

Received: 2016.11.20  
Accepted: 2017.02.01  
Published: 2017.03.10

# Elevated Expression of Immunoreceptor Tyrosine-Based Inhibitory Motif (TIGIT) on T Lymphocytes is Correlated with Disease Activity in Rheumatoid Arthritis

**Authors' Contribution:**

Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

ABDEFG 1 **Qing Luo\***  
ABCDF 2 **Zhen Deng\***  
BCD 2 **Chuxin Xu**  
BCF 2 **Lulu Zeng**  
BCF 2 **Jianqing Ye**  
BCD 2 **Xue Li**  
BCF 1 **Yang Guo**  
BCD 1 **Zikun Huang**  
ABCDEF 1 **Junming Li**

1 Department of Clinical Laboratory Medicine, The 1<sup>st</sup> Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, P.R. China  
2 College of Medicine, Nanchang University, Nanchang, Jiangxi, P.R. China

\* Equally contributed to this study

**Corresponding Authors:**

Junming Li, e-mail: [lisir361@163.com](mailto:lisir361@163.com), Zikun Huang, e-mail: [491353062@qq.com](mailto:491353062@qq.com)

**Source of support:**

This work was supported by the National Natural Science Foundation of China (81360459) and Jiangxi Provincial Natural Science Foundation of China (20151BAB215031)

**Background:**

It is well known that lymphocytes play an important role in rheumatoid arthritis (RA). T cell immunoreceptors with immunoglobulin (Ig) and immunoreceptor tyrosine-based inhibitory motif (TIGIT) have immunosuppressive co-stimulatory molecules that mediate inhibitory effects, but their roles in RA are poorly understood.

**Material/Methods:**

Were recruited 76 patients with RA and 33 healthy controls (HC). Clinical manifestations, laboratory measurements, physical examination, and medical history of RA patients were recorded. The expression of TIGIT on CD3<sup>+</sup> T lymphocytes, B lymphocytes, monocytes, neutrophils, CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes, and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was determined using flow cytometry. The expression of TIGIT on T lymphocytes in patients with RA was further analyzed to investigate its correlations with markers of autoimmune response, inflammation, and disease activity in RA.

**Results:**

Compared with HC, the expression levels of TIGIT on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes were significantly increased in patients with RA (P < 0.01). The frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was positively correlated with RF, increased ACPA, ESR, and CRP levels. The frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was positively correlated with RF and ESR levels. Furthermore, the expression level of TIGIT on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was positively correlated with the DAS28 score in RA.

**Conclusions:**

The expression levels of TIGIT on T lymphocytes were elevated and correlated with disease activity in RA.

**MeSH Keywords:**


**Arthritis • Autoantibodies • T-Lymphocytes**

**Abbreviations:**

**RA** – rheumatoid arthritis; **RF** – rheumatoid factor; **anti-CCP** – antibodies against cyclic citrullinated peptides; **PD-1** – programmed death-1; **PD-L1** – programmed death ligand 1; **PB** – peripheral blood; **DAS28** – disease activity score 28; **HCs** – healthy controls; **ESR** – erythrocyte sedimentation rate; **CRP** – C-reactive protein; **ACPA** – anticitrullinated protein antibodies; **DMARDs** – disease-modifying anti-rheumatic drugs; **MFI** – mean fluorescence intensity; **Ig** – T cell immunoreceptors with immunoglobulin; **TIGIT** – immunoreceptor tyrosine-based inhibitory motif; **Tim-3** – T cell immunoglobulin domain- and mucin-domain-containing molecule-3; **CIA** – collagen-induced arthritis

**Full-text PDF:**

<http://www.medscimonit.com/abstract/index/idArt/902454>

 2421

 1

 6

 33



## Background

Rheumatoid arthritis (RA) is characterized by persistent synovitis and systemic inflammation, and frequently leads to cartilage erosion and bone injury. Activation and recruitment of immune cells, especially lymphocytes and monocytes, into the joints are major characteristics of RA [1,2]. About 1% of the general population has RA, and many patients develop long-term joint damage, severe illness, and disability [3]. The mechanisms underlying RA are complex, including genetic and environmental factors, inflammatory cytokines, and abnormalities of both innate immunity and adaptive immunity [4,5]. Pivotal in the pathogenesis of RA is the pathogenic autoantibody production such as rheumatoid factor (RF) and antibodies against cyclic citrullinated peptides (anti-CCP) [6,7]. Evidence from both human studies and animal models have indicated that autoantibody production and RA pathogenesis are dependent upon T cells [8–10]. Indeed, recent research demonstrated that T cells with abnormal co-stimulatory molecules can activate autoantibody-producing B cells [11], suggesting the pivotal role of co-stimulatory molecules in the pathogenesis of RA. Revealing the abnormalities of co-stimulatory molecules expression on immune cells is therefore crucial for understanding the mechanisms of RA [12–16].

Co-stimulatory molecules regulate the functional outcome of T cell activation and the balance between activation and inhibitory signals, resulting in increased susceptibility to the induction of autoimmunity. T cell immunoreceptors with immunoglobulin (Ig) and immunoreceptor tyrosine-based inhibitory motif (TIGIT), also known as WUCAM, VSIG9, or VSTM3, is a newly identified co-inhibitory receptor. The poliovirus receptor (PVR, CD155) is the physical ligand of TIGIT, which is expressed mainly on antigen-presenting cells (APC). The interaction of PVR and TIGIT mediates the inhibitory effects on TIGIT-expressing cells [17]. In a mouse model, loss of TIGIT resulted in hyperproliferative T cell responses and increased susceptibility to autoimmune diseases [18], indicating the immunosuppressive role of TIGIT. As expected, TIGIT was reported to inhibit the activation of T cells and NK cells, manifested by down-regulating cytokines secretion by T cells and increasing the cytotoxicity

of NK cells [17,19–21]. Furthermore, the levels of TIGIT on NK cells was significantly decreased in patients with RA compared to healthy individuals, and is associated with the increase of IFN- $\gamma$ -producing NK cells in RA patients [22]. Zhao et al. [23] revealed that TIGIT-expressing synovial CD4<sup>+</sup> T cells were negatively correlated with the severity of RA. However, the expression of TIGIT on immune cells in the peripheral blood of patients with RA, as well as its role, is still unclear.

