

The T_H17/Treg Imbalance in Rheumatoid Arthritis and Relation to Disease Activity

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Abstract

Objectives: T regulatory cells (Treg) and proinflammatory T_H17 cells are newly identified T lymphocyte subsets, which have significant effects on autoimmunity and inflammation. Tregs play key role in the maintenance of self-tolerance. T_H17/Treg disturbed balance has been reported to contribute to several autoimmune diseases.

Our aim was to assess the Treg/T_H17 pattern and T_H17 related cytokines, in peripheral blood of Egyptian rheumatoid arthritis patients, and their relation to disease activity score (DAS). Analyzing IL17A and IL23 as indicators of T_H17 function, was to study the effect of T_H17 in RA.

Methods: 100 Egyptian rheumatoid arthritis patients, divided into Group I (14), Group II (48), Group III (38), with low <3.2, moderate 3.2 to <5.1 and high >5.1, activity DAS score respectively; and 50 healthy age and sex matched controls were enrolled. Peripheral blood T_H17 and Treg (CD4⁺CD25⁺Foxp3⁺) frequencies were analyzed by flowcytometry, and the serum levels of interleukins (IL17A), (IL23) were determined by ELISA.

Results: Active RA patients (groups II and III) revealed an obvious increase in peripheral T_H17 frequencies, and levels of T_H17-related cytokines, and a significant decrease in Treg (CD4⁺CD25⁺Foxp3⁺) frequencies in group III, when compared to healthy controls. T_H17/Treg ratios were positively correlated with serum concentrations of IL17A and IL23 cytokines. Frequencies and levels showed statistical significant correlation with DAS scoring.

Conclusions: Our study indicated that T_H17/Treg balance was disturbed in peripheral blood of RA patients, leading to an increase of proinflammatory cytokines, correlating with DAS, and suggesting an important role in the development of RA.

Keywords Tregs; IL-17; IL-23; Rheumatoid arthritis; T_H17

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that may lead to the destruction of joint architecture without effective therapy. Over the past few decades, extensive research has provided data to support the hypotheses that RA is an antigen-driven autoimmune disease [1]. T cells clearly play a central role in the initiation and perpetuation of chronic inflammation prevalent in RA. Besides Th1/Th2 cell populations, two other subpopulations were subsequently identified: regulatory T cells (Treg), producing IL-10 and transforming growth factor (TGF)- β [2], and IL-17-producing CD4⁺ T cells (T_H17) [3]. These two subsets play a key role during tolerance and inflammatory responses [4,5]. The role of T_H17 cells, during development of autoimmune diseases, has largely been described over the past few years [5]. These cell subsets can act at different stages of RA disease, at different sites, and with different intensities, to participate in the complex network of cell-cell interactions that govern the development and chronicity of rheumatoid synovitis.

T helper-type 17 (T_H17) cells can secrete interleukin (IL17) in humans [6]. IL17 is a pleiotropic cytokine that induces the expression of proinflammatory cytokines and matrix metalloproteases [7], hence participates in tissue inflammation and destruction. It has been demonstrated in many experimental arthritis models, the pathogenic effect of IL17 [8]. On the other hand, regulatory T (Treg) cells are T cell subset, whose peculiar function is to suppress autoreactive lymphocytes. They mediate immune tolerance, and attempt to maintain lymphocyte homeostasis. The two T cell subpopulations different behavior as well as their reciprocal plasticity pointed out the importance of T_H17/Treg cell disturbed balance in the pathogenesis of RA. As a result, a novel hypothesis has been proposed regarding the pathogenic mechanism of RA, that the T_H17/Treg imbalance, along with T_H1/T_H2 imbalance, may be responsible for the development and progression of RA [9-11]. In addition, the effects of currently employed RA therapeutic strategies on these lymphocyte subpopulations, have been investigated. A particular interest on the possible therapeutic targeting of these cells and their products was developed to overcome the limitation of currently employed biological therapies [12]. Blockade of critical cytokines may result in a shift in this polarization

from T_H17/T_H1 phenotypes toward Treg/T_H2 [13]. Yet, there is controversy in results [14]. Meanwhile, variations in patient response to available anti-RA therapeutics, corroborate the fact that RA is a heterogeneous and complex disease due to various immune pathways involved.

