B cell depletion treatment decreases Th17 cells in patients with rheumatoid arthritis

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Research article

Abstract

Introduction: We aimed to evaluate for any possible effects of treatment with rituximab (RTX) on the peripheral Th17 and Treg subpopulations in patients with rheumatoid arthritis (RA).

Patients and methods: We analyzed 16 patients with RA initiating RTX treatment, 11 patients with RA initiating abatacept treatment, 11 patients with RA treated with other medications, 8 patients with other autoimmune rheumatic diseases initiating RTX, and 14 healthy volunteers. Th17 cells ($CD4^+IL23R^+IL17A^+$) and Treg cells ($CD4^+CD25^{hi}FoxP3^+$) were evaluated flow-cytometrically.

Results: Th17 cells from patients treated with RTX decreased significantly at weeks 8 and 16 (mean ± SEM: 3.01% ± 0.54⍰ $CD4^+$ cells at week 0 vs. 1.53% ± 0.24⍰ at week 8 vs 1.10% ± 0.20⍰ at week 16, p = 0.0004). Reductions of Th17 cells were evident in:clinical responders (DAS28 score ≤ 3.2), ACPA (+) and RF (-) patients; circulating Tregs remained stable. Th17 and Tregs were not affected by ABA treatment or by changes in disease activity. Tregs, but not Th17 cells, decreased following treatment with RTX in patients with other autoimmune diseases (0.75% ± 0.16% at week 0 vs. 0.43% ± 0.16% at week 8, p = 0.033).

Conclusion: RTX-induced B cell depletion results in a significant reduction of circulating Th17 cell percentages, whereas it has no effect on Tregs of patients with RA. This reduction of Th17 cells was evident particularly in responders to RTX treatment, ACPA+ and RF (-) patients with RA.

Introduction

Rituximab (RTX)-mediated B cell depletion is an approved treatment for patients with rheumatoid arthritis (RA). Anti-CD20 mAb targets and eliminates B cells which are currently thought to play a significant role in disease pathogenesis. Although RTX is an approach affecting B cells exclusively, it may be reasonable to investigate whether treatment with RTX affects other cell populations besides B cells, such as T lymphocytes, through the interplay between cells that contribute to the inflammatory process in RA. Experimental studies in animals have presented data on alterations of T cells following B cell depletion [1, 2]. Additionally, a few studies have displayed evidence on the possible effect of RTX on T cells of patients with RA [7−14] and other autoimmune diseases, such as systemic lupus erythematosus (SLE) [4, 5] and systemic sclerosis [6]. The aim of this study was to investigate if treatment of patients with RA with RTX could induce any changes in the numbers of peripheral Treg and Th17 cells. To address this question, we analyzed flow-cytometrically circulating T cell subpopulations before and after treatment with RTX at defined time points. Our experimental data suggest that B cell depletion in patients with RA significantly reduces peripheral Th17 cells whereas numbers of Tregs remain unaffected.

Patients And Methods

Patients and healthy donors: We studied 5 groups of individuals: i) 16 patients [10 women, 6 men, mean age (± SD): 66 ± 12 years] with RA receiving RTX 1g biweekly for the first time (RTX group). Patients were

analyzed at weeks 0 (baseline) and at weeks 8 and 16 of treatment. ii) 11 patients with RA receiving abatacept for the first time. Patients were analyzed at weeks 0 and 8 of treatment (biologic disease-controls). iii) 11 patients with RA already under treatment either with synthetic or biologic disease modifying antirheumatic drugs (DMARDs) at 2 different time points (disease-controls), iv) 8 women with other-than-RA autoimmune diseases [5 with SLE, 2 with polymyositis and 1 with Sjögren's syndrome] receiving RTX for the first time. Analysis of T cell subpopulations was performed in this group at weeks 0 and 8 of treatment (RTX disease controls) and v) 14 healthy donors. Written informed consent was obtained from all participating individuals and the study protocol was approved by the local (Patras University Hospital) Ethics Committee. Patient demographics are depicted in Table 1.

