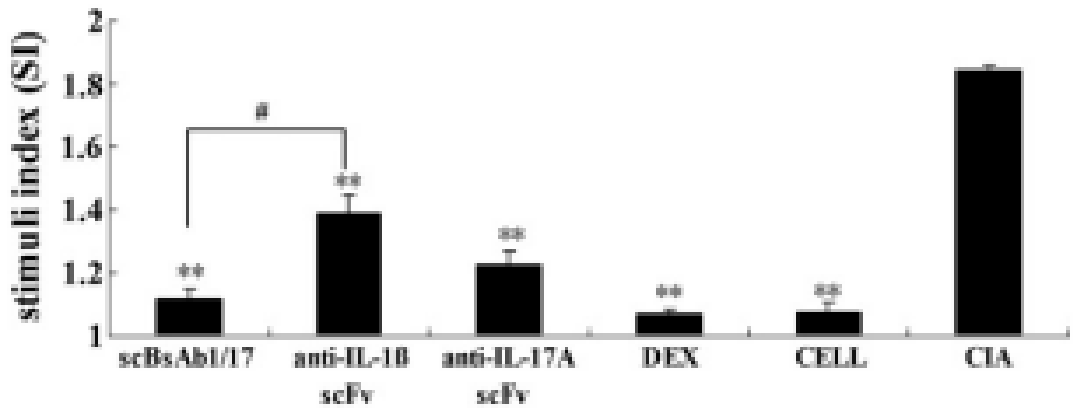
12. scBsAb1/17 reduces the expression of proinflammatory cytokines in the spleen



Mice were treated by antibodies every 2d from day 21 to 49 after the first immunization. The spleens were isolated on the 49th day. mRNA was isolated from spleens of experimental mice. cDNA was synthesized and real-time PCR was performed using primers specific for IFN-γ, TNF-α, IL-1β, IL-2 and IL-6. Expression of each gene was calculated relative to the expression of housekeeping gene, β-actin. One-way ANOVA revealed a significant effect. **p<0.01, *p<0.05, vs CIA control;#p<0.05, vs anti-IL-1β scFv or anti-IL-17A scFv.

All tested antibodies and DEX remarkably reduced mRNA expression of IFN-γ, IL-2, TNF-α, IL-6, and IL-1β. scBsAb1/17 significantly inhibited gene expression of IL-6, IL-2, IL-1β and IFN-γ except for TNF-α compared to that of anti-IL-1β scFv or anti-IL-17A scFv treatment alone, the difference was statistically significant.

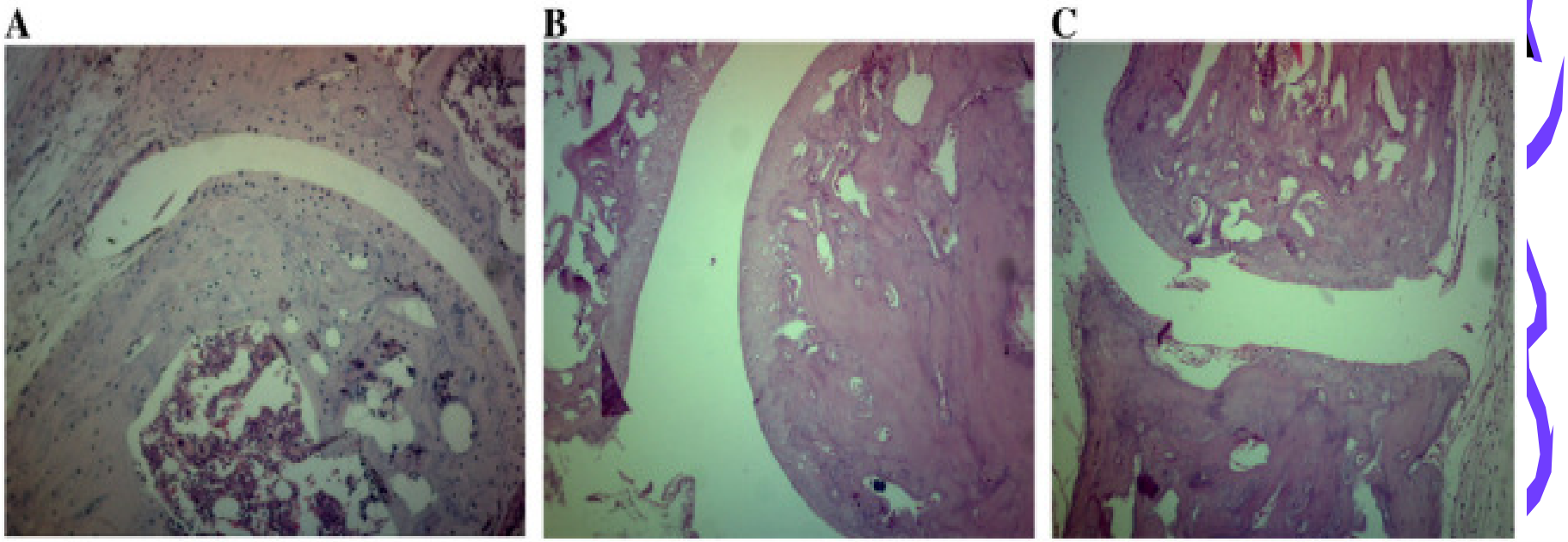
13. scBsAb1/17 reduced splenocyte proliferation response to CII stimulation