In the current study, we detected the expression of TIGIT on leucocytes in peripheral blood and quantified the proportions of TIGIT-expressing peripheral T-subset cells in RA patients. The correlation between TIGIT expression on peripheral T-subset cells and RA activity was also evaluated.

## Material and Methods

### Subjects

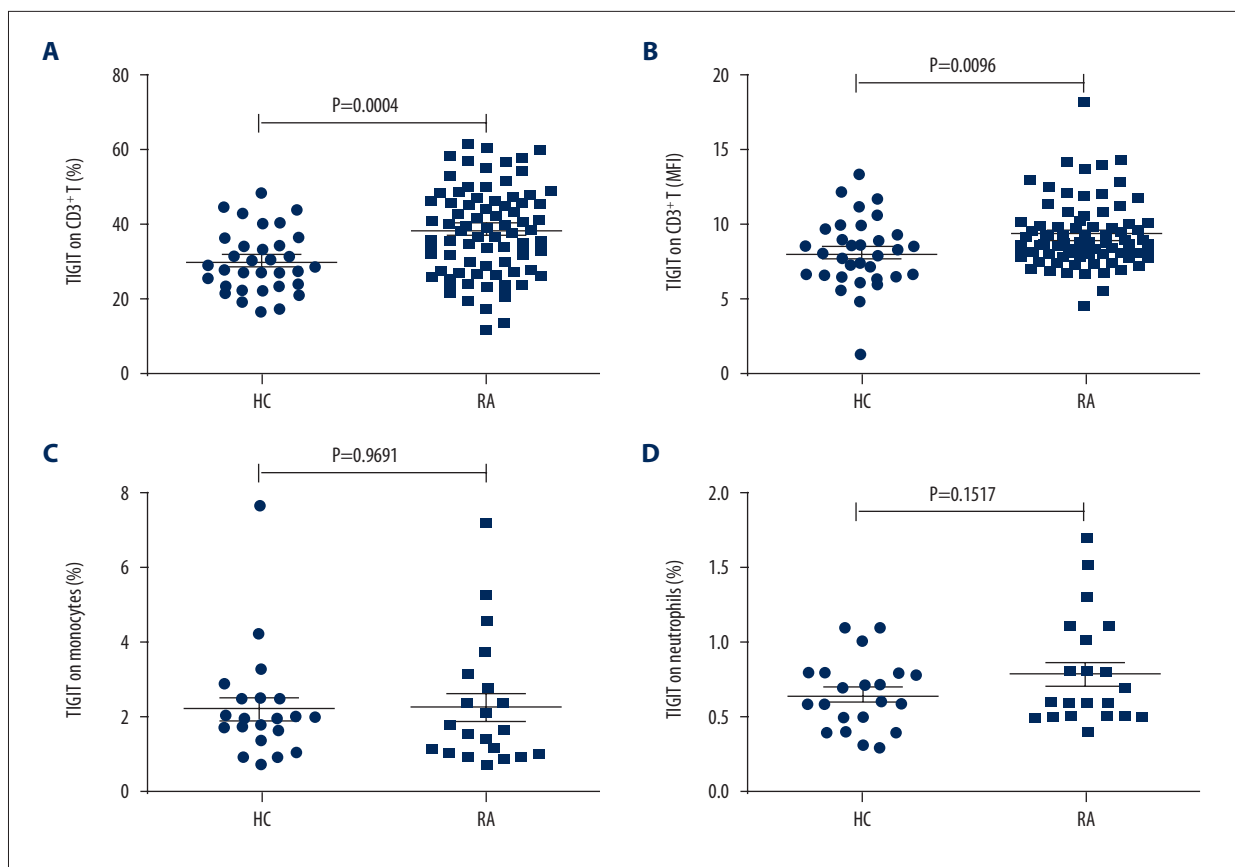
Fresh peripheral blood (PB) was collected by venipuncture from 76 patients with RA who fulfilled the American College of Rheumatology criteria for RA [24] and from 33 healthy controls (HCs) who were unrelated to the patients and who did not have infectious diseases, cancer, or inflammatory or autoimmune diseases (5 males, 28 females). The means of age of HCs was 48.6 years (Table 1). There was no statistically significant difference between RA patients and HCs regarding age or sex. None of the HCs members were currently receiving prescription drugs. Disease activity of RA was calculated using the disease activity score 28 (DAS28) [25]. The Ethics Committee of the First Affiliated Hospital of Nanchang University (Ethics Review Code: Research 2013-019) approved the study. Before the study, written informed consent were obtained from all participants.

### Flow cytometry

Fresh peripheral blood was collected from RA patients and HCs. The molecular phenotypes of leucocytes – CD3<sup>+</sup> T lymphocytes, B lymphocytes, monocytes, neutrophils, CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes,

**Table 1.** Baseline characteristics of patients with rheumatoid arthritis.

Categories	Patients with RA (n=76)	Healthy controls (n=33)
Females, %	84.2%	85%
Age (years, average $\pm$ SD)	53.7 $\pm$ 11.4	48.6 $\pm$ 11
DAS28 (average $\pm$ SD)	4.03 $\pm$ 1.9	–
RF (IU/mL, average $\pm$ SD)	425.9 $\pm$ 816.1	–
ACPA (RU/mL, average $\pm$ SD)	519.6 $\pm$ 855.3	–
CRP (mg/L, average $\pm$ SD)	17.4 $\pm$ 25.7	–
ESR (mm/h, average $\pm$ SD)	40.4 $\pm$ 33.2	–



**Figure 1.** TIGIT Expression on T lymphocytes, monocytes, and neutrophils. **(A)** Patients with RA had an elevated frequency of TIGIT-expressing T lymphocytes, as compared with HC ( $P=0.0004$ ). **(B)** Patients with RA had elevated MFI of TIGIT on T lymphocytes, as compared with HCs ( $P=0.0096$ ). **(C)** TIGIT expression on monocytes had no significant difference between HC and RA patients ( $P=0.9691$ ). **(D)** TIGIT expression on neutrophils had no significant difference between HC and RA patients ( $P=0.1517$ ).

and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes – were determined immediately using flow cytometry in a CYTOMICS FC 500 flow cytometer and analyzed using CXP software programs (BECKMAN COULTER). Fluorochrome-conjugated mAbs from BD Pharmingen and e Bioscience (San Diego, CA, USA) were used to examine the expression of CD3, CD4, CD8, CD14, CD15, CD19, and TIGIT.

