

Role of Chemotaxis of V δ 2 T Cells to the Synovium in the Pathogenesis of Acute Gouty Arthritis

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Background: This study aimed to explore the potential roles of V δ 2 T cells in the pathogenesis of acute gouty arthritis.

Methods: Patients with gout (n=86), rheumatoid arthritis (n=7), osteoarthritis (n=9), and healthy controls (n=40) were enrolled. $\gamma\delta$ T cell frequency, chemokine receptor expression and production of pro-inflammatory cytokines in cells from peripheral blood and synovial fluid samples were quantified by flow cytometry. The chemotaxis ability of V δ 2 T cells was determined by transwell migration assay. The levels of chemokines were also detected by enzyme-linked immunosorbent assay.

Results: Peripheral V δ 2 T cells had significantly lower frequencies in acute gout patients than that in healthy controls ($P<0.001$). These peripheral V δ 2 T cells were negatively correlated with inflammatory markers. V δ 2 T cells from acute gout patients accumulated in synovial fluid, as evidenced by a higher abundance of V δ 2 T cells in it than that in the serum ($P<0.01$). And V δ 2 T cells expressed a high level of C-X-C chemokine receptor 3 ($P=0.035$), and its corresponding chemokine C-X-C motif chemokine ligand 10 showed a high concentration in synovial fluid ($P<0.05$). V δ 2 T cells from synovial fluid of acute gout patients produced a high level of interleukin-17 ($P=0.033$).

Conclusion: The up-regulated expression of C-X-C chemokine receptor 3 on V δ 2 T cells potentially facilitates their infiltration into synovial fluid during acute gouty arthritis. Further production of interleukin-17 by V δ 2 T cells may contribute to the pathogenesis of gout. This study sheds new light on developing novel V δ 2 T cells-based therapeutic strategies for gout treatment.

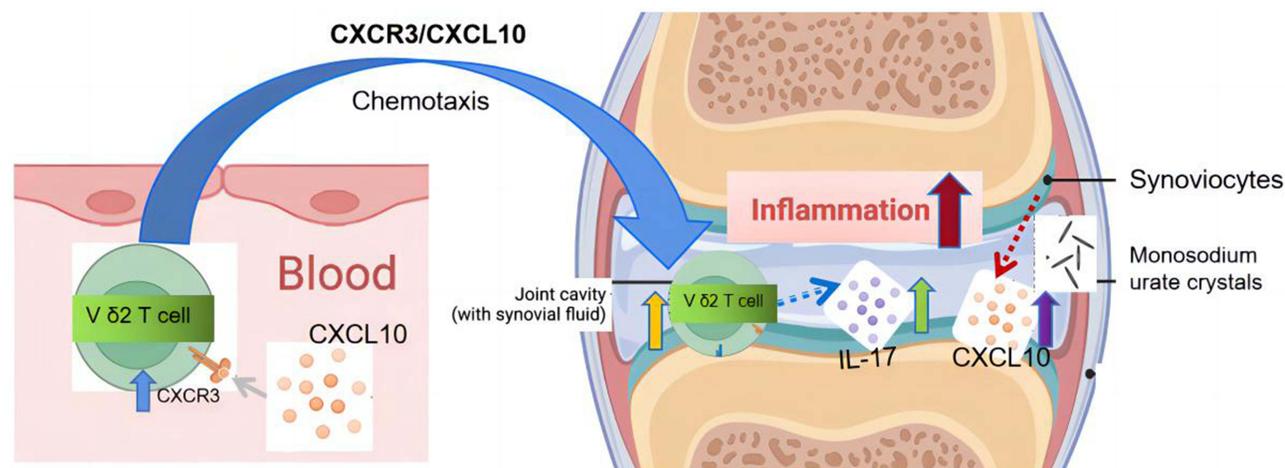
Keywords: acute gouty arthritis, $\gamma\delta$ T cells, V δ 2 T cells, Interleukin-17, CXCR3, CXCL10

Introduction

Gout, the most prevalent form of inflammatory arthritis, is caused by the deposition of monosodium urate (MSU) crystals in articular and non-articular structures.¹ MSU crystals, a kind of damage-associated molecule, can stimulate innate immune pathways. Activation of the NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome in macrophages and monocytes by MSU crystals is of particular relevance for the initiation of the gout flare, and neutrophils also play an important role in this process. Interleukin-1 β (IL-1 β) can lead to the subsequent release of various pro-inflammation cytokines and chemokines to prompt the progression of gout.²

Acute gouty inflammation is mediated mainly by the innate immune cells and corresponding cytokines.³ Innate immune cells are mainly composed of monocytes, macrophages, neutrophils, natural killer T-like (NKT-like) cells, $\gamma\delta$ T cells, and innate lymphoid cells (ILC). Among these, $\gamma\delta$ T cells are a subset of T cells with distinctive T cell receptor (TCR), which is composed of one γ chain and one δ chain. Differed by the chains expressed by $\gamma\delta$ T cells, two main subsets have been defined in human, namely V γ 9/V δ 2 and V δ 1.⁴ V γ 9/V δ 2 T cells are mainly distributed in peripheral

Graphical Abstract



blood and lymphatic system, and V δ 1 T cells are mainly distributed on epithelial and mucosal surfaces. In normal human peripheral blood, 70–90% of $\gamma\delta$ T cells are V γ 9/V δ 2 T cells, accounting for 1–5% of the total T cells.⁵ Different distributions of specific subsets of $\gamma\delta$ T cells are associated with distinct immunological functions.⁶ It is well established that these cells play regulatory roles in many physiological and pathological processes, such as diabetes, rheumatoid arthritis (RA),⁷ psoriatic arthritis (PsA),⁸ multiple sclerosis and anxiety.⁹ $\gamma\delta$ T cells are recognized for their robust anti-tumor functions, and also play a protective role during successful pregnancies through the regulation of several cytokines.^{10–12} In patients with RA, V δ 2 T cells exhibit heightened chemotaxis potential and express elevated levels of chemokine receptors C-C chemokine receptor 5 (CCR5) and C-X-C chemokine receptor 3 (CXCR3), which potentially facilitate the infiltration of V δ 2 T cells into the synovium and contributed to the disease progression of RA.⁷

Interleukin-17 (IL-17) is an effective proinflammatory cytokine, and its elevated level was found harmful in autoimmune diseases and cancer.¹³ IL-17 attracts neutrophils that induce inflammation, stimulates formation of bone-resorbing osteoclasts, and augments cytokine and enzyme production from joint resident cells.^{14–16} Over the years, more cell types, including Th17 cells, mast cells, neutrophils, cluster of differentiation (CD) 8+ cells and $\gamma\delta$ T cells, have been found to be able to produce IL-17.^{17–19} The levels of serum IL-17 elevated in patients with acute gouty arthritis (AGA), and $\gamma\delta$ T cells are one of the major sources of IL-17 in serum of patients with AGA.³ Similarly, several recent studies have demonstrated that IL-17-producing $\gamma\delta$ T-helper 17 ($\gamma\delta$ Th17) cells are a major innate cellular source of IL-17 in collagen-induced arthritis (CIA) models.^{20,21} Moreover, it has been confirmed that V δ 2 T cells from RA could infiltrated into the synovium from peripheral blood and played crucial roles in the pathogenesis of RA.⁷ However, whether and how the IL-17-producing $\gamma\delta$ T cells contribute to the disease progression of AGA has not been intensively elucidated so far.

Nowadays, the main managements of acute gouty arthritis are pain control and uric acid lowering.^{1,22,23} For gout patients, it is important to take anti-inflammatory drugs timely and use uric acid-lowering drugs regularly. Recently, several studies have newly found that both anti-inflammatory drugs (such as ibuprofen) and 5-aminoorotic acid could serve as new inhibitors to xanthine oxidase.^{24,25} However, over the past few decades, the progress in drug research to treat gout was slow. Moreover, the traditional treatment mainly focuses on the control of the clinical manifestation – high level of inflammation, but pays little attention to the immune disorders related to the pathogenesis of AGA. Therefore, it is of great interest to explore the mechanism of immune cell disorder associated with AGA, which may be act as a potential therapeutic target. In recent study, the differences in peripheral T cell populations have been shown to be associated with the development of Mediterranean fever, chronic granulomas disease and gout. As a result, peripheral T cells, such as $\gamma\delta$ T cells, may be a potential target for the treatment of gout.²⁶

This study investigated the abundance of innate immune cells, especially $\gamma\delta$ T cells, in peripheral blood and synovial fluid (SF) from patients with gout and explored their possible connections with the development of gout.

