Cytokine 113 (2019) 305–310

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Elevated levels of IL-37 correlate with T cell activation status in rheumatoid arthritis patients

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ARTICLE INFO

Keywords:
IL-37
Rheumatoid arthritis
CD26
T lymphocytes

ABSTRACT

Objective: To assess the association between serum levels of IL-37 in rheumatoid arthritis patients and percentage of peripheral blood T lymphocytes expressing the activation marker CD26 and investigate their correlation with disease activity.

Methods: The study included 48 rheumatoid arthritis patients and 42 age and sex matched healthy controls. Serum levels of IL-37 were determined using enzyme linked immunosorbent assay while percentage of $CD3^+CD26^+$T cells in peripheral blood mononuclear cells was assayed using flowcytometry.

Results: Serum levels of IL-37, as well as the percentage of $CD3^+CD26^+$T cells, were significantly higher in rheumatoid arthritis patients than in healthy controls. Also, serum IL-37 levels were higher in patients with severe disease activity than patients with moderate and low disease activity. In rheumatoid arthritis patients, both serum levels of IL-37 and percentage of $CD3^+CD26^+$T cells correlated with disease activity (DAS28), C-reactive protein levels and erythrocyte sedimentation rate. In addition, serum levels of the anti-inflammatory cytokine IL-37 positively correlated with the percentage of $CD3^+CD26^+$T cells in peripheral blood of rheumatoid arthritis patients.

Conclusion: Our results indicate a strong correlation between serum levels of IL-37 and frequency of activated T cells in peripheral blood of rheumatoid arthritis patients. Our results suggest that in an active disease status, activated T lymphocytes may be a contributing source to the elevated levels of IL-37 trying to down-regulate the active inflammatory process.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune joint disease that is associated with considerable morbidity and diminished quality of life [1]. The disease is characterized by chronic inflammation of multiple joints, infiltration of the synovium by T lymphocytes and synovial hyperplasia eventually leading to progressive joint destruction and physical disability [2]. It is well established that pro-inflammatory cytokines play a detrimental role in the pathogenesis of RA [3]. Several cytokines, mainly tumor necrosis factor- α (TNF-α), interleukin (IL)-6, IL-1, VEGF and IL-17, significantly contribute to the disease through activating different genes associated with inflammatory mediators, including additional cytokines and matrix metalloproteinases that are involved in tissue degradation, proliferation of synovial tissue and joint erosion [4].

Interleukin-37 (IL-37), a new member of the IL-1 family, exerts anti-inflammatory effects by suppressing innate immune responses through attenuating the production of pro-inflammatory cytokines such as TNF-α, IL-1α, IL-1β and IL-6 [5]. Accumulating data suggest that IL-37 might play an important role in controlling inflammation in several inflammatory diseases, including rheumatoid arthritis [6], systemic lupus erythematosus [7], osteoarthritis [8], obesity [9], inflammatory bowel disease [10] and bronchial asthma [11]. Both expression and production of IL-37 increase following stimulation with pro-inflammatory cytokines, such as IL-1, IL-18, TNF-α and IFN-γ, as well as various toll-like receptor ligands [12]. Hence, during inflammation, IL-37 tries to shift the cytokine balance away from excessive inflammation acting as a key modulator aiming to restore the inflammatory balance.

Previous studies suggested that IL-37 might participate in the development of RA. It has been reported that IL-37 levels were higher in

Abbreviations: RA, Rheumatoid arthritis; IL-37, Interleukin-37; TNF-α, Tumor necrosis factor- α; CRP, C-reactive protein; RF, Rheumatoid factor; DAS28, Disease activity score 28; PBMCs, Peripheral blood mononuclear cells; PBS, Phosphate buffered saline

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https://doi.org/10.1016/j.cyto.2018.07.027
Received 27 February 2018; Received in revised form 6 July 2018; Accepted 24 July 2018
Available online 01 August 2018

RA patients compared to levels in healthy controls and that IL-37 levels significantly correlated with the levels of pro-inflammatory cytokines, C-reactive protein (CRP), rheumatoid factor (RF) and the disease activity score 28 (DAS28) [6,13]. Also, levels of IL-37 decreased in patients responding to treatment with disease modifying anti-rheumatic drugs (DMARD) [14].

Rheumatoid arthritis is a disease that is mediated primarily by T lymphocytes mainly type 1[15] and type 17 helper T cells [16]. Recent evidence suggests that T cells are a source of IL-37 in RA patients. Ye et al. [17] have reported that expression of IL-37 mRNA was significantly higher in $CD3^+$ T cells from RA patients compared with healthy controls.