### ESR, CRP, and autoantibody assay

Erythrocyte sedimentation rate (ESR) was measured according to the instructions described by the manufacturer. Level of C-reactive protein (CRP) and RF were determined using nephelometry. Anti-citrullinated protein antibodies (ACPA) from serum IgG were measured using commercially available ELISA kits (Kexin, Shanghai, China).

### Statistical analysis

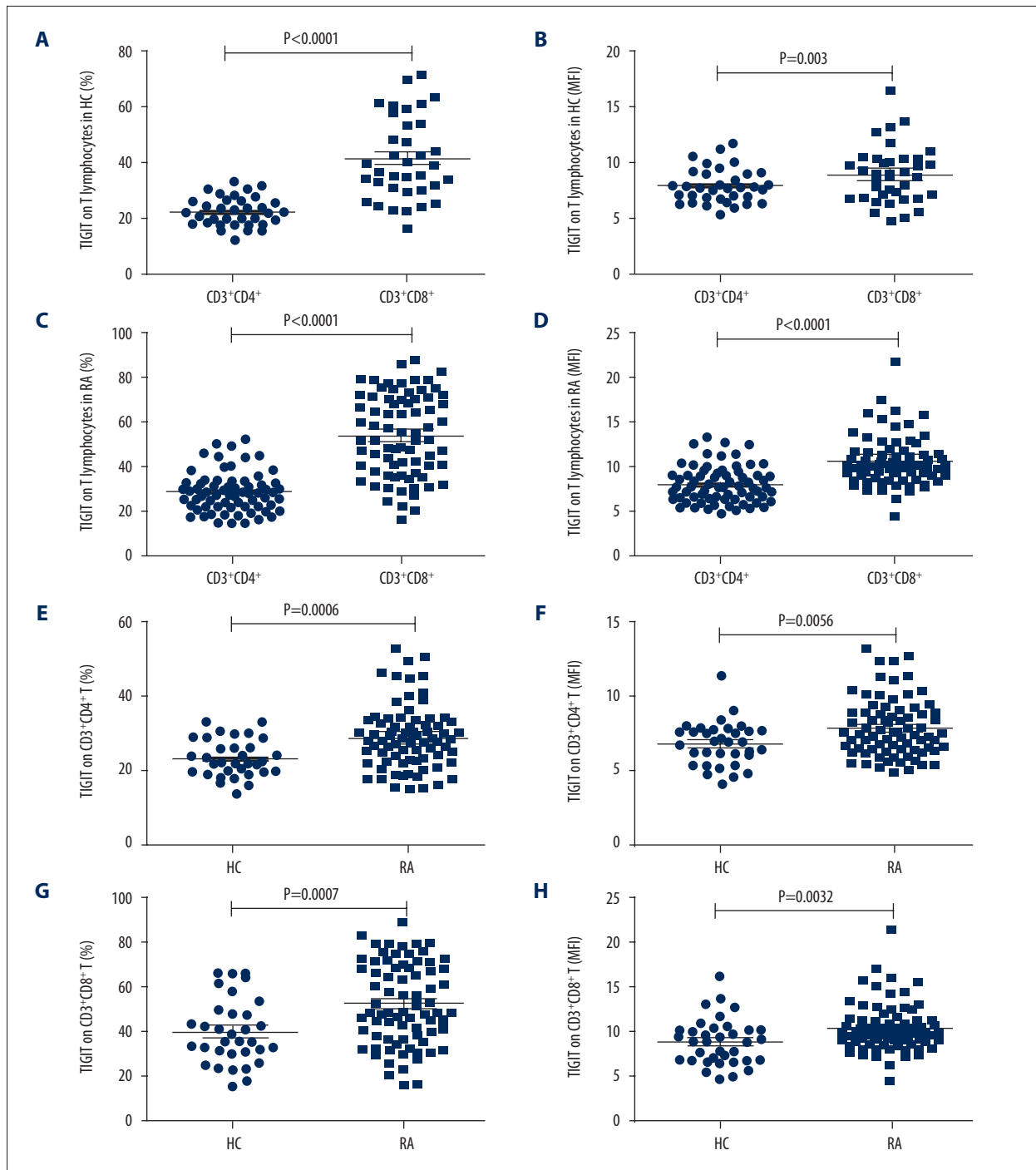
Statistical analysis and graphics presentation were conducted using Prism version 5.0 software (GraphPad Software, San

Diego, USA). Comparison between groups was analyzed by *t* test or Mann-Whitney test. Correlations were analyzed using the Pearson method or nonparametric Spearman method. A *P* value of less than or equal to 0.05 was considered significant.

