Published online: 2019-07-17

Investigating the Balance between Th17/Treg Cells in Rheumatoid Arthritis and its Association with Disease Activity

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J Child Sci 2019;9:e75–e83.

Abstract

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by articular inflammation and joint destruction. The mechanism of RA pathogenesis is not fully understood, but humoral and cellular immunity are known to be involved. $CD4^+$ T lymphocytes and cytokines released by these cells are suggested to initiate inflammation in RA. This study aimed to assess T helper 17 (Th17)/regulatory T (Treg) cell ratio and its correlation with disease activity in adult and juvenile RA. This study included 80 patients, with RA, including 40 adults (mean age: 36.4 $\pm$ 11.1 years and 40 juveniles mean age: 12.7 $\pm$ 2.2 years), and 80 healthy controls. For all patients and control subjects, patient and disease characteristics; laboratory tests for complete blood count, erythrocyte sedimentation rate, C-reactive protein (CRP), rheumatoid factor, anti-cyclic citrullinated peptide (anti-CCP), anti-nuclear antibodies (ANA), and flow cytometry to determine the numbers of Th17 and Treg cells. There was a statistically significant increase in the Th17/Treg ratio in patients with active disease compared with those with inactive disease for both adult and juvenile RA compared with controls. However, a similar significant difference was not observed between those with inactive adult and juvenile RA and controls. There were significant positive correlations between the Th17/Treg ratio and disease activity score 28 (DAS28), CRP, anti-CCP, and ANA in active adult and juvenile RA. The Th17/Treg ratio was increased in active form of adult and juvenile RA compared with inactive RA and control, indicating the Th17/Treg ratio as a potentially useful marker of disease activity.

Keywords
- Th17 cell
- regulatory T cell
- rheumatoid arthritis
- disease activity

received
February 11, 2019
accepted after revision
May 27, 2019

DOI https://doi.org/10.1055/s-0039-1693158.
ISSN 2474-5871.

Rheumatoid arthritis (RA) is a chronic inflammatory disease that primarily affects synovial membranes and causes irreversible joint damage. The prevailing pathogenic model of RA assumes an autoimmune mechanism.[1] $CD4^+$ T cells are a major component of the inflammatory infiltrate in the rheumatoid synovium in many patients, $CD4^+$ T cells and B cells in the synovial infiltrates are organized into lymphoid structures that resemble germinal centers.[2] However, a few studies focused on the role of T helper 17 (Th17) cells in arthritis, and the published results are controversial. A report suggested an important proinflammatory role for Th17 cells in various autoimmune diseases including RA.[3] Conversely, Treg cells play a major role during the inflammatory response in RA.[4,5]

Th17 cells secrete interleukin-17 (IL-17), which participates in inflammation and tissue destruction.[6,7] The pathogenic effect of IL-17 was shown in many experimental RA models.[8] In contrast, Treg cells, a distinct T cell subset, suppress lymphocyte responses, and attempt to maintain lymphocyte homeostasis. Thus, an imbalance between Th17 and Treg cells may contribute to the pathogenesis of RA. Therefore, a novel hypothesis for the pathogenic mechanism of RA states that an imbalance in the Th17/Treg ratio in combination with an imbalance in the Th1/Th2 ratio is responsible for the development and progression of RA.[9–11] Therefore, we conducted a prospective case-control study to determine if the Th17/Treg ratio could be used in new therapeutic strategies that can target these cells and their products to overcome limitations of currently used therapeutic strategies.[12]

Materials and Methods

Ethics Statement

This prospective case-control study conducted from April 2017 to May 2018 was approved by the ethics committee of the Faculty of Medicine at Bani Suef University. Written informed consent was obtained from all subjects including patients with RA and healthy controls or the legal guardians of patients with juvenile RA.

Study Design

This study prospectively enrolled 80 patients with RA including 40 patients with adult RA (mean age: 36.4 ± 11.1 years) and 40 patients with juvenile RA (mean age: 12.7 ± 2.2 years). The patients were enrolled from the Clinical Immunology Unit at the Hospital of Faculty of Medicine at Bani Suef University and the Pediatric Rheumatology Clinic at the Hospital of Faculty of Medicine, Minia University, Minia, Egypt. For all patients, the diagnosis was based on The American college of Rheumatology 2010 criteria. Based on the disease activity score (DAS28), both groups consisting of 22 and 18 patients had inactive and active disease respectively, DAS28 above 5.1 and below 3.2 indicate active and controlled disease respectively. In addition, 80 healthy individuals, including 40 adults and 40 children, who were matched for age, sex, geographical location, and ethnicity were included as controls. The study approved by ethical committee of Bani Suef University, Bani Suef and Minia University, Minia, Egypt.