Table 1
Demographic, clinical, serological and treatment characteristics of patients with RA

	RA patients RTX	RA patients ABA	RA patients control
Number of patients (n)	16	11	11
Age (Mean ± SD)	66 ± 12	60 ± 13	62 ± 12
Female	10	8	7
Men	6	3	4
Disease duration (mo)	97 ± 75	114 ± 60	175 ± 130
CRP (mg/dL)	2.35 ± 2.54	2.75 ± 4.25	0.9 ± 1.0
DAS28 score	5.58 ± 1.40	5.30 ± 1.27	3.38 ± 1.32
RF (+)	6 / 16	5 / 11	5 / 11
ACPA (+)	11 / 16	5 / 11	8 / 11
Corticosteroids	10 / 16	11 / 11	6 / 11
DMARDs	9 / 16	4 / 11	10 / 11
TNF-α blockers	0	0	4 / 11

Cells and antibodies

Heparinized whole venous blood was obtained from all participating individuals. Peripheral blood mononuclear cells (PBMC) were isolated by standard Ficoll – Hypaque gradient centrifugation. Three-colour flow cytometric analysis was employed to analyze T cell subpopulations of interest. Both Th17 and Treg T cells are expressed as percentages of $CD4^+$ T cells. Th17 T cells were phenotypically defined as $CD4^+IL-23R^+IL-17A^+$ cells and Tregs were defined as $CD4^+CD25^{high}FOXP3^+$ T cells. PBMC were stained with anti-human CD4-PE-Cy5, anti-human IL-12/IL-23 p40-PE mAb, whereas intracellular staining was performed with FITC-conjugated anti-human IL-17A. We also employed PE-conjugated anti-human CD25 mAb for surface staining and FITC-conjugated anti-human FOXP3 Staining set for intracellular staining

(all fluorochrome-conjugated mAb and the relevant isotypic controls were purchased from e-Bioscience Products).

Flow cytometry

To activate cells, PBMC from all groups were incubated with phorbol myristate acetate (PMA) 50ng/ml and 1µg/ml ionomycin for 5 hours in the presence of 10 µg/ml Brefeldin A. One million PBMCs were washed three times in ice-cold PBS. Surface staining was subsequently performed with anti-human CD4-PE-Cy5, and anti-human IL-12/IL-23 p40-PE mAb. Afterwards, fixation with 0.01% paraformaldehyde was performed for 20 min on ice. Saponin 0.1% was added and after incubation for 30 minutes, cells were stained with FITC-conjugated anti-human IL-17A mAb. Intracellular staining was fixed with paraformaldehyde 0.01%. Six patients (all women) from the RTX-group of patients with RA were also separately analysed for a different subpopulation called "surface Th17 cells" (sTh17) using only surface staining for all molecules, including IL-17A. PBMCs were stimulated with 50ng/ml PMA and 1µg/ ml ionomycin for six hours. The stimulation and surface staining were performed at different time points than intracellular IL-17A staining and Brefeldin A was not employed. Staining and analysis of such sTh17 T cells was performed as previously described [37].

All samples were also analyzed for circulating Treg T cells at all time points; Tregs were defined as $CD4^+CD25^{high}FOXP3^+$ T cells. Surface staining, fixation, permeabilization and intracellular staining was performed also as described above.

Statistical analysis

Repeated measures of ANOVA was employed for the statistical analysis our data. In some instances, we employed the unpaired two-tailed Students' t-test (GraphPad Prism Software). Values of $p < 0.05$ were considered as statistically significant.

Results

Treatment with RTX reduces circulating Th17 cells in patients with RA

RTX targets CD20 on the surface membrane of B cells and depletes them with incompletely understood mechanism(s). Since different cells of the immune system are inter-related via a multitude of pathways and mechanisms, we asked the question of whether B cell depletion treatment potentially alters T cells as well in patients with RA. To address this question we evaluated flow-cytometrically for potential alterations of different T cell subpopulations before and following RTX administration.

Blood samples were drawn from 16 patients with RA at baseline and at weeks 8 and week 16 following RTX treatment initiation. Th17 T cells, defined as $CD4^+IL-23R^+IL-17A^+$ T cells, are expressed as percentages of whole CD4 + T cells. Flow cytometric analysis disclosed a significant reduction of Th17

cells at all time points following treatment with RTX. More specifically, Th17 T cells of RA patients taking RTX (n = 14) were significantly reduced at week 8 and 16 compared to baseline percentages (mean ± SEM: 3.01% ± 0.54% CD4 + at week 0 vs. 1.53% ± 0.24% at week 8, vs 1.10% ± 0.20% at week 16, p = 0.0004). One representative experiment is shown in Fig. 1A, B and C and all patients are collectively depicted in Fig. 1D. Therefore, our data suggest that B cell depletion therapy reduces significantly the numbers of circulating Th17 T cells in patients with RA.