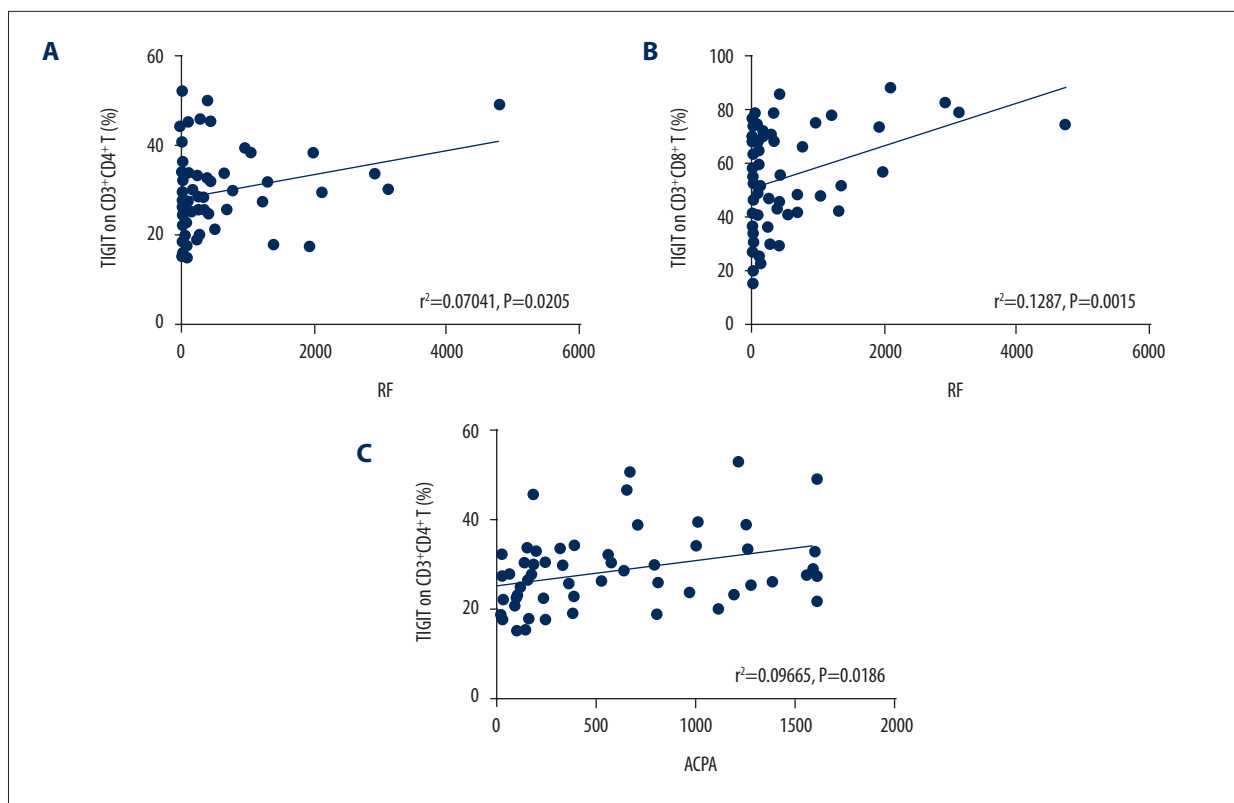
## Results

### Characteristics of study subjects

Information describing the study subjects is shown in Table 1. Patients with RA were divided into a remission group (DAS28 <2.6) and an active group (DAS28 >2.6) according to DAS28 [25]. Overall, 73.3% of the patients with RA were active patients. Among them, 9 patients had new-onset RA (<6-month disease duration) [26]. All patients were administered disease-modifying antirheumatic drugs (DMARDs).



**Figure 2.** TIGIT Expression on T lymphocytes subsets. (A) The frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was significantly elevated compared to CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes in HCs ( $P < 0.0001$ ). (B) The MFI of TIGIT on CD3<sup>+</sup>CD8<sup>+</sup> T lymphocyte was significantly elevated compared to CD3<sup>+</sup>CD4<sup>+</sup> T lymphocyte in HCs ( $P = 0.003$ ). (C) The frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was significantly elevated compared with that of CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes in RA ( $P < 0.0001$ ). (D) The MFI of TIGIT on CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was significantly elevated compared with that of CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes in RA ( $P < 0.0001$ ). (E) The frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocyte was significantly elevated in patients with RA as compared with HCs ( $P = 0.0006$ ). (F) The MFI of TIGIT on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was significantly increased in patients with RA compared with HCs ( $P = 0.0056$ ). (G) The frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was significantly increased in patients with RA compared with HCs ( $P = 0.0007$ ). (H) The MFI of TIGIT on CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was significantly elevated in patients with RA as compared with HCs ( $P = 0.0032$ ).



**Figure 3.** Correlation of frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes with autoantibody. (A) The frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was positively associated with RF level in patients with RA ( $r^2=0.07041$ ,  $P=0.0205$ ). (B) The frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was positively associated with RF level in patients with RA ( $r^2=0.1287$ ,  $P=0.0015$ ). (C) The frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was positively associated with increased ACPA in patients with RA ( $r^2=0.09665$ ,  $P=0.0186$ ).

### TIGIT expression on peripheral blood leucocytes in RA patients and HCs

To investigate the range of TIGIT expression in RA patients and HCs, the expression levels of TIGIT on peripheral blood leucocytes – T lymphocytes, B lymphocytes, monocytes, and neutrophils – were determined using flow cytometry. Results showed that both the frequency of TIGIT-expressing T lymphocytes and the mean fluorescence intensity (MFI) of TIGIT on T lymphocytes were significantly elevated in RA patients compared to HCs ( $P<0.05$ ) (Figure 1A, 1B). The frequencies of TIGIT-expressing monocytes and neutrophils had no significant difference between RA patients and HCs (Figure 1). B lymphocytes had no apparent TIGIT expression.