Data collected for all subjects included patient and disease characteristics; clinical examination, laboratory measurements including complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor(RF), anti-cyclic citrullinated peptide (anti-CCP), and anti-nuclear antibodies (ANA) and flow cytometry to determine the numbers of Th17 and Treg cells.

Sample Collection

A total of 7 mL venous blood was collected by venipuncture and divided as follows: 1 mL in an ethylenediaminetetraacetic acid-coated tube for CBC and flow cytometric identification of Th17 and Treg cells, 2 mL in a citrated tube for ESR, 4 mL in a plain tube, to allow clotting. Serum of the sample in the plain tube was separated by centrifugation at 3,000 rpm for assessment of high-sensitive CRP, RF, anti-CCP, and ANA.

Assays

CBC was determined by an automated cell counter (Sysmex KX-21-N; TAO Medical, Japan), ESR was measured by the Westergren's method, high-sensitive CRP was determined by an enzyme immunoassay from Chemux Bioscience, United States. Semi-quantitative RF was measured using latex particles coated with human gamma-globulins that agglutinated in the presence of RF in the incubated samples (Teco Diagnostics, United States). The levels of Immunoglobulin G (IgG)-class auto-antibodies against cyclic citrullinated peptides in human serum, anti-CCP,[13] were determined by enzyme immunoassay (ORGENTIC Diagnostic, Germany). ANA was measured using a kit from ORGENTIC Diagnostic by a fully automated analytical instrument (Alegria, Germany).[14] Assessment of Th17 and Treg cells was achieved by flow cytometry on a BD FACS Canto LS (United States); the kits were supplied by BioLegend, and the protocols are detailed below.

Immunofluorescence Staining for Th17 Cells

Th17 cells were detected using the BioLegend human Th17 flow kit, which includes FITC-conjugated anti-CD3, PE-conjugated anti-CD4, and PerCP-conjugated anti-IL-17 antibodies, according to the following protocol. Briefly, 100 µL blood was added into two tubes (test and negative control) and 0.5 mL fixation reagent was added for 20-minute incubation at room temperature, followed by centrifugation to remove the supernatants. Next, 2 mL intracellular staining permeabilization reagent was added, and the tubes were incubated for 20 minutes at room temperature, and the supernatants were removed by centrifugation. Next, 20 µL antibody cocktail containing PerCP-conjugated anti-IL-17, FITC-conjugated anti-CD3, and PE-conjugated anti-CD4 antibodies was added into the test tube only, whereas the isotype control antibody was added to the negative control tube. The tubes were incubated at room temperature in the dark for 30 minutes. After washing the tubes twice with 2 mL intracellular staining perm-wash buffer, the samples were resuspended in 0.5 mL phosphate-buffered saline and analyzed by flow cytometry.[15]

Table 1 Demographic data of the study groups

	Adult RA (*N* = 40)	Adult control (*N* = 40)	Juvenile RA (*N* = 40)	Juvenile control (*N* = 40)
Age (y)	37.8 ± 11.1	36.4 ± 12.5	11.7 ± 2.2	12.4 ± 1.9
Disease duration (y)	5.0 ± 2.4	–	2.6 ± 0.8	–
Sex				
Male (%)	2 (5)	2 (5)	16 (40)	16 (40)
Female (%)	38 (95)	38 (95)	24 (60)	24 (60)

Abbreviation: RA, rheumatoid arthritis.

Immunofluorescence Staining for Treg Cells

Treg cells were detected using the BioLegend One Step Staining human Treg flow kit, which includes FITC-conjugated anti-FOXP3, PE-conjugated anti-CD25, and PerCP conjugated anti-CD4 antibodies. Briefly, 100 µL blood was added into two tubes (test and negative control), and 0.5 mL fixation reagent was added for 20 minutes of incubation at room temperature, followed by centrifugation to remove the supernatants. Next, 2 mL intracellular staining permeabilization reagent was added to each tube, which were incubated for 20 minutes at room temperature, and the supernatants were removed by centrifugation. Next, 20 µL of the antibody cocktail containing FITC-conjugated anti-FOXP3, PE-conjugated anti-CD25, and PerCP-conjugated anti-CD4 was added into the test tube only, whereas the isotype control antibody was added in the negative control tube; the tubes were incubated at room temperature in the dark for 30 minutes. After washing the tubes twice with 2 mL intracellular staining perm wash buffer, the samples were resuspended in 0.5 mL phosphate-buffered saline and analyzed by flow cytometry.[16]

Statistical Analysis

All statistical analyses were conducted using the Statistical Package for the Social Science, software package version 20 for Microsoft Windows (SPSS, Chicago, IL, USA). Data were presented as means ± standard deviation or frequencies when appropriate. Spearman's correlation coefficient test was used for correlation analyses, and Student's *t*-test was used for comparison of numerical variables between two groups. *p*-Values less than 0.05 were considered statistically significant at the 95% confidence level.