The frequencies of T_H17 cells were found to increase in peripheral blood mononuclear cells (PBMCs) of RA patients compared to healthy controls [15,16]. An enhanced expression of IL-17 has been observed in the rheumatoid synovium, and synovial fluids of patients with early RA [17].

Few studies have been conducted to evaluate the role of Treg cells in blood of RA patients. Conflicting results have been reported concerning Treg cell proportion in RA peripheral blood. Most studies emphasize the presence of increased numbers of Treg cells in RA synovium [18,19]. Treg cells isolated from the synovial fluid of RA patients appear to be functionally inactive in regards to their ability to suppress pro-inflammatory cytokine production [20].

IL-23 promotes the expansion and survival of T_H17 cells. IL 23 promotes T_H17 cells producing tumour necrosis factor- α (TNF- α), IL-17, IL-6, IL-22, and GM-CSF which are associated with the induction of autoimmune inflammation in RA disease [21].

Aim

To analyze the Serum cytokine Levels (IL17 and IL23), frequency and cell surface phenotype of Treg and T_H17 cells, in a sample of Egyptian patients with rheumatoid arthritis (RA), compared to healthy control subjects, and to correlate findings with DAS.

Materials and Methods

This study has been approved by the Research Ethical Committee of Cairo University, Faculty of Medicine (Ethical protocol number: N-68-2012).

Patients and healthy controls

One hundred RA patients with variable disease activity, fulfilling the revised classification criteria of the American College of Rheumatology [22], were enrolled in this study. They were all chronic rheumatoid arthritis patients, attending the rheumatology outpatient clinic, Cairo University hospital. All had no extra-articular manifestations. They were treated with disease modifying anti-rheumatic drugs [DMARDs] or methotrexate. Patients taking corticosteroids were excluded. Disease activity was estimated according to clinical indices, as well as laboratory markers of inflammation: the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), rheumatoid factor and calculation of the disease activity score (DAS28). Patients were classified as having active or inactive disease on the basis of the DAS28: 14 patients had low active disease with DAS28 (<3.2), while 48 had moderately active disease (3.2 to <5.1) and 38 patients had high activity disease with DAS28 (>5.1). The main patients' characteristics were summarized in Table 1. Laboratory investigations included rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), complete blood count and liver and kidney functions. According to these clinical and laboratory data, indices of disease activity were calculated such as Ritchie Articular Index and Disease Activity Score. Samples were taken during day time, before administration of any drug, or co-medication.

Fifty healthy controls free of pain, acute and chronic inflammatory diseases and matched with patients for age and sex, were included in the study. Written consent was obtained from all the subjects after full explanation of the procedure.

Treg (CD4⁺CD25⁺Foxp3⁺) cells enumeration using flow-cytometry

Small aliquots of whole peripheral blood were labeled with appropriate combinations of monoclonal antibodies (mAb): 10 μ l of Mouse anti-human CD4 fluorescein conjugated FITC [Becton Dickinson, San Jose, CA, USA, BD] and Mouse anti-human CD25 (IL-2 R α) phycoerythrin PE labelled [BD] mAb were incubated for 45 min, followed by red cell lysis. Following surface staining, cells were fixed and permeabilized using BD Fixation/Permeabilization Kit. Cells were resuspended in 250 μ l of cytofix and incubated for 20 minutes at 4°C then washed. 10 μ l of polyclonal anti-human/mouse/rat Foxp3- Allophycocyanin, APC conjugated [e Bioscience, USA], were incubated with the cells overnight in the dark. Gating was done on CD4⁺ cells first then detection of CD25⁺Foxp3⁺ within the population followed. Unstained cells, as isotypic control, were used to exclude autofluorescence. Analysis was performed using a flow cytometer (Beckmann Coulter, FL, USA).