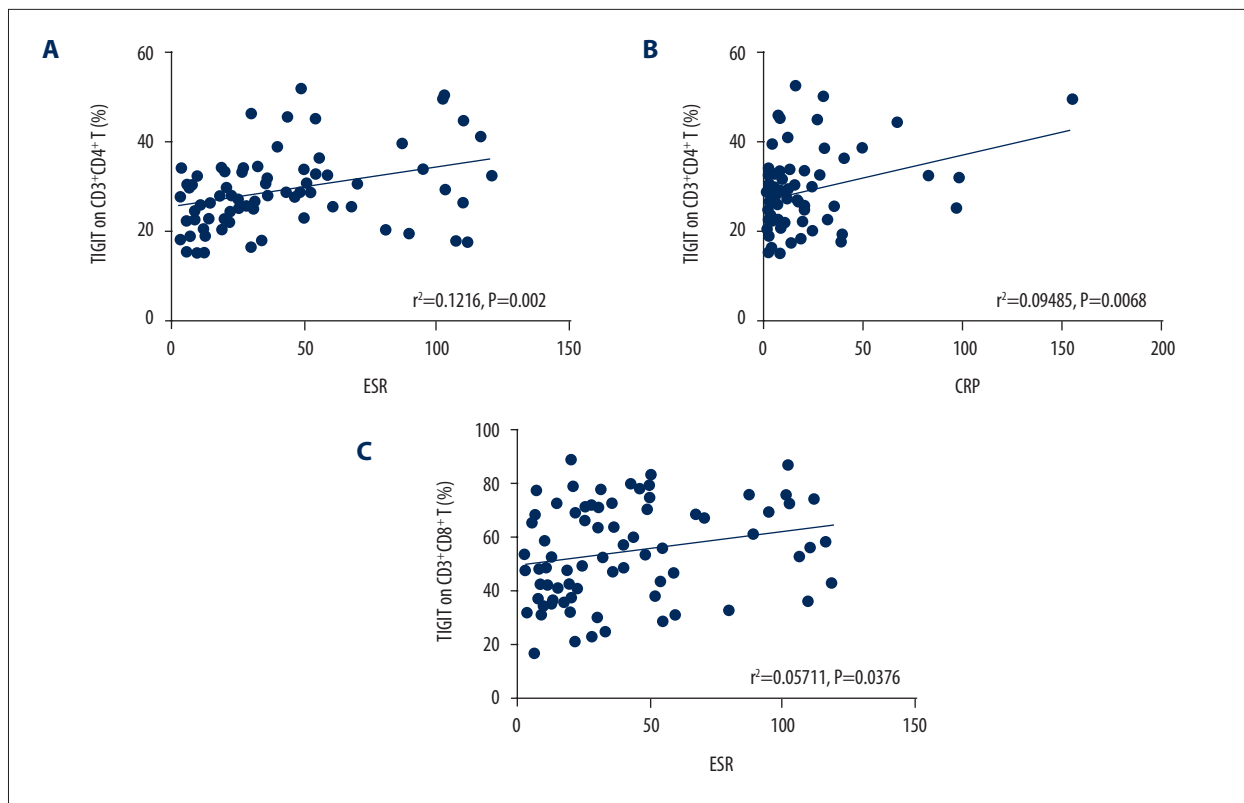
### TIGIT expression on T lymphocyte subsets in patients with RA and HCs

The aforementioned results demonstrate that TIGIT expression on T lymphocytes was significantly elevated in patients with RA as compared with HCs. To reveal the TIGIT expression profiles on T lymphocytes, the expression levels of TIGIT on T lymphocyte

subsets in patients with RA and HCs were determined and analyzed. The results showed that both the frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes and the MFI of TIGIT on CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes were significantly elevated, as compared with that of CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes in HCs ( $P<0.05$ ) (Figure 2A, 2B) and patients with RA ( $P<0.0001$ ) (Figure 2C, 2D). Moreover, we showed that the frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes and the MFI of TIGIT on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes were significantly elevated in patients with RA, as compared with that of HCs ( $P<0.05$ ) (Figure 2E, 2F). The frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes and the MFI of TIGIT on CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes were significantly elevated in patients with RA, as compared with HCs ( $P<0.05$ ) (Figure 2G, 2H).

### The frequency of TIGIT-expressing T lymphocytes is correlated with markers of autoimmune response

RA is characterized by autoantibody overproduction, such as RF and ACPA. Thus, the hallmark antibodies of RA – RF and ACPA – were detected and analyzed for their relationship with the expression of TIGIT on CD3<sup>+</sup>CD4<sup>+</sup> T and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes. Data showed that 53 patients were positive for RF and 57 patients



**Figure 4.** Correlation of frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocyte with inflammatory markers. **(A)** The frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was significantly correlated with ESR in patients with RA ( $r^2=0.1216$ ;  $P=0.002$ ). **(B)** The frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes correlated significantly with CRP level in patients with RA ( $r^2=0.09485$ ;  $P=0.0068$ ). **(C)** The frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was significantly correlated with ESR in patients with RA ( $r^2=0.05711$ ;  $P=0.0376$ ).

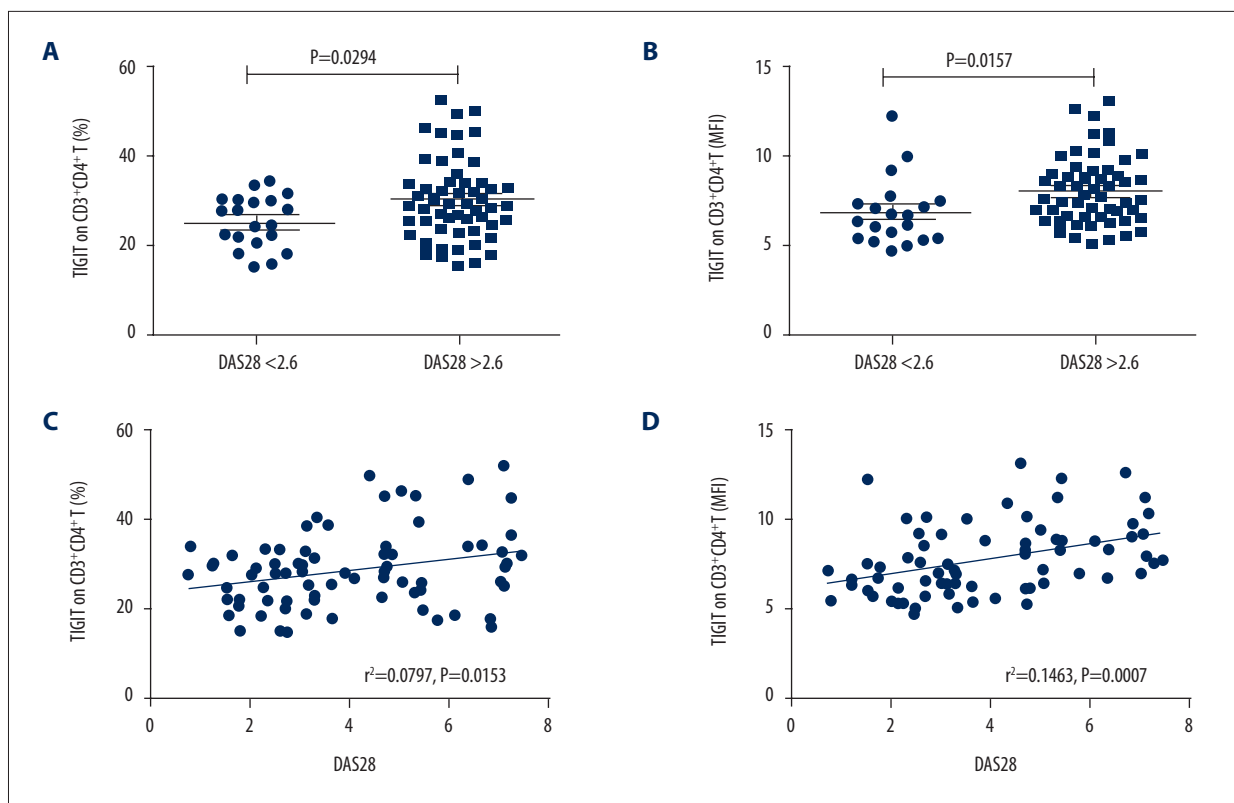
were positive for ACPA, for all patients who underwent autoantibody detection. As shown in Figure 3A and 3B, the frequencies of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes were positively correlated with RF levels. No significant correlation was found between the frequencies of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes, TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes, and serous ACPA (data not shown). However, the frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was positively associated with increased ACPA ( $r^2=0.07$ ;  $P=0.018$ ) (Figure 3C). Moreover, the correlations between the MFI of TIGIT on T lymphocytes and serum levels of RF and ACPA in RA were also investigated, but no obvious correlation was found (data not shown). These results indicate that the increased frequency of TIGIT-expressing T lymphocytes is associated with the markers of autoimmune response, suggesting that TIGIT-expressing T lymphocytes may be involved in the pathogenesis of RA.