Results

►**Fig. 1** shows the percentages of Th17 cells in the studied groups. Th17 cells were significantly higher in both active adult and juvenile RA (2.9% and 2.3% respectively) than in control adult and juvenile groups (0.4% and 0.3% respectively). ►**Fig. 2** shows the percentages of Treg cells in the studied groups. Treg cells were significantly lower in both active adult and juvenile RA (0.5% and 0.9% respectively) than in control adult and juvenile groups (2.4% and 2.1% respectively). As shown in ►**Table 1**, which summarizes the demographic characteristics of the participants of the study, the adult RA group was composed of 95% females and 5% males, whereas the juvenile RA group was composed of 40% females and 60% males. The mean disease duration was 5.0 ± 2.4 years in the adult RA group and 2.6 ± 0.8 years in the juvenile RA group.

As summarized in ►**Table 2**, the DAS28 scores were 4.1 ± 2.8 and 4.3 ± 3.5 in the adult and juvenile RA groups, respectively. Additionally, 75% of the adult patients were RF positive, whereas 35% of the juvenile patients were RF

Table 2 Laboratory data of the study groups

	Adult RA (*N* = 40)	Adult control (*N* = 40)	*p*-Value	Juvenile RA (*N* = 40)	Juvenile control (*N* = 40)	*p*-Value[a]
DAS28	4.1 ± 2.8	–	–	4.3 ± 3.5	–	–
ESR in first hour (mm/h)	50.0 ± 32.0	8.0 ± 2.0	<0.001	40.0 ± 21.0	6.0 ± 1.0	<0.001
CRP (ng/mL)	44.4 ± 15.6	4.5 ± 1.2	0.003	35.2 ± 13.3	4.2 ± 1.0	<0.020
RF (IU/mL)[b] 0 (negative %) 1 (positive %)	10(25) 30(75)	40(100) 0	<0.001	14(35) 26(65)	40 (100) 0	<0.001
Anti-CCP (U/mL)	45.4 ± 24.02	2.8 ± 1.2	<0.001	40.2 ± 20.9	2.1 ± 1.04	<0.001
ANA (IU/mL)	5.5 ± 3.7	0.3 ± 0.1	<0.001	4.8 ± 2.06	0.2 ± 0.1	<0.001

Abbreviations: RA, rheumatoid arthritis; Anti-CCP, anti-cyclic citrullinated peptide; ANA, anti-nuclear antibodies.
Note: analysis of quantitative data was performed by the independent sample *t*-test; qualitative data were analyzed by the chi-squared test.
[a]*p*-Value is nonsignificant if ≥0.05; *p*-value is significant if < 0.05; *p*-value is highly significant if <0.01; *p*-value is very highly significant if <0.001.
[b]0: negative (less than 8.0 IU/mL); 1: positive (more than 8.0 IU/mL).

Table 3 Lymphocyte subpopulation analysis

	Adult RA (N = 40)	Adult Control (N = 40)	p-Value	Juvenile RA (N = 40)	Juvenile Control (N = 40)	p-Value[a]
Absolute number of lymphocytes (cells/μL)	1,399.6 ± 321.2	1,801.5 ± 115.8	<0.001	2,414.1 ± 434.9	2,702.5 ± 418.04	<0.001
Absolute number of $CD4^+$ T cells (cells/μL)	458.3 ± 180.4	777.2 ± 81.8	<0.001	830.4 ± 190.6	1,013.8 ± 273.5	<0.001
Subpopulations of CD4+ lymphocytes (absolute counts)						
Th17 absolute count (cells/μL)	13.0 ± 1.7	3.9 ± 1.2	0.055	16.0 ± 2.8	4.7 ± 1.1	0.024
Treg (cells/μL)	7.7 ± 4.1	22.4 ± 4.1	<0.001	9.7 ± 4.5	24.4 ± 2.1	<0.001
Subpopulations of CD4+ lymphocytes (% of CD4+)						
Th17 (% of $CD4^+$ T cells)	2.2 ± 0.5	0.4 ± 0.1	<0.001	2.0 ± 0.6	0.3 ± 0.1	<0.001
Treg (% of $CD4^+$ T cells)	1.1 ± 0.6	2.8 ± 0.4	0.002	1.3 ± 0.5	2.3 ± 0.3	0.002
Th17/Treg ratio	1.35 ± 1.41	0.23 ± 0.11	0.001	1.61 ± 1.57	0.24 ± 0.08	<0.001