Materials and Methods

Patients and Samples

In this retrospective study, a total of 86 gout patients' samples (including 49 acute gouty arthritis patients' samples, 11 remission period of gout patients' samples, and 26 intercritical period of gout patients' samples), 7 rheumatoid arthritis patients' samples, 9 osteoarthritis (OA) patients' samples, and 40 healthy control subjects, were collected. Among them, SF was collected from acute gouty arthritis patients (n=18), rheumatoid arthritis patients (n=7) and osteoarthritis patients (n=9). All gout patients were male and were either hospital inpatients or clinical attendees at the Peking Union Medical College Hospital (PUMCH) between December 2019 and April 2022. All patients met the 1977 American Rheumatism Association Preliminary gout classification criteria²⁷ as well as the 2015 American College of Rheumatology/European League Against Rheumatism gout classification criteria.²⁸ Patients with infections, tumors, and other immune diseases were excluded. Acute gouty arthritis, also called gout flare, was defined as a clinically evident episode of acute inflammation induced by MSU crystals and characterized as joint pain, swelling, erythema and tenderness. Remission period was defined as the period of alleviation or absence of clinically evident inflammation after episodes of acute flare. Intercritical period was defined as the asymptomatic period after or between gout flares, despite the persistence of MSU crystals.^{29,30} Healthy controls (HCs) were defined as gout-free male subjects with no other arthritis-related diseases, and controls were age and sex matched with the gout patients. Peripheral blood samples were collected from patients after overnight fasting during different gout periods and from HCs. SF samples from patients were obtained from arthrocentesis. Written informed consent was obtained from all participants. This study was approved by the Ethics Committee of PUMCH (ethics approval number JS-2339) and conducted in accordance with the clinical guidelines of the 1975 Declaration of Helsinki.

Clinical and Laboratory Examinations

The clinical characteristics of patients with AGA, OA, RA and healthy controls are summarized in [Tables 1](#) and [2](#).

Flow Cytometry

Peripheral blood mononuclear cells (PBMCs) and synovial fluid mononuclear cells (SFMCs) from gout patients and HCs were isolated using Ficoll gradient centrifugation (Dakewe, Beijing, China).³¹ Subsequently, these cells were used for flow cytometry analysis. Fluorescein isothiocyanate (FITC)-conjugated anti-human CD14, phycoerythrin (PE)-conjugated anti-human CD16, allophycocyanine (APC)-conjugated anti-human CD192 (CCR2), PE-conjugated anti-human CD294 (CRTH2), APC-conjugated anti-human CD127 (IL-7R α), peridinin-chlorophyll-protein/cyanine5.5 (PerCP/Cy5.5) – conjugated anti-human CD117 (c-kit), FITC-conjugated anti-human Lineage Cocktail (CD3, CD14, CD19, CD20, CD56), PerCP/Cy5.5-conjugated anti-human CD3, APC-conjugated anti-human CD57, PerCP-conjugated anti-human CD8, PE/Cy7-conjugated anti-human CD56 (NCAM), FITC-conjugated anti-human CD3, PE-conjugated anti-human TCR γ/δ , APC anti-human TCR V δ 2, PerCP/Cy5.5-conjugated anti-human CD3, FITC-conjugated anti-human TCR V δ 2, APC-conjugated anti-human IL-1 β , APC-conjugated anti-human IL-17, APC-conjugated anti-human tumor necrosis factor (TNF) - α , APC-conjugated anti-human interferon (IFN) - γ , PE/Cy7-conjugated anti-human C-X-C chemokine receptor 3 (CXCR3), and APC/Fire 750-conjugated anti-human C-C chemokine receptor 5 (CCR5) antibodies were purchased from BD Biosciences (Franklin Lakes, NJ, USA). PerCP/Cy5.5-conjugated anti-human TCR V δ 1 was purchased from Thermo Fisher Scientific (Waltham, MA, USA). $\gamma\delta$ T cells were defined as CD3⁺ TCR γ/δ ⁺; V δ 1 T cells were defined as CD3⁺ TCR V δ 1⁺ TCR V δ 2⁻; V δ 2 T cells were defined as CD3⁺ TCR V δ 1⁻ TCR V δ 2⁺; innate lymphoid cells were defined as CD127⁺Lineage⁻; natural killer T-like cells were defined as CD3⁺CD56⁺; monocytes were defined as CD14⁺⁺ CD16⁻ CCR2⁺ or CD14⁺ CD16⁺ CCR2⁺ or CD14⁺ CD16⁺⁺ CCR2⁻.

For the staining of surface markers, PBMCs and SFMCs were rinsed with phosphate buffered saline (PBS) (Solarbio, Beijing, China) containing 1% bovine serum albumin (BSA) (Solarbio) and were incubated in various combinations of

Table 1 Clinical Characteristics of Gout Patients and Healthy Controls

	HC (n=40)	AGA (n=49)	Remission period (n=11)	Intercritical period (n=26)
Age (years)	31.9±8.5	38.2±13.5	41.0±13.1	35.7±11.1
BMI (kg/m ²), M (Q1-Q3)	24.8(21.4–26.3)	29.0(26.3–37.8)	26.5(25.92–8.7)	27.6(24.5–30.2)
Disease duration (months), M (Q1-Q3)		48(12–105)	24(1–108)	54(24–84)
sUA (umol/L)	367.9±64.48*	491.6±129.7	485.9±115.3	490.7±117.8
Cr (umol/L)	79.26±5.62*	89.44±18.32	92.85±7.61	91.91±17.36
Hgb (g/L)	154.35±8.6	151.5±13.62	148.5±19.79	157.4±9.99
WBC (10 ⁹ /L)	5.44±0.99*	8.04±2.18	6.76±1.51	6.748±1.39
PLT (10 ⁹ /L)	241.54±47.32	281.7±65.96	247.1±42.03	248.9±68.2
MONO (10 ⁹ /L)	0.34±0.15	0.39±0.13	0.39±0.14	0.34±0.1
NEUT (10 ⁹ /L)	3.09±0.79*	5.26±1.801	4.6±0.37	4.21±1.16*
LY (10 ⁹ /L)	1.71±0.64	2.14±0.77	1.62±0.37	1.98±0.45
γδ T cells (10 ⁵ /mL)	5.51±3.57	4.96±3.39	5.83±1.94	5.28±2.63
Vδ1 T cells (10 ⁵ /mL)	0.68±0.77	0.85±1.1	0.71±0.46	0.67±0.41
Vδ2 T cells (10 ⁵ /mL)	3.98±3.07*	1.75±1.67	3.07±1.27*	2.90±1.43*
ESR (mm/h),		23.63±21.69	16.56±16.73	6.5±4.81*
hsCRP (mg/L),		16.64±23.1	9.77±9.79	2.42±1.75*
TC (mmol/L)		4.9±0.92	5.25±1.23	4.79±0.84
TG (mmol/L)		2.2±1.13	1.98±1.33	2.46±1.71
HDL-C(mmol/L)		1.02±0.20	1.07±0.38	0.98±0.21
LDL-C(mmol/L)		3.09±0.76	3.47±1.05	2.99±0.76

Notes: *Represented statistical significance (p<0.05) compared with AGA group.