#### Frequency of TIGIT-expressing T lymphocytes is correlated with inflammatory markers

RA is characterized by synovial hyperplasia and inflammation. Patients with RA frequently have elevated levels of

inflammatory markers. To determine the relationship between the frequency of TIGIT-expressing T lymphocytes and inflammatory markers, inflammatory markers, such as ESR and CRP, were determined and analyzed for their relationship with the frequencies of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes in patients with RA. As shown in Figure 4A and 4B, positive correlations between the frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes and ESR and CRP were found. We also found that the frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was positively associated with ESR ( $r^2=0.057$ ,  $P=0.037$ ) (Figure 4C). However, no obvious relationship was found between the frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes and CRP (data not shown). The correlations between the MFI of TIGIT on T lymphocytes and inflammatory markers in RA were also investigated, but no significant correlation was found (data not shown). These results indicate that the frequency of TIGIT-expressing T lymphocytes is correlated with inflammatory markers.





**Figure 5.** Correlation of TIGIT expression on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocyte with disease activity. (A) The frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was significantly increased in active patients with RA, as compared with those in remission (P=0.0294). (B) MFI of TIGIT on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was significantly increased in active patients with RA, as compared with those in remission from RA (P=0.0157). (C) The frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was significantly correlated with DAS28 in patients with RA ( $r^2=0.0797$ ; P=0.0153). (D) The MFI of TIGIT on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was significantly correlated with DAS28 in patients with RA ( $r^2=0.1463$ ; P=0.0007).

### TIGIT expression on T lymphocytes is correlated with disease activity in RA

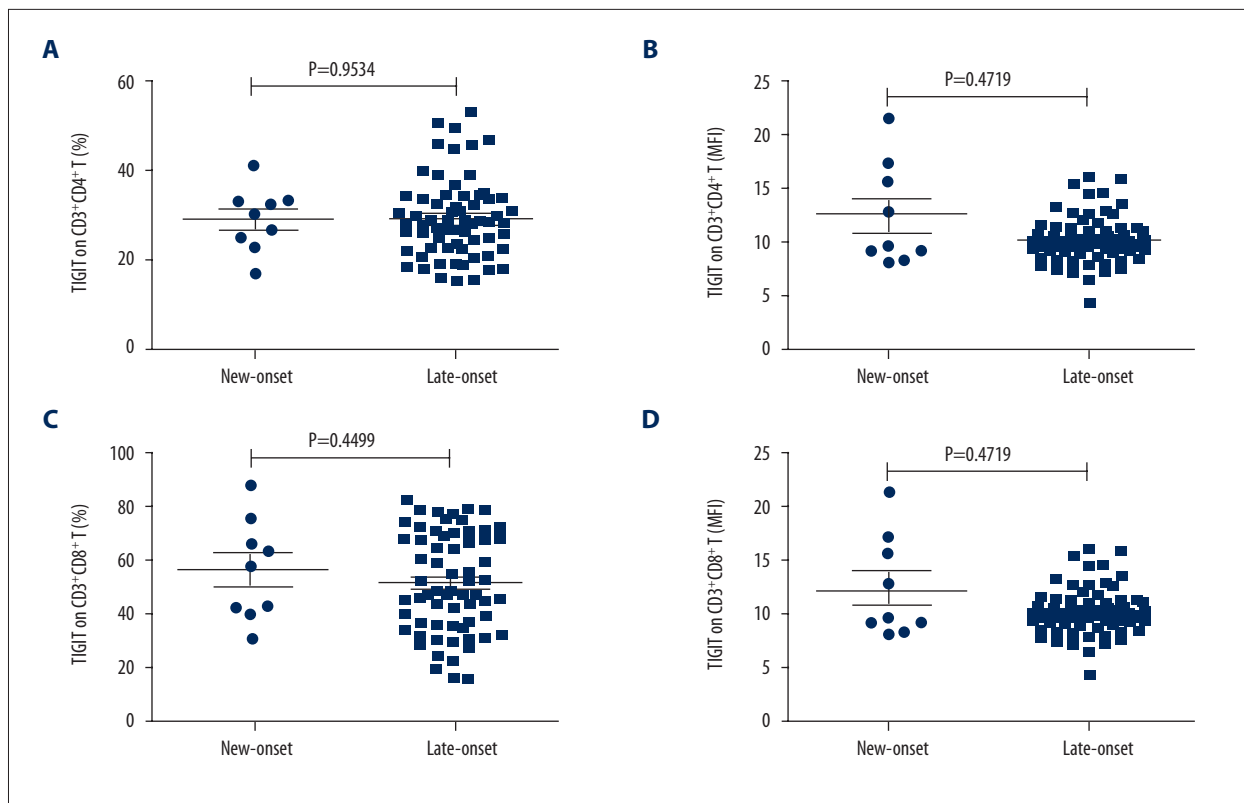
The aforementioned results demonstrate that the frequency of TIGIT-expressing T lymphocytes is associated with markers of autoimmune response and inflammation. Autoantibodies such as RF and ACPA are reported to be associated with disease activity and the severity of joint destruction in RA [27]. Further, ESR and CRP are traditionally used for calculating DAS28, a scoring system for assessing the severity of RA. Thus, patients with RA were further classified into active and remission according to their DAS28 score, and the correlation between disease activity and the expression of TIGIT on T lymphocytes was analyzed. Data indicated that the frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes and the TIGIT MFI on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes in patients with active RA were significantly increased, as compared with that of patients who were in remission (P<0.05) (Figure 5A and B). Furthermore, both the frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes and MFI of TIGIT on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes were positively correlated with DAS28 score (Figure 5C, 5D), demonstrating that TIGIT