Abbreviation: RA, rheumatoid arthritis.
[a]p-Value is nonsignificant if ≥0.05; p-value is significant if <0.05; p-value is highly significant if <0.01; p-value is very highly significant if <0.001.

negative. The absolute number of lymphocytes and $CD4^+$ T cells were significantly higher in both the adult and juvenile RA groups compared with the healthy controls (►**Table 3**). Additionally, the Th17/Treg ratio was significantly higher in both the adult and juvenile RA groups, compared with the healthy controls.

We next analyzed the changes in Th17 and Treg cell numbers in association with active disease. As presented in ►**Table 4**, there was a significant increase in the percentage of Th17 cells (p- value > 0.001) and a significant decrease in the percentage of Treg cells (p-value > 0.001), with a consequent increase in the Th17/Treg ratio, in the adult patients with active RA compared with both the adults patients with inactive RA and the healthy controls, whereas the Th17/Treg ratio was not significantly different between the adult patients with inactive RA and the healthy controls.

►**Table 5** shows the Th17/Treg ratio analysis in the patients with juvenile RA. Similar to that observed in the adult patients with RA, there was a significant increase in the percentage of Th17 cells and a significant decrease in the percentage of Treg cells, with a consequent increase in the Th17/Treg ratio, in the patients with active juvenile RA compared with both those with inactive juvenile RA and the healthy controls. Comparison of the patients with

Table 4 Comparison of Th17 and Treg cell numbers and Th17/Treg ratio in adult patients with active and inactive RA and healthy controls

	Active adult RA (N = 18)	Inactive adult RA (N = 22)	Adult control (N = 40)	p-Value [a]
Absolute number of lymphocytes (cells/μL)	1,067.2 ± 42.1	1,671.5 ± 118.5	1,801.5 ± 115.8	P1: < 0.001 P2: < 0.001 P3: 0.002
$CD4^+$ Tcells (cells/μL)	270.7 ± 40.9	611.7 ± 54.8	777.2 ± 81.8	P1: < 0.001 P2: < 0.001 P3: < 0.001
Th17 (% of $CD4^+$ T cells)	2.6 ± 0.6	1.4 ± 0.2	0.4 ± 0.1	P1: < 0.001 P2: < 0.001 P3: 0.228
Treg (% of $CD4^+$ T cells)	0.6 ± 0.1	1.7 ± 0.2	2.8 ± 0.4	P1: < 0.001 P2: < 0.001 P3: 0.643
Th17/Treg ratio	2.667 ± 1.13	0.28 ± 0.08	0.23 ± 0.11	P1: < 0.001 P2: < 0.001 P3: 0.755

Abbreviations: RA, rheumatoid arthritis.
Note: the ratio Th17/Treg was calculated for each case and mean Th17/Treg ratios were derived from these values.
[a]P1: p-Value of active RA patients and control; P2: p-Value of active RA patients and inactive RA patients; P3: p-Value of inactive RA patients and control.

Table 5 Comparison of Th17 and Treg cell numbers and Th17/Treg ratio in juvenile patients with active and inactive RA and healthy controls

	Active juvenile RA (*N* = 18)	Inactive juvenile RA (*N* = 22)	Juvenile control (*N* = 40)	*p*-Value[a]
Absolute number of lymphocytes (cells/μL)	1967.2 ± 52.1	2630.5 ± 302.5	2702.5 ± 418.0	P1: < 0.001 P2: < 0.001 P3: 0.012
CD4+ (cells/μL)	470.7 ± 40.9	841.7 ± 54.8	1013.8 ± 273.5	P1: <0.001 P2: <0.001 P3: <0.001
Th17 (% of CD4)	2.6 ± 0.3	1.5 ± 0.2	0.3 ± 0.1	P1: < 0.001 P2: < 0.001 P3: 0.020
Treg (% of CD4)	0.9 ± 0.1	1.5 ± 0.1	2.3 ± 0.3	P1: < 0.001 P2: < 0.001 P3: 0.761
Th17/Treg ratio	3.15 ± 1.01	0.35 ± 0.13	0.24 ± 0.08	P1: < 0.001 P2: < 0.001 P3: 0.492

Abbreviation: RA, rheumatoid arthritis.
Note: the ratio Th17/Treg was calculated for each case and mean Th17/Treg ratios were derived from these values.
[a]P1: *p*-value of active RA patients and control; P2: *p*-value of active RA patients and inactive RA patients; P3: *p*-value of inactive RA patients and control.