CD26 is one of the activation markers of T cells. It participates to T-cell signaling as a co-stimulatory molecule and its enzymatic activity plays an important role in enhancing cellular responses [18]. In this study, we investigated serum levels of IL-37 in patients with RA and we also evaluated the correlation of serum IL-37 with the percentage of T cells expressing CD26 as an indicator of T cell activation.

2. Materials and Methods

2.1. Patients and controls

Forty eight newly diagnosed RA patients were recruited from the Rheumatology Clinic, Demerdash Hospital, Ain Shams University. Patients were diagnosed, according to the 2010 RA classification criteria established by the American College of Rheumatology /European League Against Rheumatism [19]. Disease severity was evaluated using the DAS28 [20]. Patients were classified into 3 groups according to their DAS28: low activity (DAS28 < 3.2), moderate activity (DAS28 3.2–5.1) and severe activity (DAS28 > 5.1). None of the patients received any treatment for RA before. Patients with other rheumatic diseases, osteoarthritis, obesity, inflammatory bowel disease and bronchial asthma were excluded from the study. Forty two age and sex matched healthy individuals were studied as a control group. Demographical and clinical characteristics of patients and healthy controls are presented in Table 1.

This study was approved by the Ethical Committee of Faculty of Medicine, Ain Shams University. Written informed consents were obtained from RA patients and healthy controls participating in the study.

Table 1
Demographical and clinical characteristics of RA patients and healthy controls.

	RA patients (n = 48)	Healthy controls (n = 42)
Age, median (IQR)	49 (36–61)	48 (35–63)
Gender		
Male, *n*	8	7
Female, *n*	40	35
ESR (mm/h), median (IQR)*	49.5 (35–64.5)	7.5 (5–15)
CRP (mg/L), median (IQR)*	21.1 (12.3–38.1)	2.9 (2.1–4.0)
RF (IU/ml), median (IQR)*	47.4 (29.2–70.8)	6.3 (2.1–9.4)
DAS28, median (IQR)	4.5 (3.1–6.2)	NA
IL-37 (pg/ml), median (IQR)*	408 (243.8–545)	105.5 (59.75–140.5)
$CD3^+CD26^+$T cells (%), median (IQR)*	65.84 (54.67–74.25)	49.89 (38.9–58.35)

ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, RF: rheumatoid factor, DAS28: disease activity score 28, NA: not applicable.
* $p < 0.0001$ RA patients versus healthy controls.

2.2. Sample collection

Three venous blood samples were obtained from each participant. The first sample (3 ml) was collected into a gel vacutainer tube. Serum was separated by centrifugation at 4000 rpm for 15 min and then stored at -80 °C until used for measurement of CRP, RF and IL-37 levels. The second sample (2 ml) was collected on EDTA-K2 vacutainer tube and used for separation of peripheral blood mononuclear cells (PBMCs) to be used in flowcytometry. The third sample (2 ml) was collected on 3.2% sodium citrate vacutainer tube and used to assess erythrocyte sedimentation rate (ESR).

2.3. Laboratory tests

Serum CRP and RF levels were determined on Cobas c6000 (Roche diagnostics, Basel, Switzerland). ESR was determined by the westergren method.

2.4. Measurement of IL-37

Serum IL-37 levels were measured using enzyme-linked immunosorbent assay kit supplied by Elabscience, USA.

2.5. Isolation of PBMCs and flowcytometry

PBMCs were isolated from blood samples using Ficoll-Hypaque (Lonza, Walkersville, USA) density gradient centrifugation. Blood was first diluted 1:1 with phosphate buffered saline (PBS) (Lonza, Walkersville, USA) and then 4 ml of diluted blood were layered onto 2 ml of Ficoll-Hypaque. Samples were then centrifuged at 2400 rpm for 20 min followed by PBMCs collection and washing twice with PBS.

Freshly isolated PBMCs were resuspended (10^6 cells per ml) in PBS containing 1% BSA and 0.05% sodium azide (FACS buffer) and stained using monoclonal CD3-FITC and CD26-PE (BD Pharminogen, BD Biosciences, CA, USA). Appropriate PE isotype-matched monoclonal antibody was used to establish gating parameters. Data was collected using a Navios flowcytometer (Beckman Coulter, IN, USA) and analysis was performed using FlowJo software (TreeStar, La Jolla, USA).