We next attempted to correlate Th17 cell reductions with possible clinical responses to treatment with RTX. The subgroup of patients responding to RTX (n = 8) by achieving a DAS 28 score ≤ 3.2 at week 16 displayed a significant reduction of Th17 T cells at both time points. Results obtained from one representative patient and the group of responders are shown in Fig. 2. More specifically, Th17 cells were reduced from 3.48% ± 0.84% at week 0, to 1.66% ± 0.32% at week 8 and further reduced to 0.78% ± 0.20% at week 16 (p = 0.0014). In contrast to RTX-responders, such decreases of circulating Th17 cells were not seen following RTX treatment in non-responders.

We next asked the question if RTX-induced Th17 subpopulation changes were correlated with rheumatoid factor (RF) positivity. Percentages of circulating Th17 cells of RF negative patients (n = 8), but not of those with RF(+), were statistically significantly reduced by RTX treatment from 3.21% ± 0.69% at baseline, to 1.60% ± 0.36% at week 8 and to 0.98% ± 0.18% at week 16, (p = 0.0035) compared to baseline as seen in Fig. 3E.

However, when we evaluated for potential differential Th17 cell reductions in ACPA positive and ACPA negative patients with RA, the reverse pattern was seen. More specifically, ACPA (+) patients with RA (n = 11) displayed a significant reduction of peripheral Th17 cells (3.06% ± 0.55 % at week 0 vs. 1.38% ±0.25 % at week 8 following RTX vs.1.14% ± 0.24% cells at week 16, p = 0.0007) following B cell depletion. Representative experiments from one ACPA (+) patient with RA is shown in Fig. 3A-C and results from the subgroup of ACPA (+) patients are collectively depicted in Fig. 3D.

Treatment with RTX reduces circulating sTh17 cells in patients with RA

In some (n = 6) patients with RA initiating treatment with RTX we additionally evaluated for potential changes of a subpopulation of surface Th17-expressing (sTh17) T cells, which express IL-17A on their surface membrane following ex vivo stimulation with PMA and ionomycin as described above. The subpopulation of sTh17 T cells was identified in our experiments flow-cytometrically, employing the same fluorochrome-conjugated mAb we used for identifying Th17 cells but without previous cell permeabilization. Surface Th17 T cells (defined as $CD4^+IL-23R^+$ surface-$IL-17A^+$ cells) were also significantly reduced following B cell depletion treatment (Fig. 4A). More specifically, sTh17 T cell percentages were 0.88% ± 0.15 % at week 0 vs. 0.49% ± 0.18% at week 8; this reduction persisted later on (0.39 ± 0.10% at week 16, p = 0.004). Therefore, our data suggest that B cell depletion treatment in patients with RA correlate with a significant decrease not only of "classic" Th17 T cells in the periphery but also of the ill-understood sTh17 T cells as well.

RTX treatment does not alter percentages of Treg T cells in the peripheral blood of patients with RA

Apart from the Th17 T cell subpopulation, we also evaluated in parallel, during the same experiments, the percentages of Treg T cells in the periphery of patients with RA initiating B cell depletion therapy. In our experiments, Treg were defined by the phenotype $CD4^+CD25^{high}FoxP3^+$ T cells. Our data suggest that percentages of Treg T cells in 16 patients with RA were not significantly altered following RTX treatment initiation. More specifically, Treg percentages were: 0.78% ± 0.13% at week 0, 0.85% ± 0.25% at week 8 and 0.99% ± 0.18% at week 16, p: NS). Additional analyses disclosed that Treg T cell percentages were not significantly different in responders vs. non-responders, or in RF (+) vs. RF (-) patients at weeks 8 and/or 16 following B cell depletion treatment.

Therefore, our data suggest that B cell depletion treatment is associated with a significant decrease of circulating Th17 T cells in patients with RA. This decrease might be more pronounced in responders vs. non-responders and in ACPA (+) patients. However, in contrast to Th17 cells, Treg T cells remained unaffected; the latter finding of our experiments confirms previously published data [13].

Treatment with the T cell-targeting agent abatacept does not alter the percentages of circulating Th17 and Treg cells in patients with RA

To address the question whether RTX-induced effects on T cell subpopulations are RTX-specific, we analyzed an additional number of patients (n = 11) with RA initiating treatment with the T cell-targeting agent ABA (biologic disease controls). Th17 and Treg T cell subpopulations were analyzed at baseline (week 0) and week 8. We employed 3-colour flow cytometry to evaluate percentages of Th17 and Tregs as described in Methods. Percentages of circulating Th17 T cells did not significantly change following ABA treatment when compared to baseline values. More specifically, Th17 cells were: 2.02 % ± 0.49% vs. 1.54% ± 0.34% at weeks 0 and 8, respectively (p = NS). Likewise, Treg percentages remained similar before and 8 weeks after ABA treatment initiation (mean ± SD: 1.83% ± 1.31% vs. 1.47% ± 1.31% at weeks 0 and 8, respectively, p = NS). Therefore, our data suggest that RTX-mediated decreases of Th17 cells are not only T cell subpopulation- but also biological drug-specific.