Table 6 Correlation between Th17 cells in studied groups and disease parameters

	Adult RA		Juvenile RA	
	r^a	*p*-Value	r^a	*p*-Value
Disease duration (y)	−0.146	0.540	0.138	0.562
DAS	0.743	<0.001	0.663	0.001
ESR	0.675	0.001	0.676	0.001
CRP	0.757	<0.001	0.826	<0.001
Anti-CCP	0.660	0.002	0.721	<0.001
RF	0.423	0.063	0.758	<0.001
ANA	0.869	<0.001	0.785	<0.001

Abbreviations: DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; anti-CCP, anti-cyclic citrullinated peptide; RF, rheumatoid factor; ANA, anti-nuclear antibodies.
[a]r = 0.00–0.24: weak or no association; 0.25–0.49: fair association; 0.50–0.74: moderate association; 0.75 + : strong association.

Table 7 Correlation between Treg cells and disease parameters

	Adult RA		Juvenile RA	
	r^a	*p*-Value	r^a	*p*-Value
Disease duration (y)	0.036,5	0.114	−0.078	0.744
DAS	−0.784	<0.001	−0.770	<0.001
ESR	−0.803	<0.001	−0.742	<0.001
CRP	−0.745	<0.001	−0.867	<0.001
Anti-CCP	−0.843	<0.001	−0.748	<0.001
RF	−0.383	0.096	−0.393	0.087
ANA	−0.671	0.001	−0.741	<0.001

Abbreviations: DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; Anti-CCP, anti-cyclic citrullinated peptide; RF, rheumatoid factor; ANA, anti-nuclear antibodies.
[a]r = 0.00 to 0.24: weak or no association; 0.25 to 0.49: fair association; 0.50 to 0.74: moderate association; 0.75 + : strong association.

inactive juvenile RA and the healthy controls did not reveal a significant difference in the Th17/Treg ratio between the two groups.

Finally, we examined the potential correlations between Th17 and Treg cells and clinical parameters (►**Tables 6** and **7**). We found that there were significant positive correlations between the percentage of Th17 cells and several parameters of RA activity including DAS28, ESR, CRP, anti-CCP, RF, and ANA; however, there was no significant correlation between the percentage of Th17 cells and disease duration in both the adult and juvenile RA groups (►**Table 6**, *p*-value = 0.540 and 0.562 respectively). Furthermore, there were significant negative correlations between the percentage of Treg cells and several parameters of RA activity including DAS28, ESR, CRP, anti-CCP, and ANA, with no significant correlations with disease duration or RF in both the adult and juvenile RA groups (►**Table 7**, *p*-value = 0.114,0.744 and 0.096, 0.087 respectively)

Discussion

For over two decades, the Th1-Th2 paradigm has been used as a framework to characterize human diseases.[17,18] After activation by antigens, $CD4^+$ T cells expand and polarize into either Th1 cells that produce interferon-γ IL-2 and lymphotoxin or Th2 cells that produce IL-4, IL-5, IL-9, and IL-13.[19] The differentiation of naïve T cells into T cell subsets depends on the cytokine milieu, which are present during the T cell activation. Interferon-γ and IL-12 differentiate naïve T cells into Th1 cells, whereas IL-4 promotes differentiation into Th2