2.6. Statistical analysis

Statistical analysis was performed using Graph Pad Prism 5.0 software (San Diego CA, USA). All Data are presented as median and interquartile range. Different groups were compared using Mann–Whitney *U* test or Kruskal-Wallis test. Spearman correlation was used to assess correlations between parameters. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Serum IL-37 is elevated in RA patients

Serum levels of IL-37 were significantly higher in RA patients than in healthy controls ($p < 0.0001$) (Fig. 1A). To examine whether IL-37 was related to disease activity, we divided the patients into three groups according to their DAS28 and compared the levels of IL-37 between the three groups. Statistical analysis revealed a significant difference between the three groups ($p < 0.0001$). Serum levels of IL-37 were significantly higher in RA patients with severe disease activity (n = 19 patients) than those with moderate disease activity (n = 16 patients) ($p < 0.05$) and low disease activity (n = 13 patients) ($p < 0.001$).

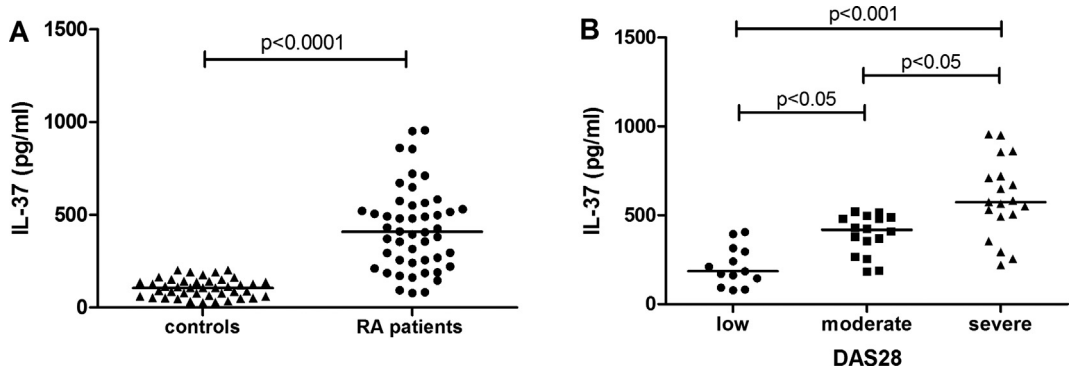

Fig. 1. Serum levels of IL-37. (A) Levels of IL-37 in healthy controls and RA patients. (B) Levels of IL-37 in RA patients with low, moderate and severe DAS28.

Also, serum levels of IL-37 were significantly higher in RA patients with moderate disease activity than those with low disease activity ($p < 0.05$) (Fig. 1B).

3.2. Elevated levels of IL-37 correlate with disease activity, CRP and ESR

To assess whether an increase in the arthritis is associated with a subsequent increase in IL-37 levels, we examined the correlation between IL-37 and indicators of disease activity and inflammation in RA patients. Serum IL-37 positively correlated with each of DAS28 ($r_s = 0.7463$, $p < 0.0001$) (Fig. 2A), CRP ($r_s = 0.7397$, $p < 0.0001$) (Fig. 2B) and ESR ($r_s = 0.4742$, $p = 0.0007$) (Fig. 2C).

3.3. High frequency of CD26 expressing T lymphocytes in the circulation of RA patients

To evaluate the percentage of circulating activated T lymphocytes in RA patients, we performed flowcytometric analysis on freshly isolated PBMCs. Cells were gated on CD3 as a marker of T cells, then the percentage of cells expressing surface CD26 was measured within the T cells as shown in the representative FACS plots (Fig. 3A–C). RA patients showed a significantly higher percentage of $CD3^+CD26^+$T cells in the circulation compared to healthy controls ($p < 0.0001$) (Fig. 4A).