Abbreviations: BMI, body mass index; sUA, serum uric acid; Cr, creatine; Hgb, hemoglobin; WBC, white blood cells; PLT, platelet; MONO, monocytes; NEUT, neutrophils; LY, lymphocytes; ESR, erythrocyte sedimentation rate; hsCRP, hypersensitive C-reactive protein; TC, triglyceride; TG, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2 Clinical Characteristics of RA and OA Patients

	RA (n=7)	OA (n=9)
Age (years)	40.3±17.8	55.75±6.18
BMI (kg/m ²)	23.2±2.05	22.35±1.4
Disease duration (months), M (Q1-Q3)	102(23–258)	96(6–120)
sUA (umol/L)	326.0±102.5	327±95
Cr (umol/L)	64.3±11.9	67.5±3.4
Hgb (g/L)	127±23	136±5.4
WBC (10 ⁹ /L)	6.08±2.21	9.18±0.36
PLT (10 ⁹ /L)	308±117	356.67±44.43
MONO (10 ⁹ /L)	0.39±0.13	0.59±0.076
NEUT (10 ⁹ /L)	3.90±1.39	6.58±0.73
LY (10 ⁹ /L)	2.05±1.04	1.95±0.45
ESR (mm/h)	59±16.94	8.67±2.5
hsCRP (mg/L)	29.06±6.352	6.89±0.75
RF (u/mL)	38.6±36.7	NA
DAS28	4.95±1.34	NA
Number of swollen joints, M (Q1-Q3)	5(4–8)	2(1–3)

Abbreviations: BMI, body mass index; sUA, serum uric acid; Cr, creatine; Hgb, hemoglobin; WBC, white blood cells; PLT, platelet; MONO, monocytes; NEUT, neutrophils; LY, lymphocytes; ESR, erythrocyte sedimentation rate; hsCRP, hypersensitive C-reactive protein; RF, rheumatoid factor; DAS28, disease activity score 28.

monoclonal antibodies (mAbs) for 30 minutes at 4°C. Then, the cells were washed and suspended in PBS. The stained cells were promptly analyzed using a Cytoflex Flow Cytometer (Beckman Coulter, CA, USA) or were fixed in 1% paraformaldehyde and analyzed within 24 hours. Data analysis was performed using FlowJo V10.8.1 Software (BD Biosciences).

For the detection of IFN- γ , TNF- α , IL-1 β and IL-17 in V δ 2 T cells, the cells were pre-treated with Leukocyte Activation Cocktail (1 μ L/mL) for 4 hours at 37°C in 5% CO₂. Then, the cells were washed twice with PBS containing 1% BSA and stained for surface markers of CD3 and V δ 2. After washing twice with PBS containing 1% BSA, the cells were fixed and permeabilized using BD Cytofix/Cytoperm solution (BD Biosciences). The cells were then washed with permeabilization solution before staining with anti-human IFN- γ , TNF- α , IL-1 β and IL-17 antibodies (BioLegend, San Diego, CA, USA). The cells were incubated at 4°C in the dark for 30 minutes, then washed twice with permeabilization solution before analysis using a Cytoflex Flow Cytometer (Beckman Coulter). Data analysis was performed using FlowJo V10.8.1 Software (BD Biosciences).

Cytokine Detection

Venous blood samples were collected into tubes containing separating gel and centrifuged at 4000 g at 4°C for 20 minutes to isolate the serum. The synovial fluids were centrifuged at 1800g at 4°C for 10 minutes to obtain the supernatant for analysis. The cytokine detection reagents were provided by Jiangxi Saiji Biotechnology Limited company (calibrator lot number: 20220101; Jiangxi, China). Ten cytokines, including interleukin (IL) -1, IL-2, IL-4, IL-5, IL-10, IL-12, IL-17, TNF- α , IFN- α and IFN- γ , were detected by multiple microsphere flow immunofluorescence according to the manufacturer's instructions. Fluorescent detection of the microsphere mixture was performed on a calibrated CytoFLEX Flow Cytometer (Beckman Coulter) for each sample in sequence. Data analysis was performed using FlowJo V10.8.1 Software (BD Biosciences).

Enzyme-Linked Immunosorbent Assay (ELISA)

The concentrations of C-X-C motif chemokine ligand (CXCL) 9, CXCL10, CXCL11, C-C motif chemokine ligand (CCL) 3, CCL4 and CCL5 in SFs and serum were analyzed by ELISA kits (Multisciences Biotech, Hangzhou, China) according to the manufacturer's instructions ([Supplementary Materials: Methods](#)). Briefly, for each test, a 96-well polystyrene microplate was used, and each well was pre-coated with monoclonal antibody specific for each cytokine. After incubation with samples, each well was added the detection antibody that can bind to the immobilized target protein. After washing away excess detection antibody and addition of horseradish peroxidase (HRP) conjugate, HRP substrate was added to wells for indirect detection of bound protein. The optical density (OD) was measured within 30 minutes after final washing, using a microplate reader set to 450 nm or 630 nm.

Transwell Chemotaxis Assay

Chemoattractant activity was assessed in a 24-well transwell or cell culture dish with 5 μ m-pore-size polycarbonate filters (Corning, NY, USA). PBMCs were resuspended in RPMI 1640 (Solarbio, Beijing, China), supplemented with 1% BSA and 1 \times 10⁶ PBMCs in 0.1 mL of the resuspension medium were added to the upper compartment of each chamber. Then, 0.6 mL of SFs or serum from AGA, RA, or OA patients were added to the lower compartment. After a 24 hours incubation at 37°C under 5% CO₂ atmosphere, cells were harvested from the lower chambers, γ δ T cell subsets that migrated to the lower chambers were counted by flow cytometry.

Statistical Analysis

The SPSS software version 23.0 (IBM Corp., Armonk, New York, US) and GraphPad Prism version 9 (GraphPad Software, Inc.; San Diego, California, US) were used for statistical analysis. Normally distributed data were judged using the Shapiro–Wilk method. The Levene method was used to test homogeneity of variance. Two sets of data that met normal distribution and homogeneity of variance were analyzed using Student's *t*-test. Multi-group comparisons of means were carried out by a one-way analysis of variance (ANOVA) test. Data that did not meet homogeneity of variance

or normal distribution were compared using Kruskal–Wallis and Mann–Whitney non-parametric tests. *P*-values <0.05 were considered statistically significant.

Results

Patients with Acute Gouty Arthritis Have a Low Percentage of V δ 2 T Cells in Peripheral Blood

We assessed the frequency of $\gamma\delta$ T cells and their sub-types among HCs and AGA patients, and the gating strategy is showed in (Figure 1A). The results revealed a significant reduction in the proportion of peripheral total $\gamma\delta$ T cells among lymphocytes in the AGA group (the absolute numbers of cells/mL were shown in Table 1) [AGA vs HC, 2.14% (1.49–3.08%) vs 3.13% (1.89–3.85%); *P*=0.026] (Figure 1B, Table 3). This decrease was due to the significant reduction in percentage of peripheral V δ 2 T cells [AGA vs HC, 30.40% (16.83–54.13%) vs 66.05% (57.45–78.93%); *P*<0.001], while the percentages of V δ 1 T cells in peripheral blood were similar between the two groups (*P*=0.2) (Figure 1C, Table 3). These findings are consistent with those of RA.⁷ No significant disparity in peripheral abundance between the AGA and HC groups was observed for the other three types of innate immune cells, including monocytes, ILCs and NKT-like cells (Figure S1). The percentages of peripheral V δ 2 T cells in the AGA group were negatively correlated with the level of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and neutrophil counts (*P*=0.037, 0.034, 0.011 respectively) (Figure 1D). No obvious correlation was observed between the percentages of total $\gamma\delta$ T cells or V δ 1 T cells and the inflammation-related indicators (Figure S2).

Compared to healthy controls, the patients with AGA had a significant reduction in the proportion of peripheral total $\gamma\delta$ T cells (*P*<0.05), while the patients with remission gout showed a significant increase in the percentage of total $\gamma\delta$ T cells in comparison to the those with AGA [Remission vs Acute, 2.89% (2.52%–3.34%) vs 2.14% (1.49%–3.08%); *P*=0.016] (Figure 1E, Table 3). The trend in the variations of peripheral V δ 2 T cell percentages mirrored that of total $\gamma\delta$ T cells (*P*<0.001; *P*=0.016, respectively) (Figure 1F, Table 3), while no significant difference was observed in the percentage of V δ 1 T cells among different disease stage groups (Figure 1G, Table 3).