Quantitation of circulating TH17 cells [23]

T_H17 cells were quantified by flow cytometry, following staining with FITC conjugated anti-CD4 (BD), and PE conjugated anti-human IL-17A (BD), according to the manufacturers protocol and through the following steps:

Stimulation of cytokine secreting cells: 1000 μ l of the sterile heparinized whole blood were stimulated for 4 hours with the polyclonal activator phorbol myristate acetate (PMA) (50 ng/ml; Sigma –Aldrich) and ionomycin (1 μ g/ml ; Sigma –Aldrich) and 10 μ g/ml brefeldin A (Sigma-Aldrich) to inhibit intracellular protein transport at 37°C in a humidified 5% CO₂ incubator. Activated cultures of blood samples were washed in wash buffer (PBS, 5% fetal bovine serum, 0.1% sodium azide, Merck).

Staining of cell surface CD4: Blood sample were stained using 10 μ l FITC-conjugated anti-CD4 (mouse IgG 1 isotype Sigma- Aldrich, Inc) for 30 min at room temperature. Cells were washed using 1 ml staining Buffer/wash and pellet by centrifugation (250 \times g). 2 ml of FACS lysing solution (Becton Dickinson, Lincoln Park NJ, USA) were added to blood samples to lyse red cells.

Fixation and permeabilization of cells using BD Fixation/ Permeabilization Kit: The cells were thoroughly resuspended in 250 μ l of BD cytofix/Cytoperm solution per tubes, and incubated for 25 min at 4°C. Cells were washed twice in 1 ml of 1x BD Perm/Wash buffer, which can be used as the wash buffer and as the antibody diluents, and then subjected to intracellular cytokine staining.

Intracellular IL-17 staining and analysis: Cells were incubated with PE –conjugated anti-human IL-17A (mouse IgG1 isotype BD) for 30 min at room temperature in the dark. FITC Mouse IgG1 and PE Mouse IgG1 (BD) isotype controls were used as negative controls. After staining, cells were washed and immediately analyzed using flow cytometry. Lymphocytes were gated on the basis of forward and side scatter properties and at least 10000 CD4⁺ cells were analyzed. Cell analysis was performed using CYTOMICS FC 500 Flow Cytometer (Beckman coulter, FL, USA) and CXP Software version 2.2.

Determination of serum IL-17 and IL-23 levels by ELISA

Serum IL17A levels were determined using ELISA Kit (e Biosciences, USA) and IL23 levels were determined using Quantikine human IL23 ELSA kit (R&D Systems, catalog number D2300B according to the manufacturers recommendations). Results were expressed as pg/ml.

Statistical analysis

All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 20 for Microsoft Windows. Data were statistically described in terms of mean ± standard deviation (± SD), frequencies when appropriate. Spearman's test was used for correlation analysis. Student t test was used for comparison of numerical variables between the study groups. P values less than 0.05 were considered statistically significant at the level of 95%.

Results

The current study included one hundred RA patients (81 females and 19 males). Their ages ranged from 19 to 70 years with a mean of

42.23 ± 12.7 years. Age of disease onset ranged from 19 to 62 years and disease duration ranged from 1 to 28 years. Fifty age and sex matched healthy individuals were included as a control group, their ages ranged from 20 to 60 years with a mean of 37.6 ± 9.8 years. The descriptive data of the patients are shown in Tables 1, 2 and 3.

Groups	Number	Male/Female	Age (years)	
			Range	Mean ± SD
I (Low)	14 (14%)	3/11	19 – 59	36.4 ± 12.3
II (Mod)	48 (48%)	8/40	20 – 66	41.8 ± 14.9
III (High)	38 (38%)	8/30	34 – 70	48.5 ± 11.5

Patients were classified as having active or inactive disease on the basis of the DAS28;
14 patients had low active disease with a DAS28 <3.2, while 48 had moderately active disease (3.2 < DAS28 <5.1) and 38 patients had high activity disease with a DAS28 >5.1

Table 1: Groups of RA patients according to Disease Activity Score.

