Article Title: Role of chemotaxis of Vδ2 T cells to the synovium in the pathogenesis of acute gouty arthritis

Author Name: Hong Di[1#], Xinxin Han[1#], Yue Yin [1], Yun Zhang[1]*, Xuejun Zeng [1]*

1 Department of Family medicine & Division of General Internal Medicine, Department of Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, State Key Laboratory of Complex Severe and Rare Diseases (Peking Union Medical College Hospital), Beijing, China.

[#]Hong Di and Xinxin Han have contributed equally to this work.

*Correspondence to: Dr. Yun Zhang, Department of Family medicine & Division of General Internal Medicine, Department of Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, State Key Laboratory of Complex Severe and Rare Diseases (Peking Union Medical College Hospital), Beijing 100730, China. Email: zhangyun10806@pumch.cn; Phone & Fax: +86010-69156098. Dr. Xuejun Zeng, Department of Family medicine & Division of General Internal Medicine, Department of Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, State Key Laboratory of Complex Severe and Rare Diseases (Peking Union Medical College Hospital), Beijing 100730, China. Email: zxjpumch@126.com; Phone & Fax: +86010-69156098.

Supplementary Materials:

Methods:

Enzyme-linked immunosorbent assay (ELISA) manufacturer's instruction in detail:

1.Determine the number of microwell strips that are required to test the desired number of samples, plus the appropriate number of blanks and standards. Assay each sample, standard, blank, and optional control sample in duplicate. Return any unused microwell strips to the provided foil bag with desiccant pack, then store tightly sealed at 2°C to 8°C.

2. Prepare Biotin-Conjugate (1:100 dilute).

3. Wash the microwell strips twice with exactly 300 µL of Wash Buffer (1x) per well, thoroughly aspirating between washes. Allow the Wash Buffer to sit in the wells for 30 seconds before aspiration. IMPORTANT! Do not scratch the inner surface of the microwells. Note: To ensure optimal test performance, we highly recommend soaking between each wash. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Do not allow wells to dry.

4. Prepare standard dilutions in the microwell plate. a. Add 100 µL of Sample Diluent, in duplicate, to all standard wells. b. Add 100 µL of the prepared standard, in duplicate, to wells A1 and A2. c. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, then transfer 100 µL to wells B1 and B2, respectively. Do not scratch the inner surface of the microwells. d. Repeat the above procedure 5 times, creating two rows of Human IL-17A Standard dilutions.

5. Add 100 µL of 1× detection buffer, in duplicate, to the blank wells.

6. Add 50 µL of 1× detection buffer to the sample wells.

7. Add 50 µL of each sample, in duplicate, to the sample wells.

8. Add 50 µL of Biotin-Conjugate to all wells.

9. Cover with an adhesive film, then incubate for 2 hours at room temperature (18–25°C) on a microplate shaker.

10. Prepare the Streptavidin-HRP (1:100 dilute).

11. Remove the adhesive film, then empty the wells. Wash the microwell strips 6 times as previously described (see step 3), then proceed immediately to the next step.

12. Add 100 µL of prepared Streptavidin-HRP to all wells, including the blank wells.

13. Cover with an adhesive film, then incubate for exactly 45minutes at room temperature (18–25°C) on a microplate shaker in the dark. Shaking is required for optimal test performance.

14. Remove the adhesive film, then empty the wells. Wash the microwell strips 6 times as previously described (see step 3), then proceed immediately to the next step.

15. Add 100 µL of Substrate Solution to all wells.

16. Incubate the microwell strips for about 10-20 minutes at room temperature (18–25°C) in the dark. Avoid direct exposure to intense light.

17. Quickly add 100 µL of Stop Solution to each well to stop the enzyme reaction, then read the results immediately. It is important to add the Stop Solution quickly and uniformly throughout the microwell plate to completely inactivate the enzyme.

18. Read the absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength (optionally, 630 nm as the reference wavelength).”