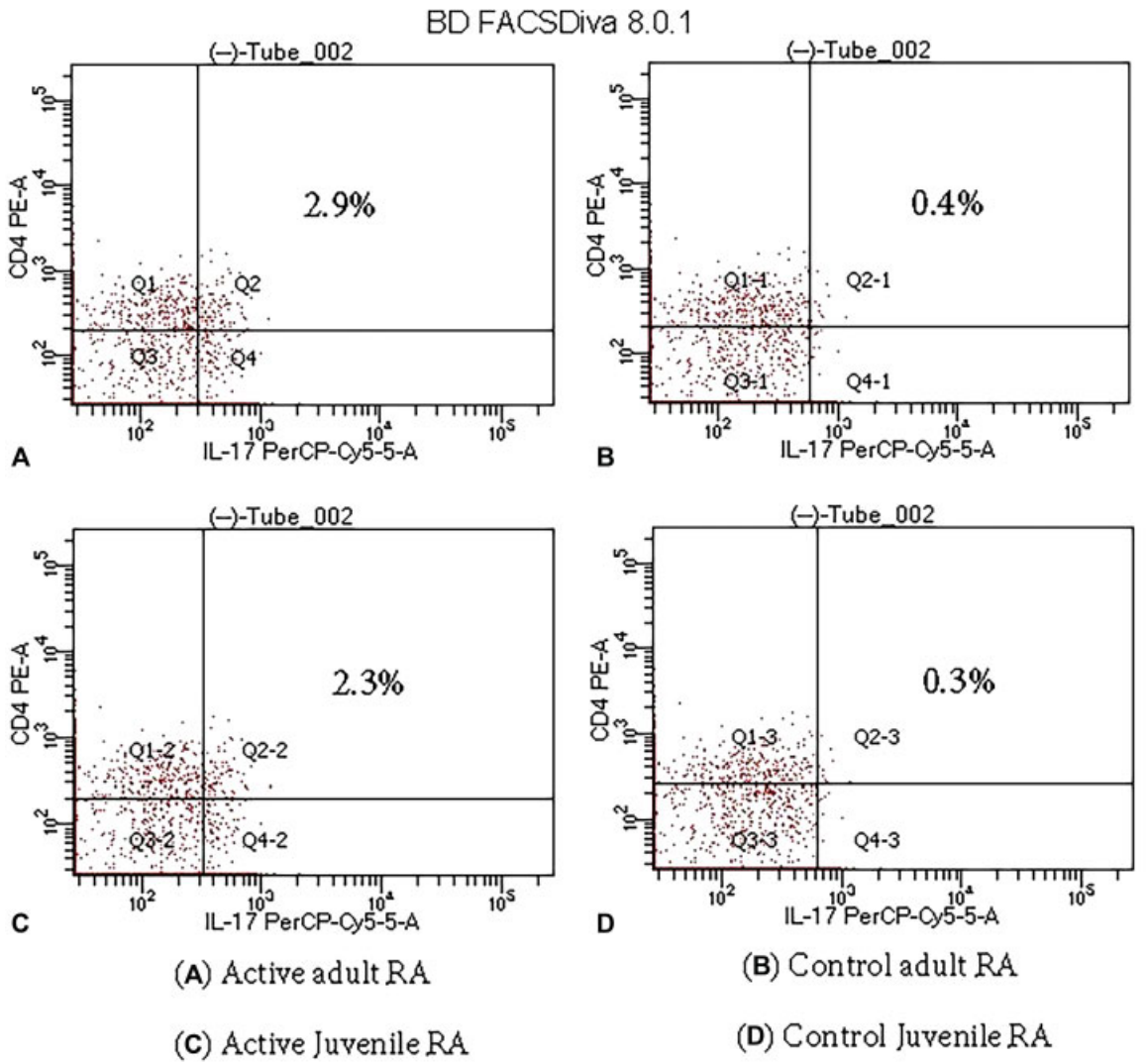

Fig. 1 Flow cytometric analysis for Th17 cells. (**A**) A patient with active adult RA (2.9%). (**B**) Control of a patient with adult RA (0.4%) (**C**) A patient with active juvenile RA (2.5%). (**D**) Control of a patient with juvenile RA (0.3%).

cells. Th1 and Th2 have different functions Th1 cells target intracellular pathogens, whereas Th2 cells target extracellular pathogens.[18,20]

RA is a systemic autoimmune disorder characterized by articular inflammation leading to joint destruction. Although the pathogenic mechanisms of RA are not fully understood, $CD4^+$ T lymphocytes and cytokines secreted by these cells were implicated in the initiation of inflammation in this disease.[21,22] This study aimed to assess the Th17/Treg ratio and its correlation with the parameters of disease activity. In patients with adult and juvenile RA, the results revealed that the absolute count of the $CD4^+$ T lymphocyte subpopulation was significantly higher in both the adult and juvenile RA groups compared with the controls. Additionally, there were significant increases in the absolute number and the percentage of Th17 cells and significant decreases in the absolute number and the percentage of Treg cells, with the consequent increase in the Th17/Treg ratio, in the patients with active adult and juvenile RA compared with those with inactive adult and juvenile RA and the healthy controls. Finally, there were no significant differences in these parameters between the patients with inactive RA and the healthy controls (p-value = 0.228, 0.643, and 0.755).