Laboratory parameters	Controls		Patients	
	Range	Mean ± SD	Range	Mean ± SD
ESR (mm)	5-13	8.3 ± 2.8	5-144	47 ± 34.9
WBC (10 ³ cell/μl)	4.4-6.1	5.1 ± 0.5	3.8-14	6.58 ± 2.3
Haemoglobin (g/dl)	11.5-14.2	12 ± 1.5	7.5-14.1	11.5 ± 2.4
S. Urea (mg/dl)	19-33	26 ± 5.5	15-40	24 ± 6.3
S. Creatinine (mg/dl)	0.45-0.8	0.65 ± 0.1	0.6-1.5	0.65 ± 0.2
ALT (U/L; N <37)	17-28	22 ± 2.6	10-45	23 ± 8.6
AST (U/L; N <37)	18-27	22 ± 3.8	11-43	26 ± 9.9
RF positive	0%		61%	
RF negative	100%		39%	

ESR: Erythrocyte sedimentation rate; WBCs: White Blood Cells; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; RF: Rheumatoid Factor

Table 2: Laboratory investigations for control and patient groups.

Parameter	Range	Mean ± SD	Group (I) Mean ± SD (Range)	Group (II) Mean ± SD (Range)	Group (III) Mean ± SD (Range)
Ritchie articular index	0–26	11.20 ± 9.35	1.80 ± 1.9 (0–5)	9.20 ± 4.9 (0–20)	16.59 ± 7.6 (4–26)
Morning stiffness (hours)	0.25–6	2.49 ± 1.55	1.36 ± 0.7 (0.25–2.5)	1.79 ± 0.94 (0.25–3.5)	3.78 ± 1.8 (0.25–6)
Disease activity score (DAS)	1.13–8.34	5.26 ± 2.64	2.89 ± 0.54 (1.13–3.08)	4.61 ± 0.5 (3.25–5.08)	6.95 ± 0.7 (5.11–8.34)
Rheumatoid factor (IU/ml)	17.4–47.8	32.6 ± 15.20			
Anti-citrullinated peptide antibody	175–275	200 ± 25			
C-reactive protein (mg/L)	18–30	24 ± 6			

Table 3: Activity and severity indices of RA patients.

Frequencies of Treg cells

Decreased frequencies of Treg cells in peripheral blood of RA patients: Table 4 shows decreased prevalence of Treg cells (CD4⁺CD25⁺Foxp3⁺) of highly active RA patients when compared with that of healthy controls (1.01 ± 0.87 and 1.72 ± 1.27%; p<0.001), while there was no obvious divergence observed between low activity and moderate activity RA patients and healthy controls (p=0.739 and p=0.293, respectively). This decrease showed a significant correlation with DAS score of patients group III (r=-0.35, p<0.05) (Figure 1). A weak insignificant correlation was found between percentage of CD4⁺CD25⁺Foxp3⁺ T cells and Ritchie Articular Index in patient's group (r=0.104).

Groups	No.	Percentage of CD4 ⁺ CD25 ⁺ FOXP3 ⁺	P value	TH 17%	P value
		Mean ± SD		Mean ± SD	
Control	50	1.72 ± 1.27		0.61 ± 0.25	
Group (I)	14	1.80 ± 0.83	>0.05	1.09 ± 0.74	>0.05
Group (II)	48	1.49 ± 1.00	>0.05	1.19 ± 0.34	<0.001*
Group (III)	38	1.01 ± 0.87	<0.001*	1.62 ± 0.73	<0.001*

*p value = 8.6 × 10⁻⁴ <0.001 Highly Significant.

Th17% p values were 0.07NS for group I; 9 × 10⁻⁴ HS for group II and 7.1 × 10⁻⁴ HS for group III

p values of each group are in comparison to control group.