V δ 2 T Cells Accumulate in Synovial Fluid of Patients with AGA

A previous study showed the accumulation of $\gamma\delta$ T cells in RA synovial effusions.³² The percentage of $\gamma\delta$ T cells in lymphocytes from SFMC of AGA patients was notably higher than that from patients with OA (*P*<0.001) (Figure 2A). In addition, we further examined the percentages of V δ 1 T cells and V δ 2 T cells within the total $\gamma\delta$ T cell population, and observed a significant increase in V δ 2 T cells (*P*<0.01), but not in V δ 1 T cells (*P*>0.05) (Figure 2B). We subsequently explored whether a correlation existed between the quantities of $\gamma\delta$ T cells in gout PBMC and SFMC. Our findings indicated that the percentage of $\gamma\delta$ T cells in lymphocytes from SFMC appeared slightly higher compared to that from PBMC of the same gout patients, though this difference was not statistically significant (Figure 2C). Moreover, the results showed a marked increase in the proportion of V δ 2 T cells in total $\gamma\delta$ T cells from SFMC, in comparison to those from PBMC [SFMC vs PBMC, 49.01% (45.1–56.53%) vs 23.58% (11.99–29.7%); *P*=0.001] (Figure 2D). Conversely, there was no significant difference in the percentages of V δ 1 T cells between gout SFMC and PBMC (Figure 2D). These findings provide evidence that V δ 2 T cells in AGA patients could migrate from peripheral blood to synovial fluid.

CXCR3 and CXCL10 Potentially Drive the Chemotaxis of V δ 2 T Cells to the Synovial Fluid of Patients with AGA

Gout SF, analogous to RA SF (utilized as a positive control for chemotaxis), significantly promoted the recruitment of $\gamma\delta$ T cells (both V δ 1 T cells and V δ 2 T cells, more remarkable for V δ 2 T cells) from AGA patients, as compared to both OA SF (used as a negative control) and gout serum (Figure 3A). These results suggested the migration of V δ 2 T cells into the synovial fluid during the disease progression of gout.

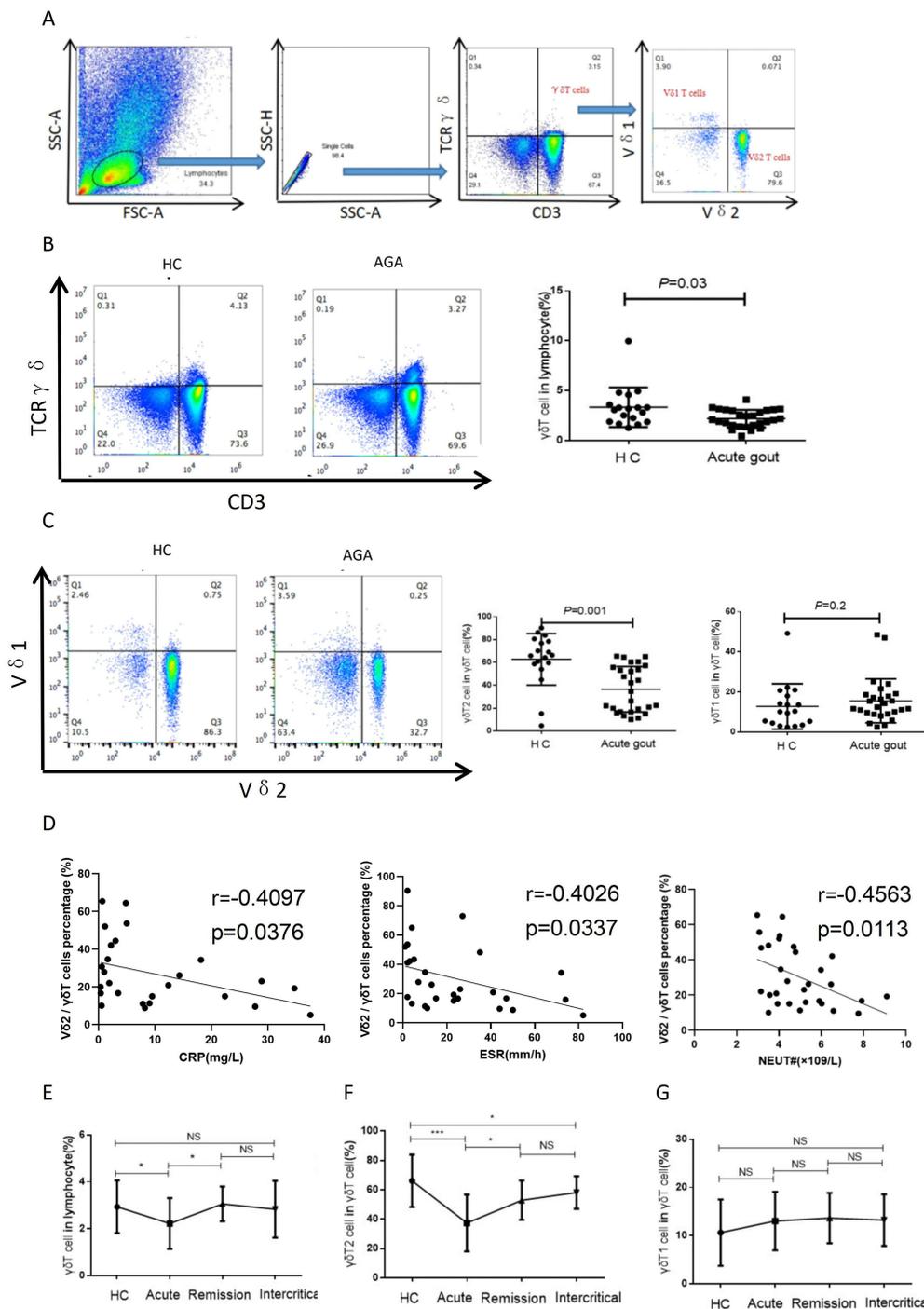


Figure 1 Patients with AGA had low levels of peripheral V δ 2 T cells. **(A)** Illustrated in this figure are representative scatter plots delineating the gating strategy employed for the detection of $\gamma\delta$ T cells, V δ 1 T cells, and V δ 2 T cells. Lymphocytes are identified by their forward scatter (FSC) and side scatter (SSC) characteristics, with single cells isolated based on side scatter area (SSC-A) and side scatter height (SSC-H) parameters. Subsequent gating is conducted for $\gamma\delta$ T cells using CD3 and TCR $\gamma\delta$ markers, and for V δ 1 and V δ 2 T cells using V δ 1 and V δ 2 markers, respectively. **(B-C)** Peripheral blood mononuclear cells derived from HCs and patients with AGA were subjected to staining with anti-CD3, anti- $\gamma\delta$ TCR, anti-V δ 1 or anti-V δ 2 monoclonal antibodies (mAbs) for subsequent flow cytometry analysis. The left panels show representative flow cytometry profiles of $\gamma\delta$ T cells **(B)**, and V δ 1 T cells and V δ 2 T cells **(C)**. The right panels show bar graphs of the percentage of the respective cell populations. The sample size consists of n=18 for the HC group, and n=27 for the AGA group. **(D)** The percentage of peripheral V δ 2 T cells in AGA shows a negative correlation with the concentration of CRP, value of ESR and the count of neutrophils (n=21). **(E-G)** Depicted herein are alternations in the percentages of $\gamma\delta$ T cells, V δ 2 T cells and V δ 1 T cells across HCs and different stages of gout. Data from HC (n=18), acute period (n=27), remission period (n=11) and intercritical period (n=26) are shown. Results are expressed as mean \pm SEM. ns, no significance; *P<0.05, and ***P<0.001 signify significance as determined through two-tailed unpaired t-test. Correlations are calculated using Spearman correlation analysis.

Abbreviations: FSC, forward scatter; SSC, side scatter; SSC-A, side scatter area; SSC-H, side scatter height; mAbs, monoclonal antibodies; NEUT, neutrophils; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; HC, healthy control; AGA, acute gouty arthritis; TCR, T cell receptor.