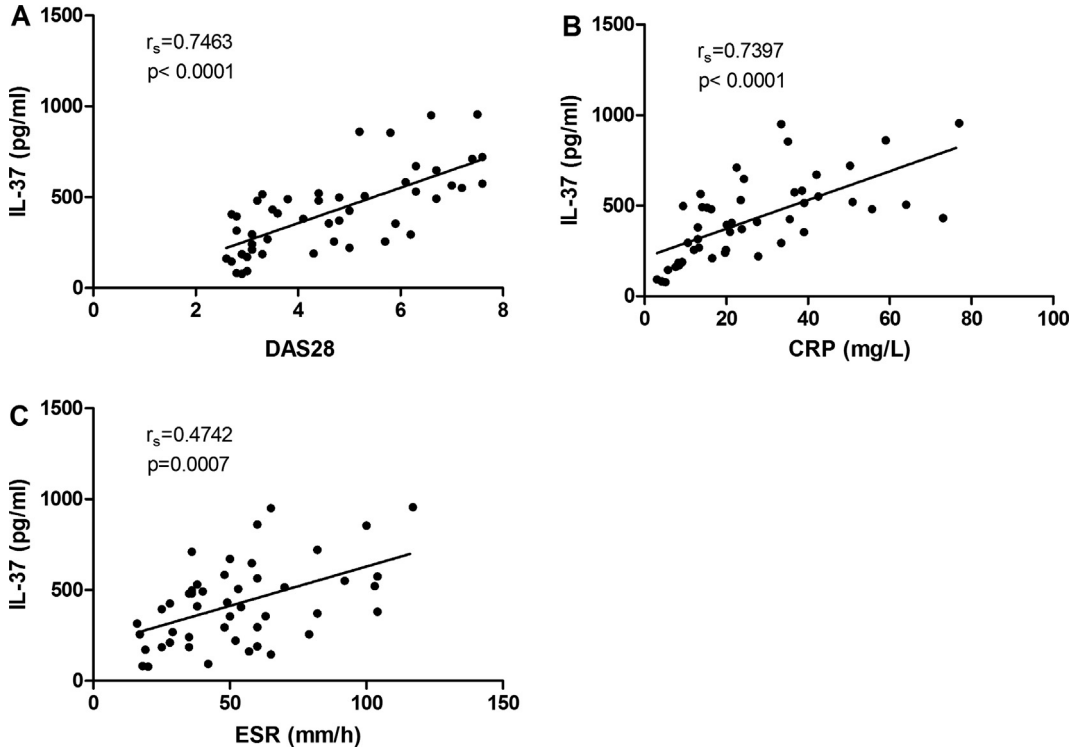

Fig. 2. Levels of serum IL-37 in RA patients positively correlated with (A) DAS28, (B) CRP levels and (C) ESR levels.

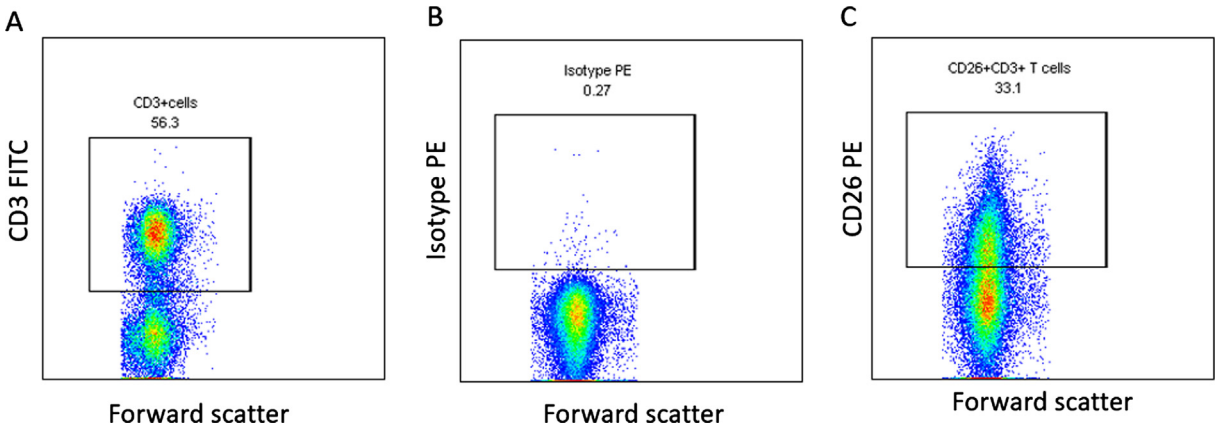

Fig. 3. Representative flowcytometry plots for gating of $CD3^+CD26^+$Tcells. (A) Representative dot-plot for gating of $CD3^+$ cells, (B) Setting the gating pararmeters using isotype PE in the $CD3^+$ cells gated in A, (C) Dot plot showing the expression of CD26 in the $CD3^+$ cells gated in A.

3.4. Positive correlations between T cell activation status and disease activity

CD26 is an activation marker of T cells. Knowing that, we wanted to examine if there is a relation between the percentage of activated T cells in peripheral blood of RA patients and disease activity as reflected by indicators of disease activity and inflammation; DAS28, CRP and ESR. In RA patients, percentage of $CD3^+CD26^+$T cells positively correlated with each of DAS28 ($r_s = 0.4520$, $p = 0.0013$) (Fig. 4B), CRP ($r_s = 0.4435$, $p = 0.0016$) (Fig. 4C) and ESR ($r_s = 0.4049$, $p = 0.0043$) (Fig. 4D).