expression on CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes is associated with disease activity in RA. The correlation between TIGIT expression on CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes and DAS28 was also investigated, but no significant correlation was found (data not shown).

Subsequently, we compared the TIGIT expression on T lymphocytes between patients with new- and late-onset RA. Data showed that the expression of TIGIT on T lymphocytes tends to be elevated in patients with new-onset RA, but a significant difference was not reached (P>0.05) (Figure 6).

### Discussion

RA is a systemic, debilitating, chronic, autoimmune disease with unclear etiology. Co-stimulatory molecules are reported to play important roles in RA [15]. Programmed cell death-1 (PD1), an immunosuppressive co-stimulatory molecule proven to play a major role in suppressing immune responses, and its ligand, programmed cell death-ligand 1, were found to be elevated in the T cells of patients with RA [12,13]. T cell immunoglobulin



**Figure 6.** TIGIT expression on T lymphocytes between patients with new-onset and late-onset RA. Data showed that the TIGIT expression on T lymphocytes tended to be elevated in patients with new-onset RA, but a significant difference was not reached ( $P>0.05$ ).

domain- and mucin-domain-containing molecule-3 (Tim-3), which are inhibitory receptors, have been showed to be expressed on T cells with high levels and to be correlated with disease activity in patients with RA [14]. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), a member of the immunoglobulin superfamily that can down-regulate T-cell function, was also reported to be decreased on T cells, and its dysfunction was identified as a potential cause for abnormal T cell activation in RA [16]. Additionally, CD40 ligand (CD40L) and many other inhibitory receptors have also been found to be expressed on T cells in RA [28,29]. TIGIT is a newly identified co-inhibitory receptor that is expressed on immune cells. The present study is the first to determine TIGIT expression on neutrophils, monocytes, B lymphocytes, and T lymphocytes in RA patients, showing that the expression of TIGIT on T lymphocytes is significantly elevated in RA patients, as compared with HCs. In addition, our study indicates that the frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T is correlated with disease activity in RA.

Lymphocytes are reported to play important roles in disease development and progression [9,10]. Our study shows that the frequency of TIGIT-expressing CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes is significantly elevated compared with that of CD3<sup>+</sup>CD4<sup>+</sup> T

lymphocytes. We also found that the TIGIT expression on CD3<sup>+</sup>CD4<sup>+</sup> T and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes was significantly elevated in patients with RA as compared with HCs. Our results support the observation that the abnormal expression of key signaling molecules on T lymphocytes plays an important role in pathogenesis of RA [30,31].

TIGIT was previously shown to be involved in interactions between T cells and follicular dendritic cells, regulating B cell responses and promoting antibody production [32,33]. RA is a systemic autoimmune disease characterized by increased auto-antibodies such as RF and ACPA. In this study, serum RF and ACPA levels were determined and analyzed for their relationship with TIGIT expression on T lymphocytes for the first time. Data indicated that the frequencies of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes were positively associated with RF in the serum of patients with RA. Furthermore, we had an interesting finding that the frequency of TIGIT-expressing CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes was positively associated with increased ACPA. These results suggest that TIGIT-expressing T lymphocytes are associated with autoimmune responses in RA.



TIGIT is an inhibitory co-stimulatory molecule that mediates inhibitory signals in immune cells [17]. Consistent with its inhibitory characteristics, the TIGIT expression on NK cells is decreased in RA patients [15], and the expression of TIGIT on CD4<sup>+</sup> T cells in synovial fluid is negatively correlated with RA disease activity [23]. Thus, from this perspective, the elevated expression of TIGIT on T lymphocytes in RA seems to be controversial. However, evidence also suggests that TIGIT is involved in regulating B cell responses and promotes antibody production [32,33]. In the present study, we found that RA-specific autoantibody levels, such as RF and ACPA, were positively correlated with the frequency of TIGIT-expressing T lymphocytes, which is consistent with the B cell-regulating profile of TIGIT. Thus, although further work is needed to clarify the exact roles and mechanisms of TIGIT-expressing T lymphocytes, it appears that in addition to functioning as an inhibitory co-stimulatory molecule, TIGIT might play other roles in RA.