Patients with RA on standard treatment have similar numbers of Th17 and Treg T cells over time

Because time, as well as changes in disease activity could be responsible for alterations in T cell subpopulation numbers, we evaluated percentages of circulating Th17 and Treg T cells in patients with RA already under other than RTX or ABA "standard" treatment regimes. Eleven patients (disease controls) with RA receiving standard treatment [synthetic DMARDs ± steroids, (n = 7) and TNF-alpha blockers ± DMARDs ± steroids, (n = 4)] were analyzed. Percentages of circulating Th17 as well as Treg T cells were not different in this disease-control group. More specifically, percentages of Th17 T cells were: (mean ± SEM: 2.38% ± 0.60% vs 2.66% ± 0.71% at time points 1 and 2, respectively, p = NS). Percentages of Treg T cells were: (mean ± SEM: 0.69% ± 0.14%, vs. 0.58% ± 0.17% at time points 1 and 2, respectively, p = NS). Both Th17 and Treg cells subpopulations did not change significantly at two different time points, despite concurrent changes in their disease activity (evaluated with the DAS28 scoring system).

Therefore, our data suggest that time on its own, and/or changes in disease activity are not sufficient to alter percentages of circulating Th17 and Treg cells in patients with RA.

Treatment with B-cell depletion of patients with other-than-RA autoimmune diseases has no effect(s) on the percentages of circulating Th17 cells

To further investigate if RTX provokes changes on Th17 and Tregs in patients with other autoimmune diseases, we also evaluated T cells subpopulations at week 0 before treatment and at week 8 of treatment with RTX in 8 women with rheumatic autoimmune diseases other than RA such as SLE (n = 5), polymyositis (n = 2) and Sjogren's syndrome (n = 1). Circulating Th17 cells in patients with these diseases were not altered after RTX administration as in patients with RA (2.48% ± 0.74% at week 0 vs 2.46% ± 0.52% at week 8, p:NS). In contrast, peripheral Tregs of these patients decreased significantly after RTX administration (0.75% ± 0.16% at week 0 vs. 0.43% ± 0.16% at week 8, p = 0.033). Therefore, our data suggest that in contrast to patients with RA, Th17 cells are not reduced after RTX treatment in patients with other autoimmune diseases.

Healthy donors

Fourteen healthy donors were analyzed as well, for their Th17 and Treg T cell subpopulations. Mean ± SEM for Th17 cell percentages were: 1.19% ± 0.32% and for Treg were: 0.84% ± 0.12%, in the peripheral blood. All 38 patients of all 3 groups with RA were compared with healthy donors for both T cell subpopulations. Significant differences in the percentages of circulating Th17 cells were seen; percentages of Th17 cells were significantly higher in patients with RA [mean ± SE: 2.52% ± 0.31% (n = 36)] when compared to the Th17 T cells of healthy donors [1.19%± 0.32% (n = 14), p = 0.019]. Results are depicted in Fig. 4B. No significant differences were recorded in the percentages of Tregs of 38 patients with RA [1.06% ± 0.15%] vs. those of 14 healthy donors [0.84% ± 0.12% (n = 14), p = 0.42].

Discussion

There are several lines of experimental evidence that B cell depletion treatment may be related, directly or indirectly, with alterations of T cell subpopulations as well. Experimental animal studies have shown that B cell depletion in mice with collagen-induced arthritis, a T cell mediated disease, delayed arthritis [1]. In murine proteoglycan-induced arthritis, B cell-depleting therapy resulted in reduced CD4^{+} T cell reactivity [2], or in an increased number of Tregs and an enhanced suppressive capacity of Tregs [3]. In humans, B cell depleting therapies have also provoked alterations of T cells in patients with autoimmune diseases [4]. Patients with lupus nephritis receiving RTX displayed an enhanced expression of genes such as FoxP3 [5]. A study from our department suggested that patients with scleroderma developed reduced numbers of CD4^{+}IL4^{+} T cells following B cell depletion [6].

In patients with RA, RTX reduced the number of circulating CD4^{+} T cells [7] particularly after multiple RTX cycles [8]. Lymph node resident T cells decreased in patients with RA following RTX administration [9]. Peripheral CD20^{+} T cells were also depleted in patients with RA receiving RTX [10]. RTX induced the

expression of the previously decreased chemokine receptor CCR5 on the surface of CD4 + T cells in patients with RA [11].