3.5. IL-37 levels correlate with the percentage of activated T cells

The relationship between IL-37 and T cell activation status in RA patients has not been previously investigated. To address this point, we examined the correlation between the percentage of peripheral blood $CD3^+CD26^+$T cells in RA patients and serum levels of IL-37. Serum levels of IL-37 positively correlated with the percentage of $CD3^+CD26^+$T cells ($r_s = 0.6499$, $p < 0.0001$) (Fig. 5).

4. Discussion

IL-37 has been the focus of a considerable number of studies focusing on autoimmune and inflammatory diseases through the past decade [5]. It is one of the members of the IL-1 cytokine family that has an anti-inflammatory function [21]. IL-37 has been shown to be affect cell proliferation, differentiation and to suppress both acquired and innate immunity [22]. The fact that IL-37 suppresses the production of pro-inflammatory cytokines has directed the attention to its potential role in RA, a disease where pro-inflammatory cytokines play a profound role [23,24]. Pro-inflammatory cytokines are in turn a key inducer of

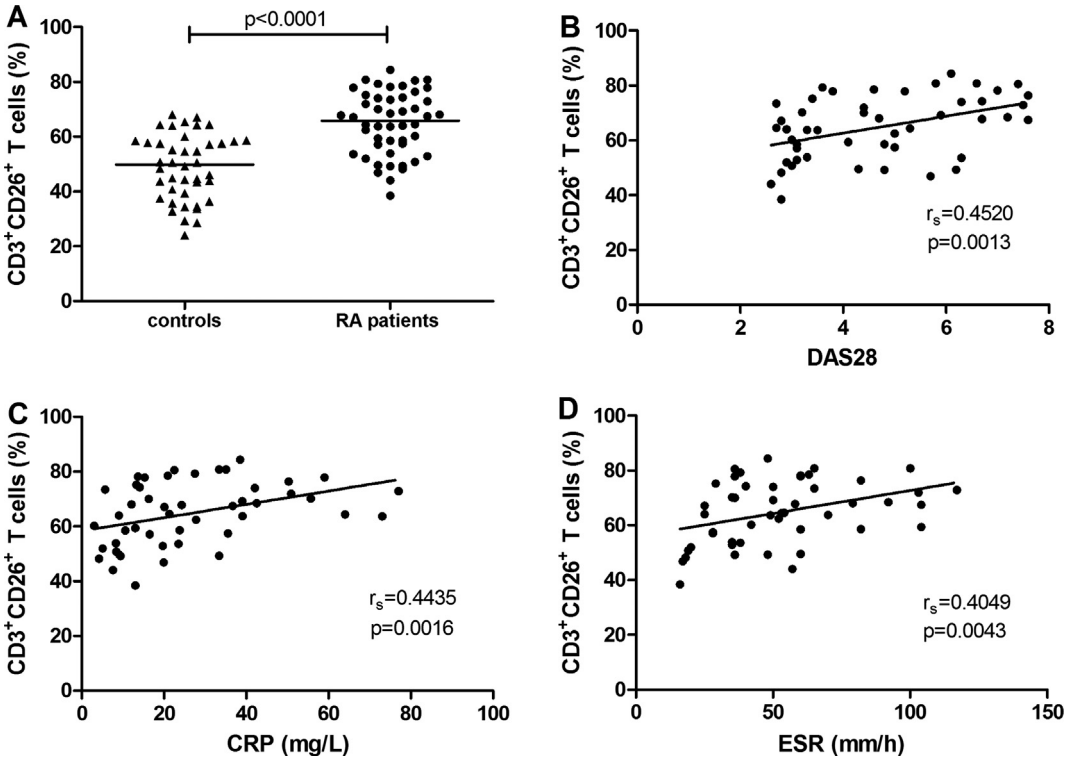

Fig. 4. (A) Higher percentage of $CD3^+CD26^+$T cells in RA patients than healthy controls. Percentage of $CD3^+CD26^+$T cells in RA patients positively correlated with (B) DAS28, (C) CRP levels and (D) ESR levels.