These results are in agreement with the findings of recent studies.[23,24] Chen and his colleagues found that the baseline percentages of circulating Th17 cells and serum levels of IL-6, IL-17, IL-21, IL-23, and tumor necrosis factor (TNFα) were higher in patients with active RA compared with those with inactive RA and healthy controls ($p < 0.001$ for all).[25] Treg cells develop in the thymus or lymphoid tissues such as spleen, lymph nodes, and intestinal mucosa, and require CD28 co-stimulation during positive selection in the thymus in the presence of transforming growth factor-β, IL-2, and IL-15.[24] In patients with RA, Treg cells are functionally defective, and restoring Treg cell function is important for the control of inflammation and restoring tolerance in RA.[26–28]

However, the implications of changes in the absolute number of Treg cells and its relation to RA disease remain unclear.[29] Some studies reported a decrease in Treg cell numbers,[27,30,31] whereas others showed no changes[32] or increases in the number of Treg cells in the peripheral blood of RA patients.[33,34]

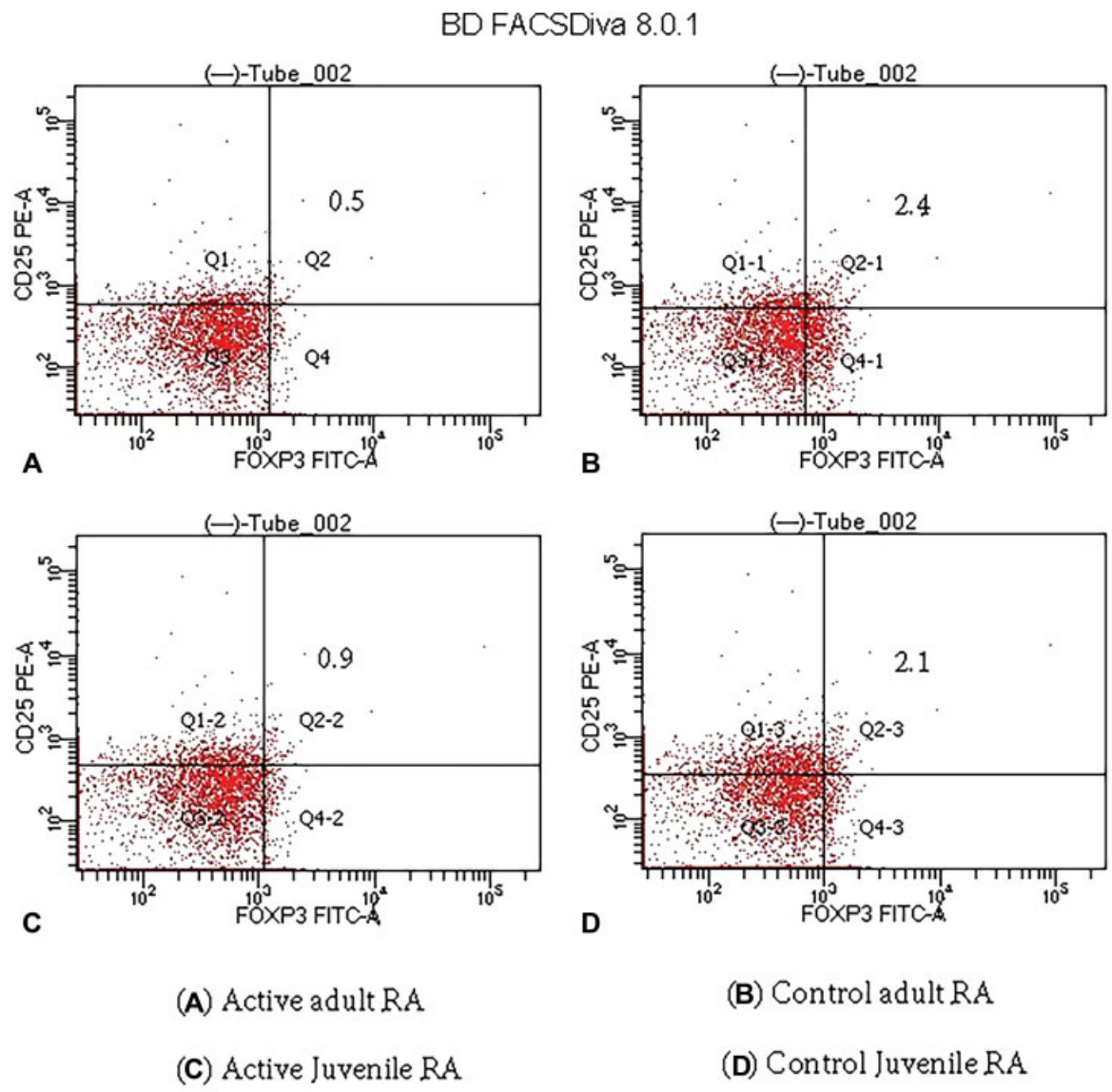

Fig. 2 Flow cytometric analysis for Treg cells. (**A**) A patient with active adult RA (0.5%). (**B**) Control of a patient with adult RA (2.4%). (**C**) A patient with active juvenile RA (0.9%). (**D**) Control of a patient with juvenile RA (2.1%).

In general, imbalance in the Th17/Treg ratio plays an important role in pathogenesis of RA, in which a decrease in the Treg cell number may be associated with an increase in the severity of arthritis. Interestingly, most of the treatment strategies such as nondepleting anti-CD4 antibody,[35] IL-21,[36] and anti-IL-6 receptor antibody[37] treatments in animal models of RA, as well as anti-IL-6 receptor antibody (tocilizumab),[38] anti-TNFα antibody,[39,40] and combination therapy using methotrexate and etanercept[30] in RA patients were reported to correct the imbalance in the Th17/Treg ratio.[33]

In the present study, we also observed increased frequencies of peripheral Th17 cells in the patients with active RA compared with those with inactive RA among the patients with adult RA;Th17 cell frequencies were positively correlated with DAS28, ESR, ANA, CRP, anti-CCP, and RF. Chen et al showed that the Th17cell levels in the peripheral blood were correlated positively with DAS28 before therapy and that there were a significant decrease in the Th17 cell levels in peripheral blood after therapy with anti-TNF-α concomitant with a reduction in DAS28.[25] Our findings support that Th17 cell levels in peripheral blood might affect inflammatory status and reflect disease activity in adult RA.