Giollio et al studied 51 patients with RA treated with RTX for 4 years. The previously reduced numbers of peripheral natural killer cells in patients with RA were reportedly increased following RTX treatment during the 1st and even further during the 2nd year of treatment [12]. B cell depletion therapy did not alter the numbers of Tregs in the circulation of patients with RA [13]. RTX was reportedly capable of reducing Th17, but not Th1 and Treg T cells in the synovial tissue of patients with RA [14]. Our study analyzed peripheral Th17 cells and Tregs in patients with RA following RTX treatment.

We report herein that circulating Th17 cells were significantly decreased after RTX treatment. A potential downside of our study is the small number of patients enrolled as well as the relatively short follow-up period (16wk), that might not be entirely representative of the longer-lasting effects of RTX. The previously mentioned study of van de Veerdonk et al examined for potential changes of synovial but not circulating Th17 cells after RTX treatment in a small number of patients with RA (n = 12) after 12 weeks of treatment [14]. The authors reported that synovial Th17 cells (defined as CD3$^+$IL17A$^+$ T cells) were reduced following RTX administration and, at the functional level, the production of cytokines IL-17A, IL-21 and IL-22 was similarly reduced.

We also evaluated for potential changes in sTh17 cells in our patients with RA receiving RTX. Surface Th17 cells are a subset of "bona fide" Th17 cells expressing IL-17A on their surface membrane [37]. They represent a small subpopulation of the "classical phenotype" Th17 cells. Such sTh17 T cells express quantitatively less IL-17A on their surface compared to the IL-17A expressed intracellularly. Nevertheless, sTh17 cells are considered as potent effector cells because of the high expression of costimulatory, adhesion and activation molecules. In our study, evaluating a small number of patients, RTX significantly reduces this small but potentially "inflammatory" subset as well.

In order to address the question of specificity of our results assigned to RTX treatment, we chose to evaluate 3 different patient groups: apart from the RTX group, we included in our study 2 additional control populations. Biological disease-controls in our study were RA patients treated for the first time with the T cell-targeting agent ABA and disease-control group consisted of patients with RA already receiving standard treatment. Our data suggest that percentages of Th17 cells decrease in patients with RA treated with B cell depletion; nevertheless, we did not directly ask questions regarding their functional status or the production and/or secretion of relevant cytokines.

We further analyzed our results and report that responders had a more pronounced reduction of Th17 cells when compared to non-responders. In addition, our data suggest that ACPA (+) patients had a significantly greater reduction of Th17 cells, unlike ACPA (-) patients; such a differential response was also seen in RF (-) but not in RF (+) patients.

In contrast to our Th17 data, our experiments suggest that no changes in Treg numbers are seen following B cell depletion. Our Treg cells data are in agreement with previously published results. In their

uncontrolled study, Feuchtenberger et al. measured Tregs at 6 and 12 months after treatment with RTX in 17 patients with RA [13]; $CD4^+CD25^+FoxP3^+T$ cells were analyzed and were reportedly unchanged in numbers after RTX treatment. It has been suggested in other studies that Tregs in patients with RA may be enriched either in the periphery [15] or in the synovial fluid [17]. In contrast, other studies have reported diminished Treg numbers in the periphery of patients with early RA [16] and diminished expression of CTLA4 and hence decreased Treg suppressive capacity in patients with RA [18]. In our study, the comparison of Treg cell numbers between patients with RA and healthy volunteers revealed no differences.

The experimental design of our study clearly suggests that RTX-induced reductions of peripheral Th17 are RTX-specific. In contrast to B cell depletion, treatment with other biologicals, such as ABA did not change the T cell subpopulations that we analyzed. In addition, the disease itself, being a process with remissions and relapses, is not responsible for the changes of Th17 cells reported herein, as suggested by the comparisons with our disease-control group of patients, because neither Th17 nor Tregs were different in numbers over time in patients with RA already receiving standard treatment. Our data depict that such T cell subset percentages remained similar at 2 different time points, despite changes of the DAS28 scores of our patients, i.e despite changes in their disease activity and therefore of their inflammatory status.

Potential changes in T lymphocyte subpopulations have been evaluated in patients with RA being treated with other biological agents. For instance, Nakayamada et al. studied 108 patients with RA receiving for the first time TNF-α blockers (n = 42), abatacept (n = 40) and tocilizumab (TOCI) (n = 22) [19]. Peripheral T cells were examined at 24 weeks post treatment; TNF-α inhibitors were associated with increased Th17 cells. ABA reportedly significantly reduced Th17 and Tregs. TOCI treatment had no significant impact on numbers of the T cell subpopulations examined in that study.