Table 4: Frequencies of circulating CD4⁺ CD25⁺ FOXP3⁺, Th17⁺ cells in RA patients and controls.

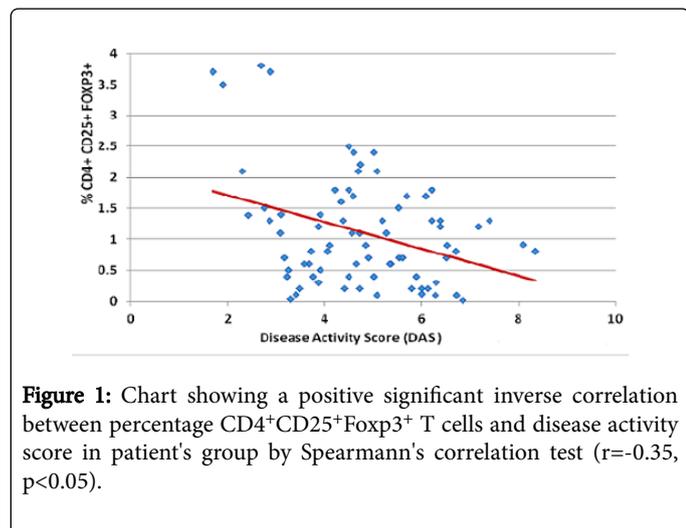


Figure 1: Chart showing a positive significant inverse correlation between percentage CD4⁺CD25⁺Foxp3⁺ T cells and disease activity score in patient's group by Spearmann's correlation test (r=-0.35, p<0.05).

Frequencies of circulating TH17 cells in RA patient: Representative example of flow cytometry chart of TH17 cells obtained from the PB of one RA patient and from a healthy control is shown in Figure 2. Significantly higher mean frequencies of circulating (CD4⁺ IL-17A⁺) TH17 cells were observed in RA patients groups II and III compared to healthy controls (p<0.001, Table 4). Consistently, the number of TH17 cells per volume of RA compared to healthy controls peripheral blood was significantly higher after stimulation with phorbol myristate acetate, ionomycin for 4 h.