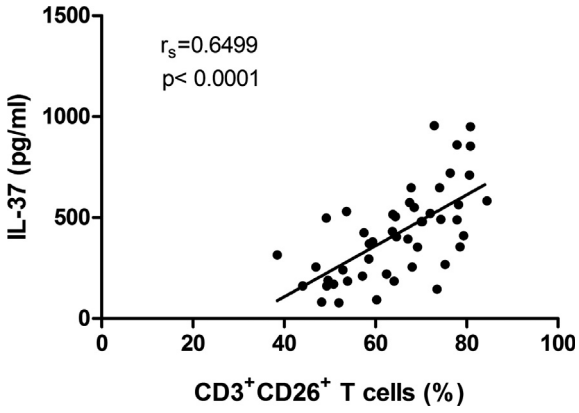

Fig. 5. Levels of IL-37 in RA patients positively correlated with percentage of $CD3^+CD26^+$T cells in peripheral blood.

IL-37 expression elucidating the crucial role of IL-37 as an important negative feed-back inhibitor of the inflammatory process triggering its production [12].

In this study, levels of serum IL-37 were significantly higher in RA patients than healthy controls. In addition, serum IL-37 were significantly higher in patients with severe activity of the disease than patients with moderate and low activity. Furthermore, serum IL-37 levels correlated with DAS28, a measure of disease activity, and with markers of inflammation; CRP and ESR. Similarly, Xia et al. [13] reported higher levels of IL-37 in Chinese RA patients and demonstrated that IL-37 levels correlated to both DAS28 and levels of pro-inflammatory cytokines. Our findings are also supported by the observations reported by Zhao et al. [14] who found that IL-37 is increased in RA patients and reported that levels of IL-37 significantly decreased in RA patients receiving DMARD compared to IL-37 levels before treatment. These studies, together with our study, suggest that IL-37 is associated with the pathogenesis of RA.

It is well established that T lymphocytes play a pivotal role in the pathogenesis of RA and it has been recently demonstrated the $CD3^+$ lymphocytes from RA patients express high levels of IL-37 mRNA and that lipopolysaccharide stimulation induced IL-37 expression in $CD4^+$ T cells under Th1-polarizing conditions [17]. Together these data suggest that T lymphocytes are a source of IL-37 in RA patients.

In this study, we wanted to investigate whether elevated levels of IL-37 are associated with T cell activation in RA. We assessed the percentage of T cells expressing CD26, a known activation marker of T cells [18], in peripheral blood of both RA patients and healthy controls and assessed its correlation to IL-37 levels, disease activity and markers of inflammation in RA patients. We found a significantly higher percentage of $CD3^+CD26^+$T cells in RA patients compared to healthy controls. Also, the percentage of $CD3^+CD26^+$T cells correlated positively to CRP, ESR and DAS28 in RA patients. This is consistent with the notion that an increased activity of the T cells is associated with a more profound inflammatory process and hence higher disease activity.

CD26 is a membrane-associated glycoprotein molecule that participates in T cell activation enhancing T cell proliferation and cytokine production [25]. Interestingly, we found that IL-37 levels positively correlated to the percentage of $CD3^+CD26^+$T cells in RA patients. Thus, it is reasonable to speculate that an active disease status with an active inflammatory process upregulates CD26 resulting in active T lymphocytes that in turn might be a contributing source to the elevated levels of IL-37 trying to down-regulate the active inflammatory process.

Muscat et al. [26] also reported higher percentage of $CD3^+$ T cells expressing CD26 in RA patients and explained that this is mostly a reflection, in the peripheral blood, of the inflammatory processes which occur at the joint level. Similarly, Cordero et al. [27] also reported higher percentage of $CD4^+CD26^+$ cells in RA patients that strongly correlated with DAS 28 scores. Also, Ellingsen et al. [28] reported elevated CD26 membrane density on $CD4^+$ T lymphocytes in active chronic RA patients.

To our knowledge, this is the first study that investigates the association of IL-37 and T cell activation status in RA patients. Also, this is the first study to assess levels of IL-37 in Egyptian RA patients. Future studies are needed to investigate combined flowcytometric staining of surface CD26 and intracellular IL-37 in different T lymphocyte subsets in RA patients.

In conclusion, we report higher serum levels of IL-37 and percentage of $CD3^+CD26^+$ T cells in RA patients that correlate with DAS28, CRP and ESR. In addition, serum levels of IL-37 positively correlated with the percentage of $CD3^+CD26^+$ T cells. On the basis of our findings, it could be hypothesized that in-vivo activation of T cells in RA, which strongly express CD26, could result in production of IL-37 by these activated T cells. Our findings may provide new insights into unravelling the immunopathogenesis of RA.

Declarations of interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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