Treatment with ABA in a study of Alvarez-Quiroga et al in 30 patients with RA revealed a decline of Treg number but, in contrast, an enhancement of their suppressive capacity at week 12 compared to baseline [20]. Adding to the confusion, the exact opposite findings came from another study. In that, Bonelli et al enrolled 15 patients with RA to receive ABA [21]. The study concluded that ABA augmented Treg numbers and inhibited activity of Tregs in patients with RA at weeks 2 and 4 after administration of ABA. Picchianti et al studied 25 patients with RA that received ABA due to unresponsiveness to TNF-alpha blockers [22]. The results suggested an improvement of suppressive function of Treg T cells although there were no significant changes in their numbers following ABA treatment. In another study of Pesce et al., TOCI was infused monthly in a rather small number of patients with RA (n = 8) [23]. T cells were evaluated before and every 8 weeks after treatment. Th17 were not significantly altered by week 24. Tregs and "Th1/Th17 cells" (a subpopulation commonly found in inflamed tissues of RA) were increased after treatment with TOCI. Samson et al also concluded that TOCI corrects the disturbed balance of the ratio Tregs/Th17 in patients with RA [24]. Results obtained from 15 patients revealed that TOCI decreased Th17 and increased Tregs after 2 months compared to baseline. The small number of patients (n = 15) and the early evaluation of the T cell subpopulations (at 2 months) could potentially be considered as

limitations of this study. Perhaps more importantly, one cannot aim towards the restoration of the imbalance between Tregs and Th17 (expressed as the Treg/Th17 ratio), since this a clearly arbitrary value.

Previous studies have implied the positive effect of TNF-alpha blockers on increasing and restoring Treg numbers and function in patients with RA [25]. Szalay et al studied 32 patients with early RA unresponsive to DMARDs that received TNF-α inhibitors [26]. Tregs were counted 4 and 8 weeks later and they were reportedly increased when compared to baseline values, whereas the number of peripheral Th17 cells was practically unchanged. However, the potential effects of TNF-α blockers on Treg and Th17 cells can be judged as controversial at best [27–32].

Our group of RTX-treated disease-control patients clearly displayed no changes in the number of Th17 cells. Tregs though were decreased after B-cell depletion. The effects of RTX on Tregs in patients with SLE have been previously reported. Tregs have been reportedly decreased in the periphery in patients with active SLE [33, 34]. Tregs were significantly increased after RTX treatment in patients with SLE [5, 35, 36]. These studies, in contrast to ours, included patients with lupus nephritis. In the study by Sfikakis et al, Tregs were evaluated by mRNA extraction and not by flow-cytometric phenotyping [5]. Other studies employed flow cytometry evaluations and analyzed Tregs either at months 1, 2 and 3 of treatment with B cell depletion [35] or at certain time points after treatment with a special emphasis on time of B-cell depletion and B cell repopulation [36]. In our study we enrolled in our RTX-treated patients only 5 patients with SLE; none had nephritis. The comparative analysis was performed at week 8 after RTX treatment. Therefore, previous studies of RTX effects on Tregs in other autoimmune diseases are not directly comparable with our study. A limitation of all studies mentioned above, including our own, is that studies are not longitudinal and analyze subgroups of patients who share certain characteristics.

To summarize all the above, it is evident that the numbers of patients with RA in each study, the time intervals the T cell subpopulations are evaluated after the administration of a biologic agent and even the very definition of the phenotype of the T cell subpopulations of interest, display a great heterogeneity among different studies resulting perhaps in heterogeneous and perhaps even contrasting results.

Conclusions

Therefore, in this communication we report that B cell depletion, an established treatment approach for patients with RA, might alter the numbers of the circulating Th17 T cell subpopulation in a biological agent-specific, inflammatory status-independent, and autoimmune disease-specific manner. Because of their inflammatory function, we propose that such decreases in Th17 numbers might potentially correlate with decreased inflammatory function in patients with RA, although not directly addressed herein.

Abbreviations

RTX : Rituximab

RA : Rheumatoid arthritis

SLE : Systemic lupus Erythematosus

ABA : abatacept

DMARDs : disease modifying anti rheumatic drugs

PBMCs : peripheral blood mononuclear cells

PMA: phorbol myristate acetate

TOCI : tocilizumab

Th17 cells : T helper 17 cells

Treg cells : regulatory T cells

RF: rheumatoid factor

ACPA : anti-citrullinated protein antibodies

TNF : Tumor necrosis factor

Declarations

- Ethical Approval and Consent to participate :

Written informed consent was obtained from all participating individuals and the study protocol was approved by the local (Patras University Hospital) Ethics Committee

- Consent for publication :

Written informed consent was obtained from all participating individuals. **Availability of supporting data :**

There are no supporting data

- Competing interests :

There are no competing interests. Both authors have nothing to declare.