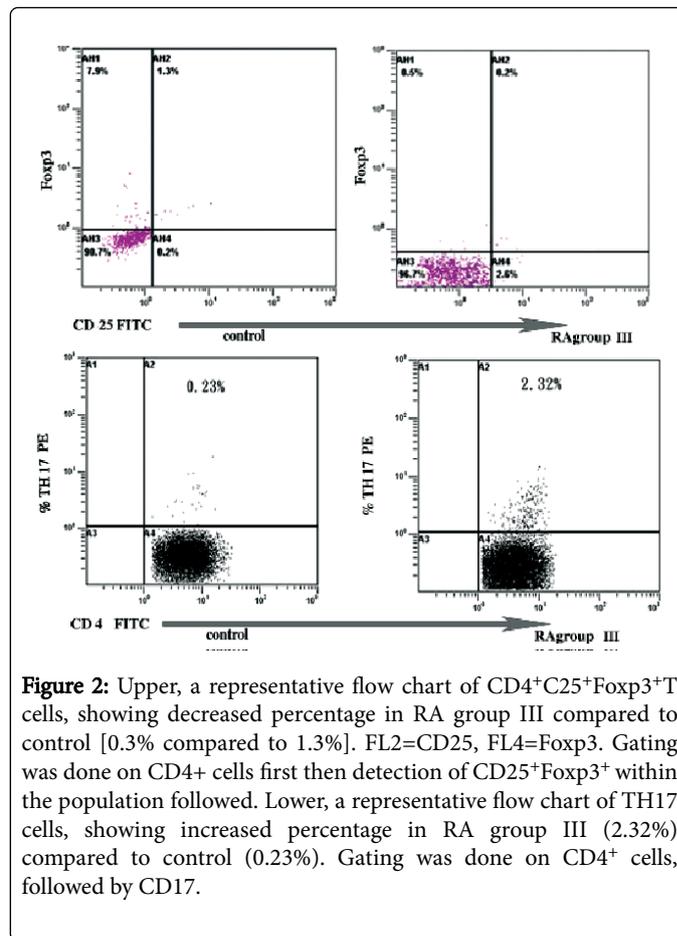


Figure 2: Upper, a representative flow chart of CD4⁺CD25⁺Foxp3⁺T cells, showing decreased percentage in RA group III compared to control [0.3% compared to 1.3%]. FL2=CD25, FL4=Foxp3. Gating was done on CD4⁺ cells first then detection of CD25⁺Foxp3⁺ within the population followed. Lower, a representative flow chart of TH17 cells, showing increased percentage in RA group III (2.32%) compared to control (0.23%). Gating was done on CD4⁺ cells, followed by CD17.

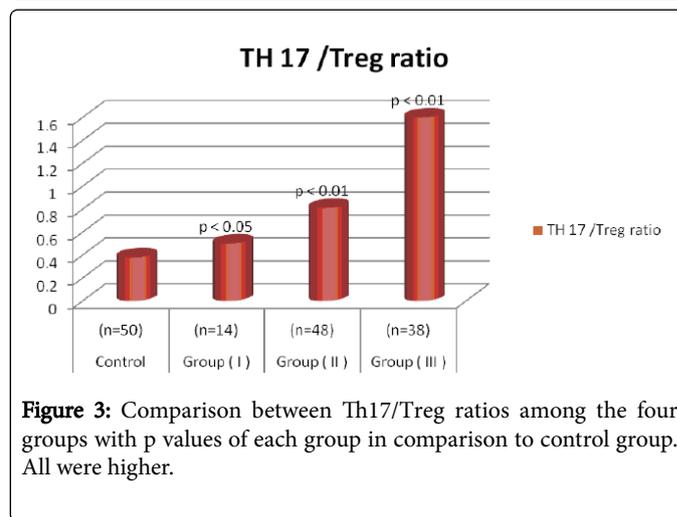


Figure 3: Comparison between Th17/Treg ratios among the four groups with p values of each group in comparison to control group. All were higher.

The significance of lower Treg cells and higher TH17 cells was further explored by calculation of TH17/ Treg ratio of individual RA patients and healthy controls. The TH17/Treg ratio was compared among four groups, as shown in Figure 3. Low activity RA group exhibited higher TH17/Treg ratio (0.51 ± 0.91) than that of healthy controls (0.38 ± 0.4, p<0.05). Moreover, there was a sharp elevation of TH17/Treg ratio in moderately and highly active RA patients (0.87 ± 0.74 and 1.58 ± 0.59, respectively) compared with those of low activity group and healthy controls (both p<0.01). Such results indicate that

there would be a T_H17/Treg dysbalance in peripheral blood of RA patients, especially in active state.

Increased TH17-related cytokines in the serum: Two T-helper cell-related cytokines as components in serum cytokine environment of RA patients and healthy controls were measured using ELISA. As shown in Table 5, RA group I exhibited higher levels of serum IL-17A and IL-23 than those of healthy controls ($p > 0.05$ NS; $p < 0.05$ S) respectively but lower than those of RA groups II & III. Active RA group had higher levels of serum IL-17A as well as serum IL-23 when compared to healthy controls (groups II and III $p < 0.001$ and $p < 0.05$) respectively. Collectively, these results suggest that there would be an increased T_H1- and T_H17-related cytokines profile in the serum from active RA patients (groups II and III DAS).