Mice were treated by antibodies every 2d from day 21 to 49 after the first immunization. The spleens were isolated on the 49th day. The spleen cells were stimulated with chick CII for 72 h. The cell proliferation was measured by MTT method. Data were shown as mean±S.D. of triplicate samples. One-way ANOVA revealed a significant effect. **p<0.01, *p<0.05, vs CIA control. #p<0.05, vs anti-IL-1β scFv or anti-IL-17A scFv. SI=OD570 collagen/OD570 medium.

The stimulus index of scBsAb1/17 was comparable with that of DEX and close to that of the normal cells. The inhibiting effect of scBsAb1/17 was stronger than that of anti-IL-1β scFv or anti-IL-17A scFv.

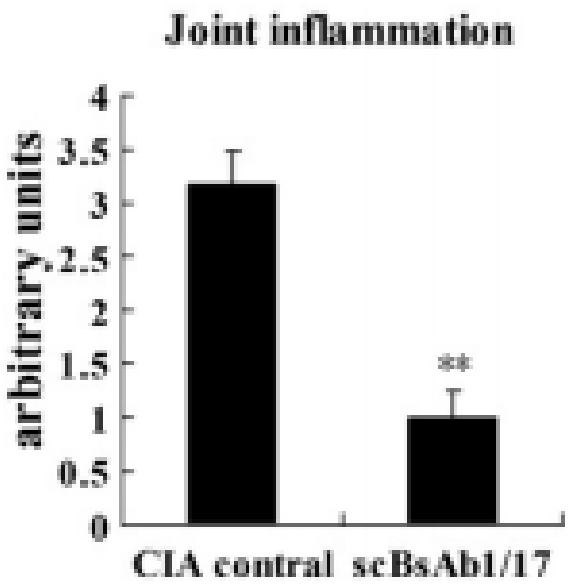
14. scBsAb1/17 ameliorates histopathological lesion in CIA mice.

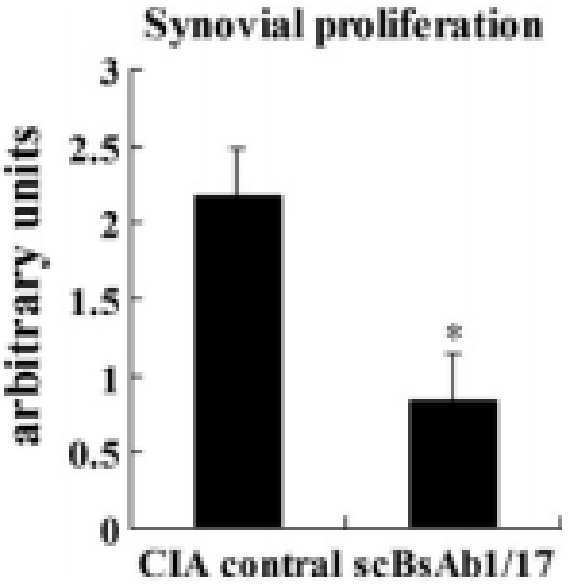


(A) The joints of CIA mice. The histologic result shows apparent synovitis, erosions, and loss of joint integrity. (B) Normal joints of mice. (C) The joints of scBsAb1/17-treated CIA mice.

There is a reduction in synovial hyperplasia, inflammatory cell infiltration and destruction of cartilage and bone in comparison with the incidence of these findings in the joints of CIA mice.

15. scBsAb1/17 treatment reduced joint degeneration in mice with CIA



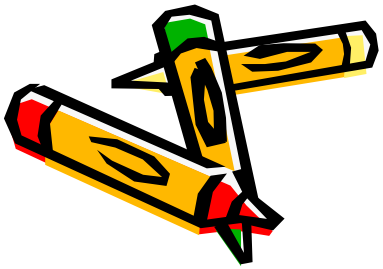


Joints were harvested from untreated-CIA control and scBsAb1/17-treated mice with CIA at the end of the experiment (on day 49). The joints were fixed in formalin, decalcified with formic acid and then stained with hematoxylin-eosin to evaluate inflammation, synovial proliferation and erosion. Results shown are based on examination of 6 sections for each treatment group. *p<0.05, **p<0.01 vs CIA control (Tukey Kramer test).

Fewer infiltrating cells and a decrease in joint synovial proliferation in scBsAb1/17-treated joints, compared to CIA controls

Conclusion

scBsAb1/17 shows more beneficial in treatment of CIA mice than monovalent single-chain antibody molecules.

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