- Funding :

None

- Authors' contributions :

SNC Liossis designed the study and wrote the paper. CA Bounia performed all the experiments, analyzed data and wrote the paper.

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Figures
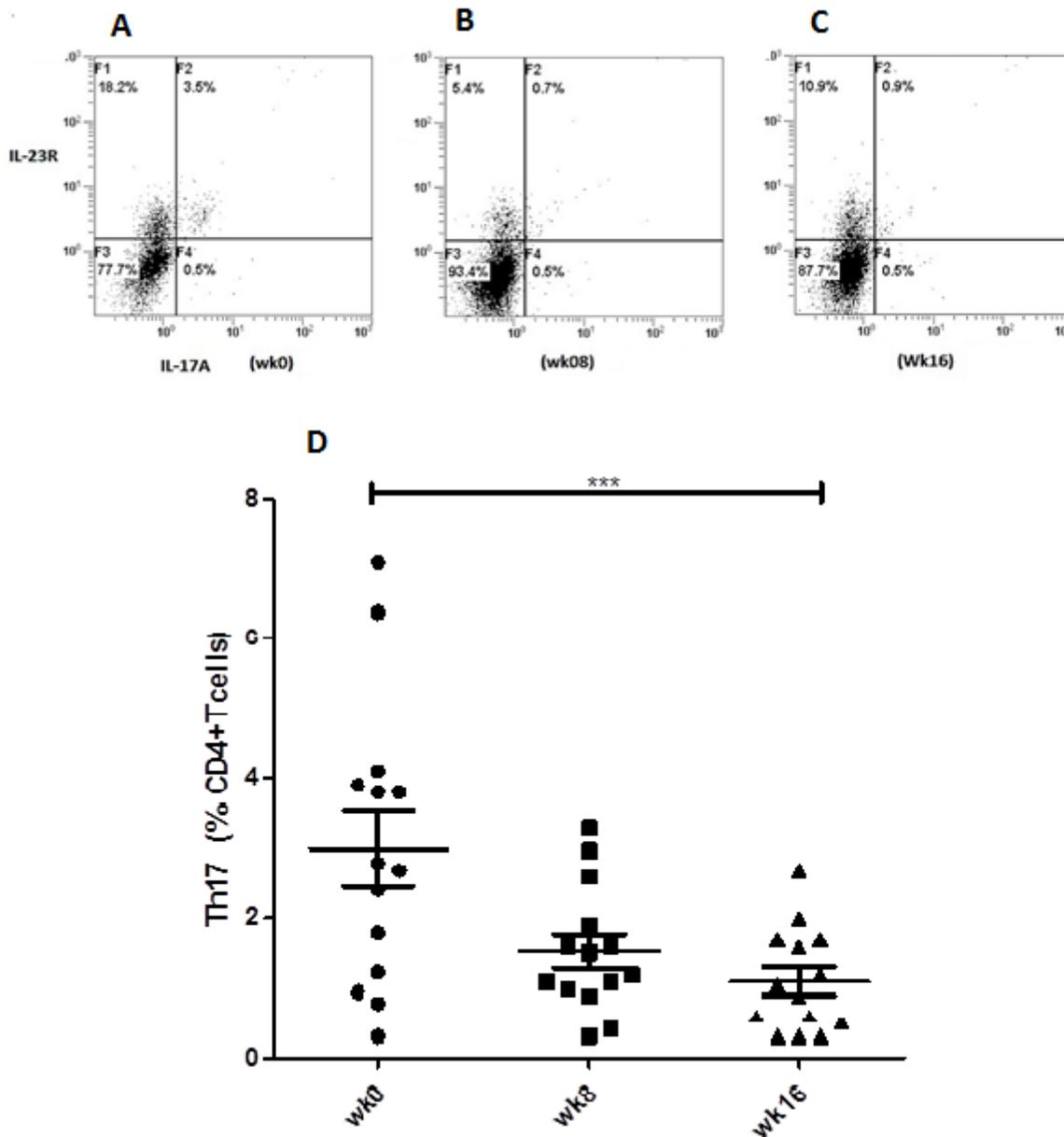
Figure 1