Table 3 The Proportions of $\gamma\delta$ T Cells and Their Subtypes in Gout Patients and Healthy Controls

	HC (n=18)	AGA (n=27)	Remission Period (n=11)	Intercritical Period (n=26)
δ T cells /lymphocytes (% , Q1-Q3)	3.13 (1.89–3.85)*	2.14 (1.49–3.08)	2.89 (2.52–3.34)*	2.91 (1.88–3.56)
V δ 1 T cells/ $\gamma\delta$ T cells (% , Q1-Q3)	10.25 (4.21–18.7)	11.6 (8.97–17.16)	12.58 (10.33–16.63)	13.5 (9.89–19.12)
V δ 2 T cells/ $\gamma\delta$ T cells (% , Q1-Q3)	66.05 (57.45–78.93)*	30.4 (16.83–54.13)	52.55 (46.97–61.83)* [§]	59.89 (50.23–65.2) [§]

Notes: *Represented statistical significance ($p < 0.05$) compared with AGA group. [§]Represented statistical significance ($p < 0.05$) compared with HC group.

The recruitment of leucocytes to specific target tissues is regulated by chemokines and their corresponding receptors.³³ Considering that the previous study had showed higher expressions of CCR5 and CXCR3 on V δ 2 T cells in RA patients compared to OA patients and HCs,⁷ we compared the expressions of CCR5 and CXCR3 on V δ 2 T cells derived from AGA patients and HCs. However, we found a notable increase in CXCR3 expression on V δ 2 T cells from AGA patients ($P=0.0005$, [Figure 3B](#)) along with a comparable expression of CXCR5 ([Figure 3D](#)), when compared with those from HCs. Additionally, V δ 2 T cells from AGA patients showed a higher expression of CXCR3 compared to V δ 1 T cells from AGA patients ($P=0.0014$, [Figure 3C](#)). Subsequently, we quantified the concentrations of various chemokines (ligands of CXCR3 or CCR5) in SF and serum samples from patients with AGA, employing SF samples from RA patients and OA patients as controls for comparison. We found that CXCL10 was the sole chemokine displaying a significantly heightened concentration within gout SF, when compared to its concentration in gout serum ($P < 0.05$, [Figure 3E and F](#)). The cut-off values of different ELISA kits were showed in [Table 4](#).³⁴ Collectively, these findings indicated that the up-regulation of CXCR3 on V δ 2 T cells in AGA patients, in combination with a higher level of its ligand CXCL10 in SF environment, may work cooperatively to promote the recruitment of V δ 2 T cells to the affected joints in patients with AGA.

V δ 2 T Cells from Patients with AGA Produce Aberrantly High Levels of IL-17

V δ 2 T cells exhibit highly cytotoxic activity and produce proinflammatory cytokines, including IFN- γ , TNF- α , IL-1 β and IL-17.^{10,32} Initially, we found a higher proportion of IL-17-producing V δ 2 T cells relative to total V δ 2 T cells in AGA patients compared to HCs ($P=0.004$, [Figure 4A](#)). However, no significant difference was observed in the proportions of TNF- α +, IFN- γ + and IL-1 β + V δ 2 T cells between blood samples from HCs and patients with AGA ([Figure S3](#)). Subsequently, we quantified diverse cytokine levels in SF samples from AGA patients as well as serum samples from both AGA patients and HCs. In comparison to serum samples from HCs, those from AGA patients exhibited a minor and non-significant elevation in IL-17 concentrations; while compared to AGA serum samples, AGA SF samples revealed significantly elevated IL-17 concentration (AGA SF vs AGA serum, 33.92 ± 20.76 (pg/mL) vs 12.4 ± 4.97 (pg/mL); $P=0.033$) ([Figure 4B](#)). Furthermore, higher concentrations of IL-2, IL-4, IL-10, TNF- α , IFN- γ , IL-1 and IL-12 were discerned in AGA SF samples compared to AGA serum ([Figure 4B](#)). To elucidate further the role of V δ 2 T cells in the increased IL-17 secretion within gout SF, we separately quantified both the proportion of IL-17-producing V δ 2 T cells relative to total V δ 2 T cells and IL-17-producing Th17 cells relative to Th17 cells within gout SF and AGA peripheral blood by intracellular staining. The results heightened a higher proportion of IL-17-producing V δ 2 T cells within gout SFMC compared to PBMC ($P=0.008$, [Figure 4C](#)). In contrast, no significant difference was observed in the proportion of IL-17-producing cells among Th17 cells from gout SFMC and PBMC ([Figure 4D](#)). These findings suggest that V δ 2 T cells from AGA patients can excessively secrete elevated levels of IL-17, potentially contributing to the pathogenesis of AGA.

Discussion

$\gamma\delta$ T cells, contributing a minority of the total human circulating T-cell pool, are associated with other immune cells and have potential regulatory roles in various diseases, such as infection, autoimmunity, cancer and other diseases.¹⁰ In our study, we initially observed a markedly diminished proportion of peripheral $\gamma\delta$ T cells, predominantly the V δ 2 T cell subset, among patients with AGA. This phenomenon could potentially arise from their substantial accumulation within synovial tissues, rather than being attributed to aberrant cellular apoptosis or compromised proliferative capacity. This