Parameter	Mean ± SD			
	Control	Group (I)	Group (II)	Group (III)
Serum IL-17 level pg/ml	7.96 ± 3.05	11.31 ± 5.08	17.31 ± 5.67	30.70 ± 18.77
Serum IL-23 level pg/ml	277.10 ± 49.04	313.5 ± 40.47	301.08 ± 57.54	402.71 ± 79.36
IL-17 p values: Group I >0.05 NS, Group II < 0.001 H.S., Group III <0.001 H.S in comparison to control group.				
IL-23 p values: Group I <0.05 S., Group II <0.05 S., Group III <0.05 S. in comparison to control group.				

Table 5: Serum levels of IL-17 and IL23 in RA patient and controls.

IL-17A showed approximately four-fold increase among patients while IL23 two-fold increase in serum level as compared to controls. The serum level of IL17 and IL23 correlated well with the T_H17 percentages in the blood of RA patients ($p = 0.01$).

Correlation between the frequencies of circulating T_H17 cells, serum levels of IL17 and IL23 and disease activity Frequencies of circulating T_H17 cells in RA patients were positively correlated with DAS28 score ($r = 0.63$, $p < 0.001$), IL17A levels ($r = 0.48$, $p < 0.05$), IL 23 ($r = 0.67$ $p < 0.001$). There is strong direct correlation between DAS28 and inflammatory T_H17.

Correlation of T_H17/Treg ratio with T_H17-related cytokines in active RA patients: T_H17/Treg ratios were positively correlated with serum concentrations of IL17A ($r = 0.650$, $p < 0.05$), IL-23 ($r = 0.713$, $p < 0.01$) of highly active RA patients. These data indicate that the serum environment characterized by increased T_H17-related cytokines may contribute to the dysbalance of circulating T_H17/Treg in active RA patients.

Discussion

Dysregulation in the immune system plays an important role in the pathogenesis of rheumatoid arthritis. The persistent nature of arthritis strengthens the suggestion of immune dysfunction.

In this study, flow cytometric analysis of RA patients peripheral blood, revealed a significant decrease in the frequency of circulating F_{oxp3}CD4⁺ CD25⁺ Tregs as compared to healthy controls. The low frequency of Treg cells was only observed in group III highly active RA patients, while there was no difference between low activity RA patients and healthy controls, which supported a protective role for Treg cells in periods of inactive RA disease.

This result is in agreement with other groups [20-26]. The exact mechanisms that reduce the level of Tregs are not clear. It was suggested that regulatory cells are recruited to sites of inflammation in an attempt to suppress disease, resulting in a relative reduction in the peripheral blood population [24]. Reports of a higher number of Tregs present in synovial fluid than that in the peripheral blood in RA patients support this explanation. Cell migration to local inflammatory tissue is enhanced by expressing particular chemokine receptors [27]. Moreover, Tregs were found to display increased tendency to undergo spontaneous apoptosis in active RA [28]. Chavele and Ehrenstein proposed that in an inflammatory condition like RA, it is quite possible that Tregs in the presence of the different proinflammatory cytokines will become unstable and convert to pathogenic T-cells [29]. Serum cytokine environment of active RA state is not in favor of the differentiation of Treg cells. This decline in regulatory T-cell numbers may predispose to persistent auto-immune diseases including RA [13]. Contrary to our results there are reports of higher Tregs in peripheral blood of RA patients [30] but in their research they assessed different CD4⁺CD25⁺ T cell subtypes (including CD4⁺ T cells expressing low levels of CD25 and those expressing F_{oxp3} and those not expressing it) which could account for the discrepancy in the results. We used CD4⁺CD25⁺F_{oxp3}⁺ as specific markers for Treg cells, and our results were similar to the reports using CD25^{bright} CD4⁺ as specific markers. Among CD4⁺CD25⁺ cells, only those expressing large amounts of CD25 (CD4⁺CD25^{bright}) exert suppressive effects. Cells expressing intermediate amounts of CD25, in contrast, are effector CD4 cells [31].

It has been documented that the frequencies of T_H17 cells were higher in PBMCs of RA patients compared to healthy controls [15,16,32]. Our study as well confirmed such finding, especially with the highly and moderately active RA patients. The difference was noted suggesting a potential predominant role of T_H17 cells in the inflammatory process and chronic progression in RA. Our observations supported the idea that T_H17 cells contributed to the pathogenesis of RA.