Supplementary Figures:

Figure S1

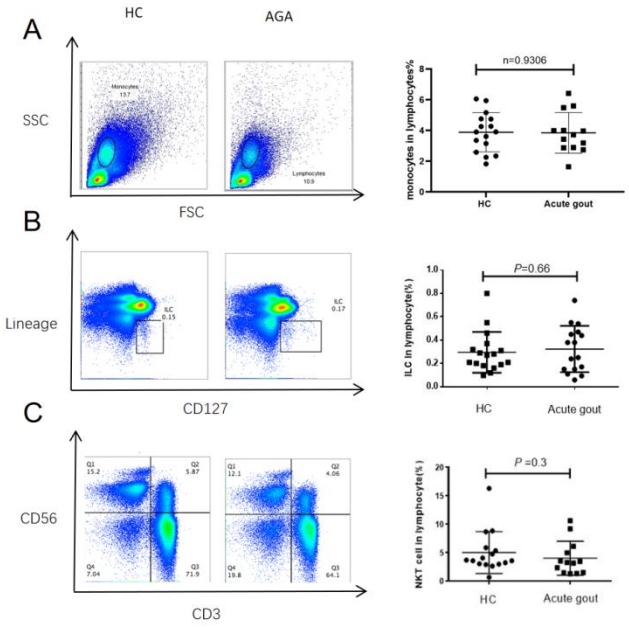

Figure S1. The percentages of monocytes, ILCs, NKT-like cells in PBMCs showed no changes between patients with AGA and HCs.

(A-C) The left panels show representative flow profiles of monocytes (**A**), ILCs (**B**), or NKT-like cells (**C**). The right panels show the percentage of positively stained cells in lymphocytes. n=16 for the HC group, and n=13 for the AGA group. ILC, innate lymphoid cells; NKT-like cells, natural killer T-like cells; PBMC, peripheral blood mononuclear cells; AGA, acute gouty arthritis; HC, healthy control.

Figure S2

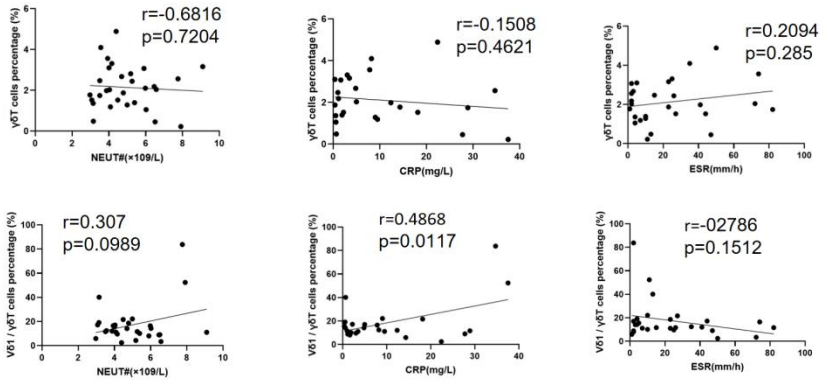

Figure S2. The correlations between the percentage of peripheral γδT cells or Vδ1 T cells in patients with AGA and the count of neutrophils, concentration of CRP and value of ESR.

Summarized data were from 21 AGA patients. NEUT, neutrophils; CRP, C reactive protein; ESR, erythrocyte sedimentation rate.

Figure S3

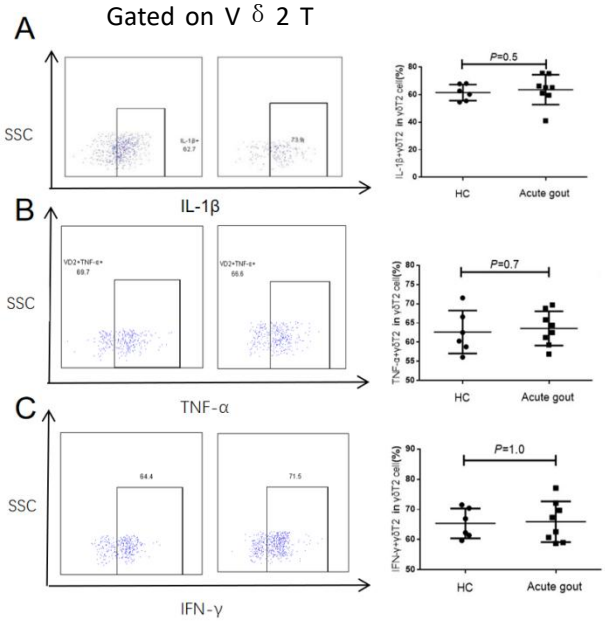

Figure S3. Vδ2 T cells from PBMCs of AGA patients and HCs produced comparable levels of IL-1β, TNF-α, and IFN-γ.

(A-C) Flow cytometry analysis of the intracellular staining of IL-1β (**A**), TNF-α (**B**), and IFN-γ (**C**) in Vδ2 T cells from PBMCs of patients with AGA and HC was performed. The right panels show bar graphs of the percentage of positively stained cells. Summarized data of HCs (n=6) and AGA (n=8) are shown. Results are expressed as mean ±SEM.