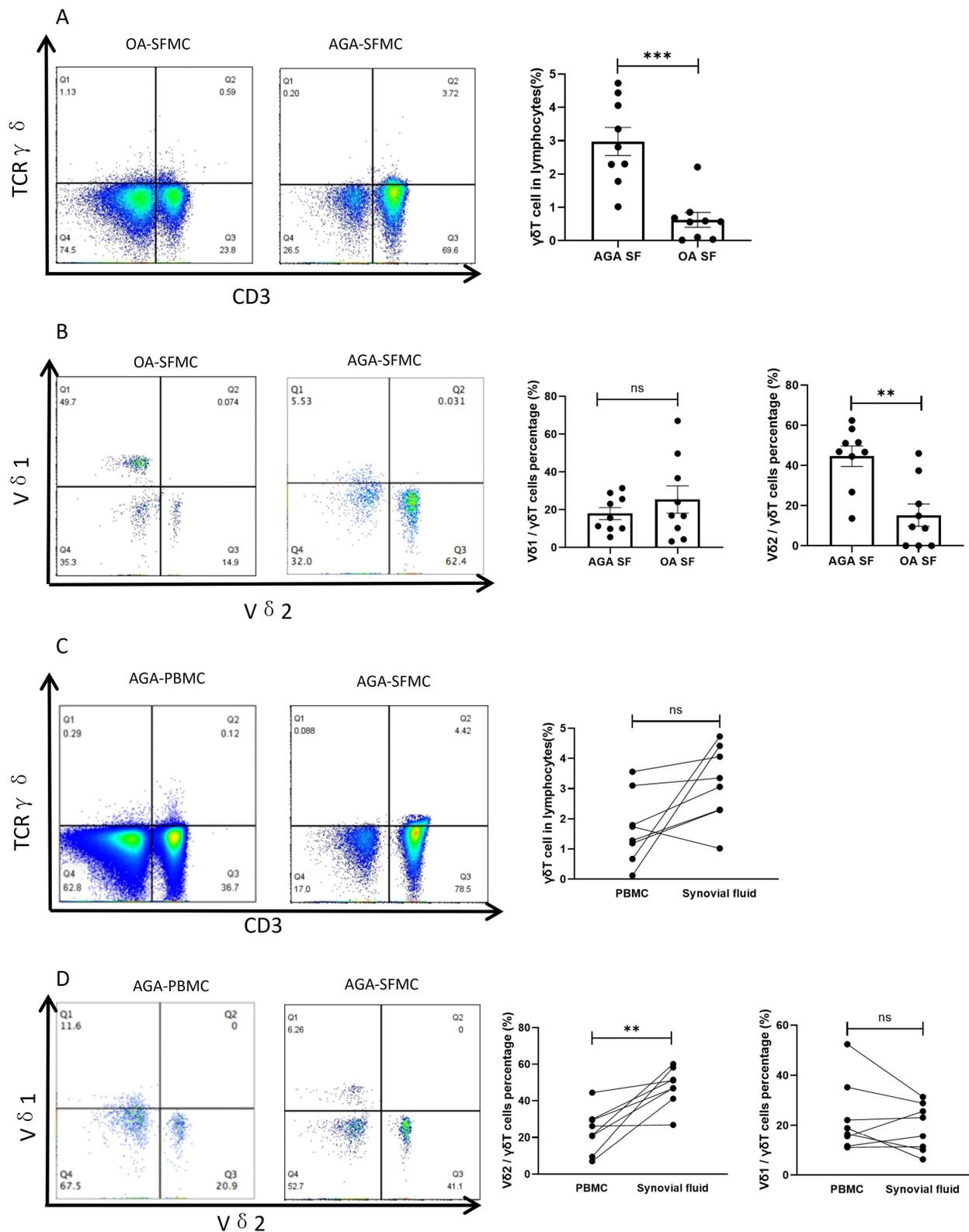


Figure 2 $V\delta 2$ T cells accumulated in gout synovial fluid. **(A and B)** The proportions of $\gamma\delta$ T cells within the lymphocytes subset **(A)** and the ratios of $V\delta 2$ T cells and $V\delta 1$ T cells among the total $\gamma\delta$ T cells populations **(B)** were quantified through flow cytometry analyses for OA-SFMC samples ($n=9$) and AGA-SFMC samples ($n=9$). Representative flow profiles are shown and the percentages were summarized. **(C and D)** The left panels show representative flow profiles of $\gamma\delta$ T cells **(C)**, $V\delta 1$ T cells and $V\delta 2$ T cells **(D)** derived from both PBMC, and SFMC from patients with gout ($n=8$). The right panels show bar graphs of the percentage of corresponding cells within the indicated populations. Results are expressed as mean \pm SEM. ns, no significance; ** $P<0.01$, and *** $P<0.001$ signify significance as determined through two-tailed paired t -test.

Abbreviations: TCR, T cell receptor; PBMC, peripheral blood mononuclear cell; SFMC, synovial fluid mononuclear cell; OA, osteoarthritis.

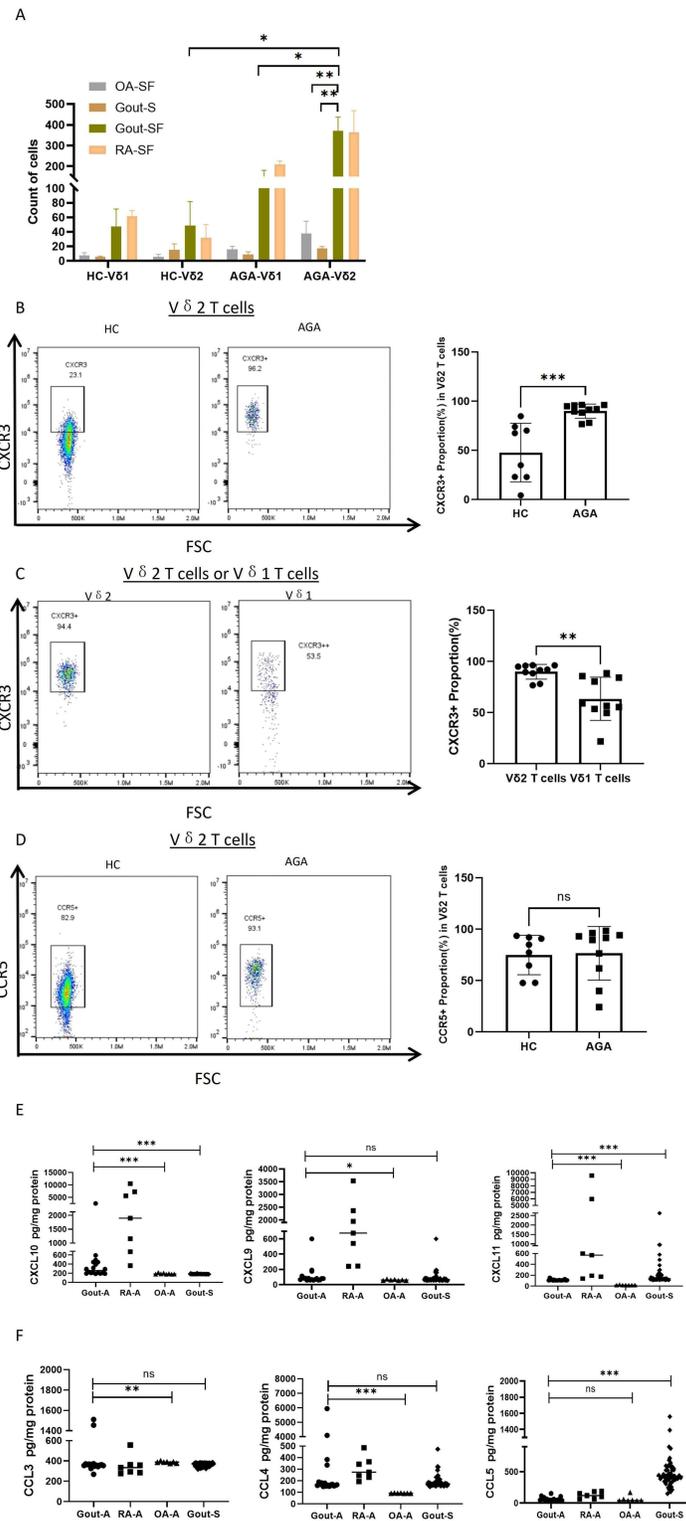


Figure 3 CXCR3 and CXCL10 may be associated with the chemotaxis of Vδ2 T cell in AGA. **(A)** Transwell migration assay demonstrated a pronounced capacity of chemotaxis in Vδ2 T cells isolated from AGA patients. Data were pooled from three independent experiments. **(B)** Comparison of the proportion of CXCR3-positive cells in Vδ2 T cells from HCs and AGA patients. **(C)** Comparison of the proportion of CXCR3-positive cells in Vδ2 T cells and Vδ1 T cells from AGA patients. **(D)** Comparison of the proportion of CCR5-positive cells in Vδ2 T cells from HCs and AGA patients. Data of HC (n=8) and AGA (n=10) are shown. **(E and F)** The concentrations of established ligands of CXCR3 and CCR5 were quantified within SF of gout patients (n=18), RA patients (n=7), OA patients (n=7), and serum of gout patients (n=49). Results are expressed as mean±SEM. *P<0.05, **P<0.01, and ***P<0.001 signify significance as determined through the Mann–Whitney U-test **(A and E)** and two-tailed unpaired t-test **(B–D)**.

Abbreviations: AGA, acute gouty arthritis; HC, healthy control; OA, osteoarthritis; RA, rheumatoid arthritis; SF, synovial fluid; CXCR 3, C-X-C chemokine receptor 3; CCR5, C-C chemokine receptor.