In contrast to our study, Yamada et al. reported that the frequency of T_H17 cells was neither increased in RA ($n = 123$), nor correlated with the DAS28 disease activity score. In addition, such cells were significantly decreased in the joints compared with PBMC of the same patients, whereas T_H1 cells were more abundant in the joints than in PBMC in these established RA patients [33]. The reason for this discrepancy is unclear, but may be due to methods for assessing disease activity and detecting T_H17 cells.

Considering contradictory results of frequency of T_H17 cells and Treg cells, we calculated the T_H17/Treg ratio for each sample. Healthy controls exhibited a low T_H17/Treg ratio, while RA patients, especially active RA patients, have a high T_H17/Treg ratio. This result indicated that the balance between T_H17 and Treg characterized by low T_H17/Treg ratio may be compromised in RA disease. Such T_H17/Treg imbalance may play a pivotal role in the development of RA, because predominant T_H17 cells can exert strong pro-inflammatory effects by producing IL-17. The imbalance of TH17/Treg has been also reported in several inflammatory disorders, such as primary biliary cirrhosis and inflammatory bowel disease [34].

We next investigated serum proinflammatory T_H17 cytokine profile in patients with RA, two related cytokines were measured; IL17 and IL23. IL-17 can promote inflammation by inducing the production of pro-inflammatory mediators, including cytokines, chemokines and other mediators of bone and cartilage destruction such as metalloproteinases [7]. IL-23 promotes the expansion and survival of

T_H17 cells. IL 23 promotes T_H17 cells producing tumour necrosis factor- α (TNF- α), IL-17, IL-6, IL-22, and GM-CSF which are associated with the induction of autoimmune inflammation in RA disease [21]. IL-17 showed a four-fold increase among patients while IL23 showed approximately two-fold increase in serum level as compared to controls. The serum level of IL17 and IL23 correlated well with the T_H17 percentages in the blood of RA patients (p=0.01). Considering higher frequencies of T_H17 cells and its two related cytokines in active RA in our study, these results are in accordance with the findings of other groups [35-38].

Nevertheless, our results also demonstrated that T_H17/Treg ratios were positively correlated with serum concentrations of T_H17 related cytokines, which further confirmed the T_H17/Treg dysbalance in peripheral blood of RA patient. Skapenko et al., reported that cytokine profile in peripheral blood of active RA patients may provide suitable environment for the differentiation of T_H1/T_H17 cells but not suitable for Treg cells [39].

We reported a significant correlation between DAS score and the decrease in frequency of Treg cells on one hand, and the increase in T_H17 percentages, serum IL17 and serum IL23 of RA patients on the other hand. The lower the frequency of Tregs, the higher the DAS score reflecting higher disease activity. Several studies also investigated possible correlations between IL17A or T_H17 cell proportion and disease activity. Most studies reported a good correlation with DAS score for T_H17 and related cytokines, or showed that IL17 was significantly higher among severely active patients, as compared to controls or to patients with inactive disease [35,36,38-40]. Others didn't report any correlation between Treg cell frequencies or cytokines with DAS score [41].

In summary, our results indicated that there would be a T_H17/Treg imbalance in peripheral blood of RA patients, and such disturbed balance may partly be due to the serum cytokine effects on microenvironment, which correlated with Disease Activity Score, especially with RA group III.

Our results were in line with the study of Niu et al. [42] and Wang et al. [43] who indicated that development of RA is associated with peripheral T_H17/Treg imbalance, and characterized by continuing generation of T_H17 cells. T_H17/Treg balance may be modified to restore homeostasis. The combination of the treatment that manipulates key cytokines with the treatment that target T_H17/Treg imbalance, may lead the road to development of novel and effective immunotherapy for RA.

Declaration of interest

Authors declare that they have no conflict of interest.

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