## References:

- Li X, Yuan FL, Lu WG et al: The role of interleukin-17 in mediating joint destruction in rheumatoid arthritis. *Biochem Biophys Res Commun*, 2010; 397: 131–35
- Yuan FL, Li X, Lu WG et al: IL-33: A promising therapeutic target for rheumatoid arthritis? *Expert Opin Ther Targets*, 2011; 15: 529–34
- Goekoop-Ruiterman YP, Huizinga TW: Rheumatoid arthritis: Can we achieve true drug-free remission in patients with RA? *Nat Rev Rheumatol*, 2010; 6: 68–70
- McInnes IB, Schett G: The pathogenesis of rheumatoid arthritis. *N Engl J Med*, 2011; 365: 2205–19
- Polgár A, Brózik M, Tóth S et al: Soluble interleukin-6 receptor in plasma and in lymphocyte culture supernatants of healthy individuals and patients with systemic lupus erythematosus and rheumatoid arthritis. *Med Sci Monit*, 2000; 6(1): 13–18
- Rech J, Hueber AJ, Finzel S et al: Prediction of disease relapses by multi-biomarker disease activity and autoantibody status in patients with rheumatoid arthritis on tapering DMARD treatment. *Ann Rheum Dis*, 2016; 75(9): 1637–44
- Agrawal S, Misra R, Aggarwal A: Autoantibodies in rheumatoid arthritis: association with severity of disease in established RA. *Clinical Rheumatology*, 2007; 26: 201–4
- He J, Tsai LM, Leong YA et al: Circulating precursor CCR7loPD-1hi CXCR5+ CD4+ T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity*, 2013; 39: 770–81
- Bennett JC: The role of T lymphocytes in rheumatoid arthritis and other autoimmune diseases. *Arthritis Rheum*, 2008; 58: S53–57
- Holmdahl R, Klareskog L, Rubin K et al: Role of T lymphocytes in murine collagen induced arthritis. *Agents Actions*, 1986; 19: 295–305
- Godefroy E, Zhong H, Pham P et al: TIGIT-positive circulating follicular helper T cells display robust B-cell help functions: Potential role in sickle cell alloimmunization. *Haematologica*, 2015; 100: 1415–25
- Raptopoulou AP, Bertisias G, Makrygiannakis D et al: The programmed death 1/programmed death ligand 1 inhibitory pathway is up-regulated in rheumatoid synovium and regulates peripheral T cell responses in human and murine arthritis. *Arthritis Rheum*, 2010; 62: 1870–80
- Moret FM, van der Wurff-Jacobs KM, Bijlsma JW et al: Synovial T cell hyporesponsiveness to myeloid dendritic cells is reversed by preventing PD-1/PD-L1 interactions. *Arthritis Res Ther*, 2014; 16: 497
- Li S, Peng D, He Y et al: Expression of TIM-3 on CD4+ and CD8+ T cells in the peripheral blood and synovial fluid of rheumatoid arthritis. *APMIS*, 2014; 122: 899–904
- Wang F, Hou H, Wu S et al: TIGIT expression levels on human NK cells correlate with functional heterogeneity among healthy individuals. *Eur J Immunol*, 2015; 45: 2886–97
- Flores-Borja F, Jury EC et al: Defects in CTLA-4 are associated with abnormal regulatory T cell function in rheumatoid arthritis. *Proc Natl Acad Sci USA*, 2008; 105: 19396–401
- Stanietsky N, Simic H, Arapovic J et al: The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc Natl Acad Sci USA*, 2009; 106: 17858–63
- Joller N, Hafner JP, Brynedal B et al: Cutting edge: TIGIT has T cell-intrinsic inhibitory functions. *J Immunol*, 2011; 186: 1338–42
- Stanietsky N, Rovis TL, Glasner A et al: Mouse TIGIT inhibits NK-cell cytotoxicity upon interaction with PVR. *Eur J Immunol*, 2013; 43: 2138–50
- Li M, Xia P, Du Y et al: T-cell immunoglobulin and ITIM domain (TIGIT) receptor/poliiovirus receptor (PVR) ligand engagement suppresses interferon-gamma production of natural killer cells via beta-arrestin 2-mediated negative signaling. *J Biol Chem*, 2014; 289: 17647–57
- Joller N, Lozano E, Burkett PR et al: Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. *Immunity*, 2014; 40: 569–81
- Wang F, Hou H, Wu S et al: TIGIT expression levels on human NK cells correlate with functional heterogeneity among healthy individuals. *Eur J Immunol*, 2015; 45: 2886–97
- Zhao W, Dong Y, Wu C et al: TIGIT overexpression diminishes the function of CD4 T cells and ameliorates the severity of rheumatoid arthritis in mouse models. *Exp Cell Res*, 2016; 340: 132–38
- Arnett FC, Edworthy SM, Bloch DA et al: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthr Rheum*, 1988; 31: 315–24
- Prevo ML, van 't Hof MA, Kuper HH et al: Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum*, 1995; 38: 44–48
- Wang J, Shan Y, Jiang Z et al: High frequencies of activated B cells and T follicular helper cells are correlated with disease activity in patients with new-onset rheumatoid arthritis. *Clin Exp Immunol*, 2013; 174: 212–20
- Wright HL, Moots RJ, Edwards SW: The multifactorial role of neutrophils in rheumatoid arthritis. *Nat Rev Rheumatol*, 2014; 10: 593–601
- Kosmaczewska A, Ciszak L, Swierkot J et al: Alterations in both the activating and inhibitory potential of peripheral blood CD4+ T cells in rheumatoid arthritis patients correlate with disease progression. *Pathol Oncol Res*, 2014; 20: 235–43

## Conclusions

To the best of our knowledge, for the first time, we showed the characteristics of TIGIT-expressing T lymphocytes from a systemic (peripheral blood) perspective regarding RA. Our data established a relationship between the frequency of TIGIT-expressing T lymphocytes and RA disease activity, which contribute to understanding the role of T lymphocytes in RA.

## Acknowledgments

We would like to acknowledge help from Dr. Rui Wu in the Department of Rheumatology, the First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China.

## Conflicts of interest

None.

29. Chen SY, Hsu WT, Chen YL et al: Lymphocyte-activation gene 3(+) (LAG3(+)) forkhead box protein 3(-) (FOXP3(-)) regulatory T cells induced by B cells alleviates joint inflammation in collagen-induced arthritis. *J Autoimmun*, 2016; 68: 75–85
30. Carruthers DM, Arrol HP, Bacon PA, Young SP: Dysregulated intracellular Ca<sup>2+</sup> stores and Ca<sup>2+</sup> signaling in synovial fluid T lymphocytes from patients with chronic inflammatory arthritis. *Arthritis Rheum*, 2000;43: 1257–65
31. Malemud CJ: Dysfunctional immune-mediated inflammation in rheumatoid arthritis dictates that development of anti-rheumatic disease drugs target multiple intracellular signaling pathways. *Antiinflamm Antiallergy Agents Med Chem*, 2011; 10: 78–84
32. Boles KS, Vermi W, Facchetti F et al: A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC. *Eur J Immunol*, 2009; 39: 695–703
33. Godefroy E, Zhong H, Pham P et al: TIGIT-positive circulating follicular helper T cells display robust B-cell help functions: Potential role in sickle cell alloimmunization. *Haematologica*, 2015; 100: 1415–25