Table 4 The Cut-off Values of Different ELISA Kits

	Mean (OD)	Standard Deviation (SD)	Cut-off OD Values	Cut-off Values (pg/mL)
CXCL10	0.0321	0.021	0.0951	229.95
CXCL9	0.1406	0.06548	0.33704	113.58
CXCL11	0.0218	0.01252	0.05936	63.98
CCL3	0.0214	0.00936	0.04948	556.64
CCL4	0.0233	0.01111	0.05663	132.29
CCL5	0.0278	0.01392	0.06956	88.93

Abbreviations: OD, optical density; CXCL, C-X-C motif chemokine ligand; CCL, C-C motif chemokine ligand; SD, standard deviation.

trend resonates with analogous observations in the context of RA,⁷ chronic inflammatory bowel disease³⁵ and chronic human immunodeficiency virus (HIV) infection.³⁶ Since no significant difference in population abundance was observed in monocytes, NKT-like cells, nor ILC between AGA patients and HCs, V δ 2 T cells seem to represent a previously unrecognized innate immune cell subset that undergoes modulation during the progression of gout. Consequently, we conducted further comparisons of $\gamma\delta$ T cell proportions across varying gout stages. The proportion of total $\gamma\delta$ T cells and V δ 2 T cells in peripheral blood decreased during the acute stage of gout, while increased during the remission stage, indicating that $\gamma\delta$ T cells, notably V δ 2 T cells, were involved in acute gouty inflammation. Significantly, during the intermittent phase, the proportion of $\gamma\delta$ T cells surged to a level insignificantly different from that of HCs, although the proportion of V δ 2 T cells remained below that of HCs. It is speculated that V δ 2 T cells may have played a certain role in the development of acute gouty arthritis into chronic gouty arthritis (CGA). In line with our observations, V δ 2 T cells represent the predominant sub-populations within $\gamma\delta$ T cells in chronic arthritis diseases including RA,⁷ psoriatic arthritis,³⁷ and ankylosing spondylitis,³⁸ actively participating in the occurrence and progression of these diseases. Collectively, our findings propose that gout disrupts equilibrium within circulating $\gamma\delta$ T-cell subsets, characterized by a specific depletion of circulating V δ 2 T cells, and targeting V δ 2 T cells might be a promising approach to treat gout.

Chemokine receptors are expressed in various types of cells and interact with chemokines to promote homing of cells to target tissues.³³ Chemokines are implicated in a wide range of diseases with prominent inflammatory components. For instance, elevated levels of C-C chemokines, particularly CCL2, CCL3, and CCL5, within the joints of RA patients coincide with the recruitment of monocytes and T cells into synovial tissues.³⁹ In context of asthma, the chemokine CCL11 (eotaxin) and its receptor, CCR3, contribute to the recruitment of eosinophils to the pulmonary environment. Furthermore, CXCR3 has also been implicated in the recruitment of T cells to inflamed skin.⁴⁰ Considering the analogous phenomenon observed in $\gamma\delta$ T cells in both gout and RA, our focus shifted to the investigation of two specific chemokine receptors: CXCR3 and CCR5, which exhibited elevated expression in V δ 2 T cells in RA.⁷ However, we found that only CXCR3, which characterizes subsets of T cells associated with certain inflammatory reactions,⁴¹ exhibited pronounced expression on V δ 2 T cells. Conversely, CCR5 demonstrated a slight elevation but not statistically significantly up-regulation on V δ 2 T cells of AGA patients. This difference might stem from the distinct pathogenic mechanisms underlying AGA and RA, especially the unique feature of MSU deposition in AGA that could contribute to the divergent observations on chemokine receptor expressions between AGA and RA patients.

CXCR3 is a receptor predominantly expressed on the surfaces of monocytes, T cells, NK cells, dendritic cells, and cancer cells. Additionally, we confirmed that the corresponding agonistic ligand CXCL10 was significantly elevated in SF of gout patients compared to their serum levels. This elevation presumably facilitates the migration of circulating V δ 2 T cells. CXCL-10 is mainly secreted by monocytes, endothelial cells, fibroblasts, mesenchymal stromal cell (MSC)⁴² and cancer cells in response to IFN- γ , which is synergistically enhanced by TNF- α . The activation of IFN-regulatory factor 3, toll-like receptors (TLR), retinoic acid-inducible gene (RIG)-I, and melanoma differentiation-associated gene (MDA)-5 synergistically collaborates with IFNs to induce CXCL10 induction.^{43,44} As we did not observe an augmented production of TNF- α and IFN- γ from peripheral blood V δ 2 T cells, it is likely that a multiple of mechanisms, rather than an autocrine-mediated feedback loop, contribute to the increased

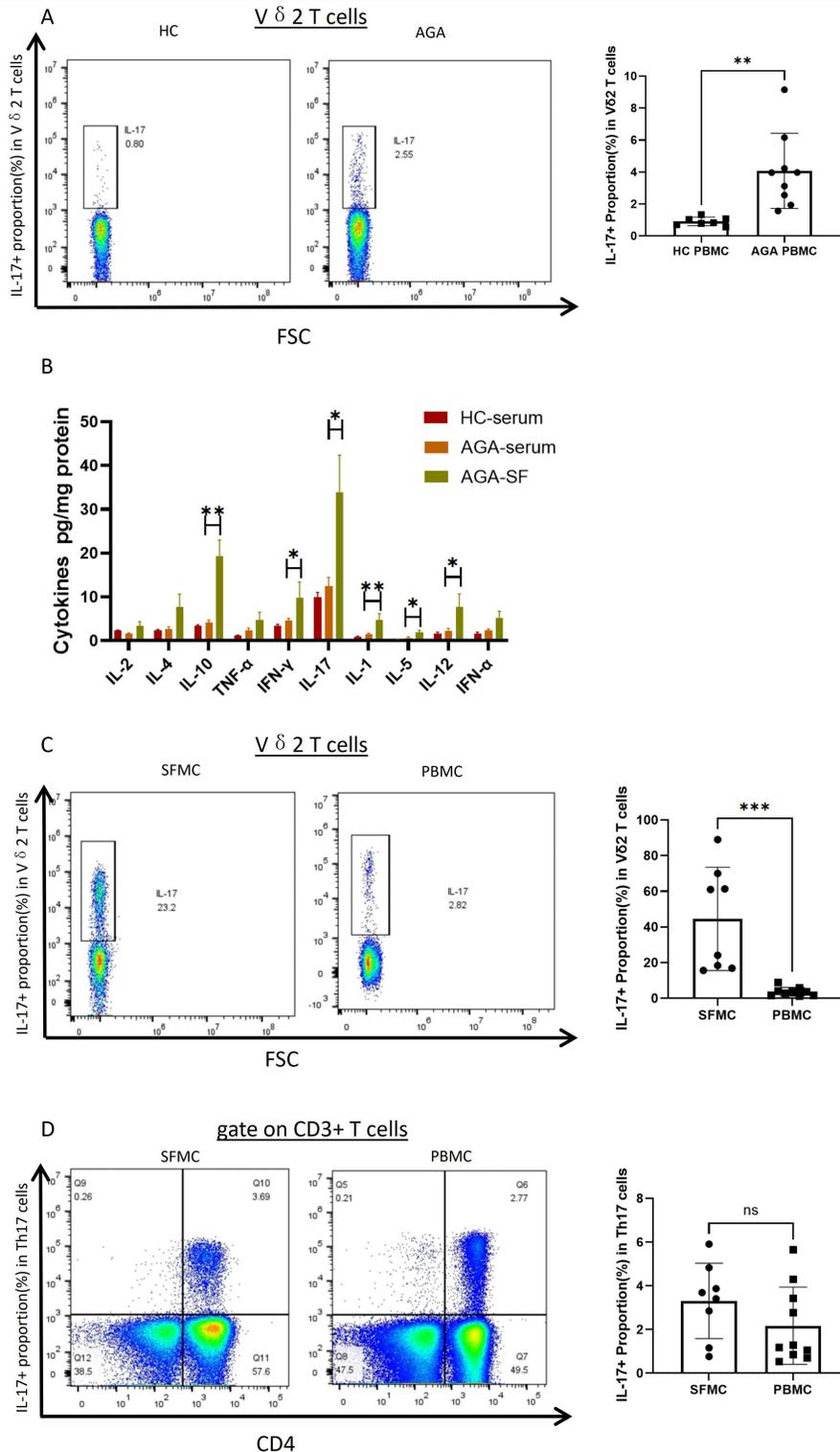


Figure 4 Vδ2 T cells in patients with AGA secreted high levels of IL-17. **(A)** Flow cytometry analyses were conducted to assess intracellular IL-17 staining in Vδ2 T cells sourced from peripheral blood samples of both HCs and AGA patients. The right panels show the percentage of positively stained cells among Vδ2 T cells. Data of HCs (n=7) and AGA (n=9) are shown. **(B)** Statistical graph showed the levels of various types of cytokines in gout SF (n=6), serum from patients with AGA (n=7) and serum from HCs (n=7). **(C and D)** Flow cytometry analyses of the intracellular staining of IL-17 in Vδ2 T cells **(C)** and in Th17 cells **(D)** of PBMC and SFMC from patients with gout were performed. The right panels show the percentage of positively stained cells among the indicated populations. Data of both groups (n=8) are shown. Results are expressed as mean±SEM. ns, no significance; *P<0.05, **P<0.01, and ***P<0.001 signify significance as determined through by two-tailed unpaired t-test.

