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

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

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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 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+ T lymphocytes, B lymphocytes, monocytes, neutrophils, CD3+CD4+ T lymphocytes, and CD3-CD19+ B lymphocytes, CD3-CD16+CD56+ NK cells – were evaluated.

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 ±SD)    | 53.7±11.4               | 48.6±11                 |
| DAS28 (average ±SD)         | 4.03±1.9                | –                       |
| RF (IU/mL, average ±SD)     | 425.9±816.1             | –                       |
| ACPA (RU/mL, average ±SD)   | 519.6±855.3             | –                       |
| CRP (mg/L, average ±SD)     | 17.4±25.7               | –                       |
| ESR (mm/h, average ±SD)     | 40.4±33.2               | –                       |
and CD3+CD8+ 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⁺CD8⁺ T lymphocytes was significantly elevated compared to CD3⁺CD4⁺ T lymphocytes in HCs (P<0.0001). (B) The MFI of TIGIT on CD3⁺CD8⁺ T lymphocytes was significantly elevated compared to CD3⁺CD4⁺ T lymphocytes in HCs (P=0.003). (C) The frequency of TIGIT-expressing CD3⁺CD8⁺ T lymphocytes was significantly elevated compared with that of CD3⁺CD4⁺ T lymphocytes in RA (P<0.0001). (D) The MFI of TIGIT on CD3⁺CD8⁺ T lymphocytes was significantly elevated compared with that of CD3⁺CD4⁺ T lymphocytes in RA (P<0.0001). (E) The frequency of TIGIT-expressing CD3⁺CD4⁺ T lymphocytes was significantly elevated in patients with RA as compared with HCs (P=0.0006). (F) The MFI of TIGIT on CD3⁺CD4⁺ T lymphocytes was significantly increased in patients with RA compared with HCs (P=0.0056). (G) The frequency of TIGIT-expressing CD3⁺CD8⁺ T lymphocytes was significantly increased in patients with RA compared with HCs (P=0.0007). (H) The MFI of TIGIT on CD3⁺CD8⁺ T lymphocytes was significantly elevated in patients with RA as compared with HCs (P=0.0032).
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^+CD4^+ T lymphocytes and the MFI of TIGIT on CD3^+CD4^+ T lymphocytes were significantly elevated, as compared with that of CD3^+CD4^+ 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^+CD8^+ T lymphocytes and the MFI of TIGIT on CD3^+CD8^+ 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^+CD8^+ T lymphocytes and the MFI of TIGIT on CD3^+CD8^+ 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^+CD4^+ T and CD3^+CD8^+ T lymphocytes. Data showed that 53 patients were positive for RF and 57 patients positive for ACPA.

**Figure 3.** Correlation of frequency of TIGIT-expressing CD3^+CD4^+ T lymphocytes with autoantibody. (A) The frequency of TIGIT-expressing CD3^+CD4^+ 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^+CD8^+ T lymphocytes was positively associated with RF level in patients with RA (r^2=1.1287, P=0.046). (C) The frequency of TIGIT-expressing CD3^+CD4^+ T lymphocytes was positively associated with increased ACPA in patients with RA (r^2=0.09665, P=0.0186).
were positive for ACPA, for all patients who underwent autoantibody detection. As shown in Figure 3A and 3B, the frequencies of TIGIT-expressing CD3+CD4+ T lymphocytes and CD3+CD8+ T lymphocytes were positively correlated with RF levels. No significant correlation was found between the frequencies of TIGIT-expressing CD3+CD4+ T lymphocytes, TIGIT-expressing CD3+CD8+ T lymphocytes, and serous ACPA (data not shown). However, the frequency of TIGIT-expressing CD3+CD4+ 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+CD4+ T lymphocytes and CD3+CD8+ T lymphocytes in patients with RA. As shown in Figure 4A and 4B, positive correlations between the frequency of TIGIT-expressing CD3+CD4+ T lymphocytes and ESR, and CRP level in patients with RA ($r^2=0.1216; P=0.002$) and ($r^2=0.09485; P=0.0376$) respectively (Figure 4). However, no obvious relationship was found between the frequency of TIGIT-expressing CD3+CD8+ 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.
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\(^+\)CD4\(^+\) T lymphocytes and the TIGIT MFI on CD3\(^+\)CD4\(^+\) T lymphocytes in patients with active RA were significantly increased, as compared with those in remission (P=0.0294). (B) MFI of TIGIT on CD3\(^+\)CD4\(^+\) 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\(^+\)CD4\(^+\) 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\(^+\)CD4\(^+\) T lymphocytes was significantly correlated with DAS28 in patients with RA (r\(^2\)=0.1463; P=0.0007).

**Figure 5.** Correlation of TIGIT expression on CD3\(^+\)CD4\(^+\) T lymphocyte with disease activity. (A) The frequency of TIGIT-expressing CD3\(^+\)CD4\(^+\) 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\(^+\)CD4\(^+\) 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\(^+\)CD4\(^+\) 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\(^+\)CD4\(^+\) 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\(^+\)CD4\(^+\) T lymphocytes and the TIGIT MFI on CD3\(^+\)CD4\(^+\) T lymphocytes in patients with active RA were significantly increased, as compared with those who were in remission (P<0.05) (Figure 5A and B). Furthermore, both the frequency of TIGIT-expressing CD3\(^+\)CD4\(^+\) T lymphocytes and MFI of TIGIT on CD3\(^+\)CD4\(^+\) T lymphocytes were positively correlated with DAS28 score (Figure 5C, SD), demonstrating that TIGIT expression on CD3\(^+\)CD4\(^+\) T lymphocytes is associated with disease activity in RA. The correlation between TIGIT expression on CD3\(^+\)CD8\(^+\) 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...
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+CD4+ 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, serous 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+CD4+ T and CD3+CD8+ 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].

Lymphocytes are reported to play important roles in disease development and progression [9,10]. Our study shows that the frequency of TIGIT-expressing CD3+CD8+ T lymphocytes is significantly elevated compared with that of CD3+CD4+ T lymphocytes. We also found that the TIGIT expression on CD3+CD4+ T and CD3+CD8+ 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, serous 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+CD4+ T and CD3+CD8+ T lymphocytes was significantly elevated in patients with RA as compared with HCs. Furthermore, we had an interesting finding that the frequency of TIGIT-expressing CD3+CD4+ 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+ 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.

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.

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Conflicts of interest

None.

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