We also found a significant decrease in the percentage of Treg cells and a significant increase in the Th17/Treg ratio in the adult patients with active RA compared with those with inactive disease ($p < 0.001$). Furthermore, there was a significant negative correlation between the Treg cells percentage and DAS28, ESR, CRP, and anti-CCP, which might be associated with an increase in RA severity.

In the juvenile group included in the current study, we observed that percentages of peripheral Th17 cells were higher in those with active RA than those with inactive RA and that the frequencies were positively correlated with all disease parameters except disease duration. There was a significant decrease in Treg cells and a significant increase in the Th17/Treg ratio in those with active juvenile RA compared with the inactive juvenile RA group ($p < 0.001$), as well as significant negative correlations between Treg cells

and DAS28, ESR, CRP, anti-CCP, and ANA. Numerous studies reported either an inverse relationship or no association between ESR and CRP in the acute phase of RA. Finally, there was no association between the Treg cell percentage and age, sex, disease duration, RF positivity, or bone erosions.[33,41–43]

To our knowledge, this is the first study comparing Treg and Th17 cells in patients with adult and juvenile RA, revealing that relationships of Treg and Th17 cells with disease parameters were comparable between the adult-onset and juvenile RA. Importantly, disease duration did not exhibit significant correlations with Th17 or Treg cell numbers in either group, implicating that the disease manifested similarly in adults and children.

Many drugs, which are capable of modifying the Th17/Treg ratio were shown to be effective in autoimmune diseases and approved for treatment of some of the autoimmune diseases.[44] The complexity of autoimmunity may explain why certain therapeutic strategies that are effective for certain diseases, such as IL-6 receptor blockade in RA, have no effect in other diseases such as AS.[45] A recent study revealed that Th17 cells are not a uniform subset but comprise pathogenic and nonpathogenic cell populations.[46] Therefore, therapeutic strategies that target only pathogenic Th17 cells may be the next step for shifting the Th17/Treg ratio. In summary, there are several challenges in identifying specific Th17/Treg targeted therapeutic strategies with efficient treatment of autoimmune diseases as well as RA.

Conclusion

In the current study, we found that the increase in the proinflammatory Th17 cells and the decrease in Treg cells were related to RA disease activity in both adult and juvenile RA patients. Further investigation is necessary to identify well-tolerated and potent compounds to induce the diversion of Th17 cells to Treg cells, and therapeutic targeting of the Th17/Treg ratio may open new lines for RA treatment.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Funding

None.

Conflicts of Interest

None declared.

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