Abbreviations: FSC, forward scattering; PBMC, peripheral blood mononuclear cell; SFMC, synovial fluid mononuclear cell; IFN-α, interferon-α; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-10, interleukin-10; IL-12, interleukin-12; IL-17, interleukin-17; TNF-α, tumor necrosis factor-α.

concentration of CXCL10 in synovial fluid during the onset of AGA. Furthermore, a prior investigation indicated that the accumulation of $\gamma\delta$ T cells, which represents an autoimmune response to infections or cancers, was regulated by CXCL9/CXCR3 axis-dependent mechanisms.⁴⁵ This implies that different chemokines binding to CXCR3 can assume distinct roles across various diseases, although inflammation-associated immune responses are involved in all these diseases. Intriguingly, we noticed that CXCL11 and CCL5 had significantly higher levels in gout serum than in gout SF, and their roles in the pathogenesis of gout need further investigation in the future. Collectively, these findings suggest that the up-regulated CXCR3 expression on V δ 2 T cells in AGA potentially orchestrated with elevated chemokine CXCL10 to promote the migration of V δ 2 T cells to the synovial fluid.

Peripheral blood V δ 2 T cells from AGA patients exhibited a higher proportion of IL-17+ V δ 2 T cells than those from HCs. However, due to the decreased V δ 2 T cell count, the IL-17 level in AGA serum did not display a significant increase compared with that in serum from HCs. A previous study showed that serum IL-17 levels in AGA patients rise as early as 8 hours after an acute gout attack,³ yet our results show no significant difference in serum IL-17 levels between AGA patients and HCs. These disparate outcomes could stem from divergent criteria for study group inclusion or variations in the acute stages at the time of sample collection. Regrettably, the mechanisms behind enhanced IL-17 secretion in V δ 2 T cells remain unexplored in our study. Recent literature, however, has unveiled the participation of the IL-23/IL-17 axis in several inflammatory arthropathies, such as psoriasis, PsA and spondyloarthritis (SpA).⁴⁶ It has been elucidated that V δ 2 T cells are able to generate IL-17-type cytokines dependent on IL-23/IL-23R signaling.⁴⁷ Moreover, the expression of IL-23R on $\gamma\delta$ T cells in crystal-induced arthritis patients surpasses that in healthy controls,³⁸ hinting that the augmented IL-17 secretion by V δ 2 T cells in AGA might also hinge on the IL-23/IL-17 axis, which is worthy refining in future studies. IL-17 alone is often poorly active, but it can synergize with other inflammatory cytokines such as TNF- α , IL-1 β , IL-22, IFN- γ , and granulocyte-macrophage colony-stimulating factor (GM-CSF), fostering increased production of inflammatory mediators like IL-6 and IL-8.^{48–50} In line with this, SF samples from AGA patients showed significantly increased levels of IL-4, IL-10, TNF- α , IFN- γ , IL-1, and IL-12, in contrast to their serum sample. This suggests that the potential synergistic effects of IL-17 and other soluble factors might collectively contribute to the progression of AGA. Additionally, IL-17-producing $\gamma\delta$ T cells might prime $\alpha\beta$ T cells to produce IL-17, thus augmenting the function of Th17 cells, which helps establish a positive feedback loop to amplify the production of IL-17. In congruence with this hypothesis, our previous study suggested that gout patients with a lengthier disease duration and involvement of a large number of joints were more prone to exhibit elevated serum IL-17 levels.⁵¹ Hence, these collective findings indicated that AGA distorts the cytokine production in $\gamma\delta$ T cells directly, and IL-17-producing V δ 2 T cells might wield a pivotal role in the pathogenesis of gout.

The principle strength of our study lies in its pioneering exploration of the role of V δ 2 T cells in gout, especially in context of AGA. Furthermore, our study is grounded in translational research, thus facilitating the application of these findings in clinical diagnostics and treatment. However, our study is not without limitations. Initially, the precise functional contributions of V δ 2 T cells to the pathogenesis of acute gouty arthritis remain incompletely elucidated. Nevertheless, we have undertaken a comprehensive depiction of the dynamic shifts in V δ 2 T cell proportions within peripheral blood across distinct phases of gout. Furthermore, we have conducted experiments to investigate the underlying mechanisms driving these alterations and the functional role of V δ 2 T cells in this context. Subsequent investigations will delve deeper into uncovering the precise functional attributes of V δ 2 T cells in AGA. Additionally, we have not exhaustively examined the expression of all potential chemokines that could prompt the migration of V δ 2 T cells, owing to the challenges associated with procuring synovial fluid from gout patients within clinical practice. Nevertheless, we have validated the participation of the CXCR3/CXCL10 axis in the migration of V δ 2 T cells in AGA through the assessment of presumed chemokines and their corresponding receptors. The involvement of other chemokines will be subjected to further scrutiny in subsequent studies.

Conclusions

In summary, our study unveils that $\gamma\delta$ T cells, predominantly V δ 2 T cells, exhibit diminished presence in peripheral blood but accumulate within gout-affected joints during acute phases. Intriguingly, their levels exhibit a partial recovery toward normalcy during intermittent phases. Furthermore, V δ 2 T cells exhibit an escalation in the secretion of pro-inflammatory cytokines, notably IL-17, suggesting their potential involvement in the pathogenesis of AGA. The chemotactic migration of V δ 2 T cells towards gout patients' synovial fluid is associated with the heightened expression of surface CXCR3 and the increased concentration of its ligand, CXCL10, within the synovial fluid. The elucidation of the roles played by V δ 2 T cells in gout not only advances our comprehension of the intricate pathogenic mechanisms underpinning gout but also promotes the exploration of novel avenues for developing promising therapeutics centered around V δ 2 T cells for the treatment of gout patients.

Abbreviations

AGA, acute gouty arthritis; IL-17, interleukin-17; MSU, monosodium urate; NLRP3, NOD-like receptor thermal protein domain associated protein 3; IL-1 β , interleukin-1 β ; NKT-like cells, natural killer T-like cells; ILC, innate lymphoid cells; TCR, T cell receptor; RA, rheumatoid arthritis; PsA, psoriatic arthritis; $\gamma\delta$ Th 17, $\gamma\delta$ T-helper 17; CIA, collagen-induced arthritis; SF, synovial fluid; HCs, healthy controls; PBMCs, peripheral blood mononuclear cells; SFMCs, synovial fluid mononuclear cells; FITC, fluorescein isothiocyanate; PE, phycoerythrin; APC, allophycocyanin; PerCP/Cy5.5, peridinin-chlorophyll-protein/cyanine5.5; PBS, phosphate buffered saline; BSA, bovine serum albumin; CD, cluster of differentiation; CXCR, C-X-C chemokine receptor; CCR, C-C chemokine receptor; IFN- γ , interferon- γ ; TNF- α , tumor necrosis factor- α ; HRP, horseradish peroxidase; OD, optical density; OA, osteoarthritis; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; CXCL, C-X-C motif chemokine ligand; CCL, C-C motif chemokine ligand; MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; HIV, human immunodeficiency virus; CGA, Chronic gouty arthritis; MSC, mesenchymal stromal cell; TLR, toll-like receptors; RIG, retinoic acid-inducible gene; MDA, melanoma differentiation-associated gene; GM-CSF, granulocyte-macrophage colony-stimulating factor.

Data Sharing Statement

The corresponding author will, on request, detail the restrictions and any conditions under which access to some data may be provided.

Ethics Approval

This study complies with the Declaration of Helsinki, and human specimen experiments were approved by the Ethics Committee of Peking Union Medical College Hospital, with informed written consent provided by all participants (JS-2339).

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

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Disclosure

The authors declare that they have no conflicts of interest.

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