Circulating Th17 cells in patients with RA receiving RTX. Gating on CD4+ cells has been performed. Th17 cells are IL23R+IL17A+ and in all experiments are depicted as % of CD4+ T cells. Th17 cells from a representative patient with RA treated with RTX at baseline (week 0) (A), at week 8 after RTX (B) and at week 16 after RTX (C) are presented. The significant decrease of Th17 cells following RTX administration in the group of patients with RA (n=14) is depicted in D.
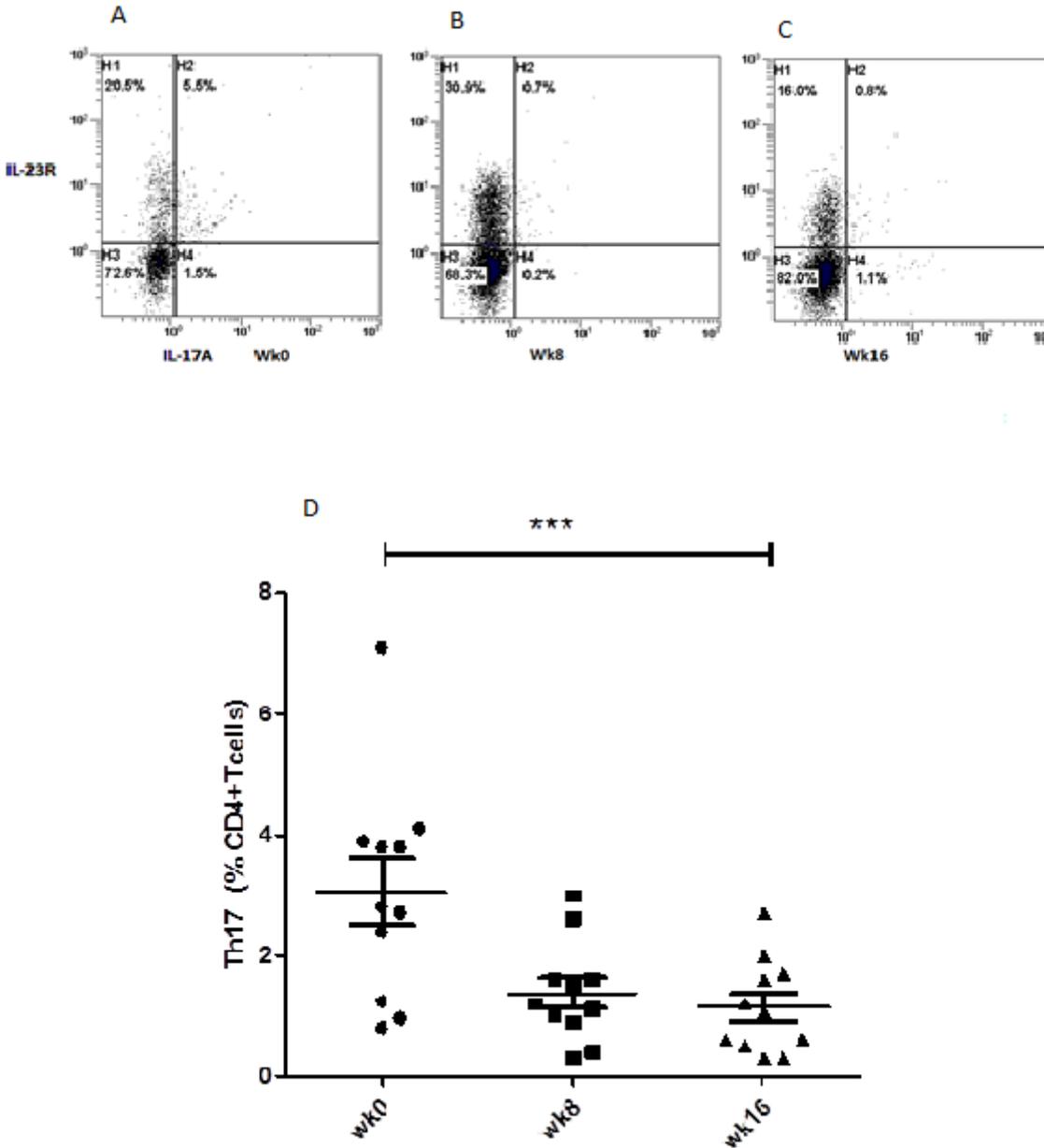

Figure 2

Th17 cells in the circulation of patients with RA achieving a clinical remission following treatment with RTX. Th17 cells from one representative patient are presented at baseline (week 0) (A), week 8 (B) and

week 16 (C) after RTX treatment. The significant decrease of peripheral Th17 cells following RTX administration in the group of patients with RA achieving a remission (n=8) is depicted in D.
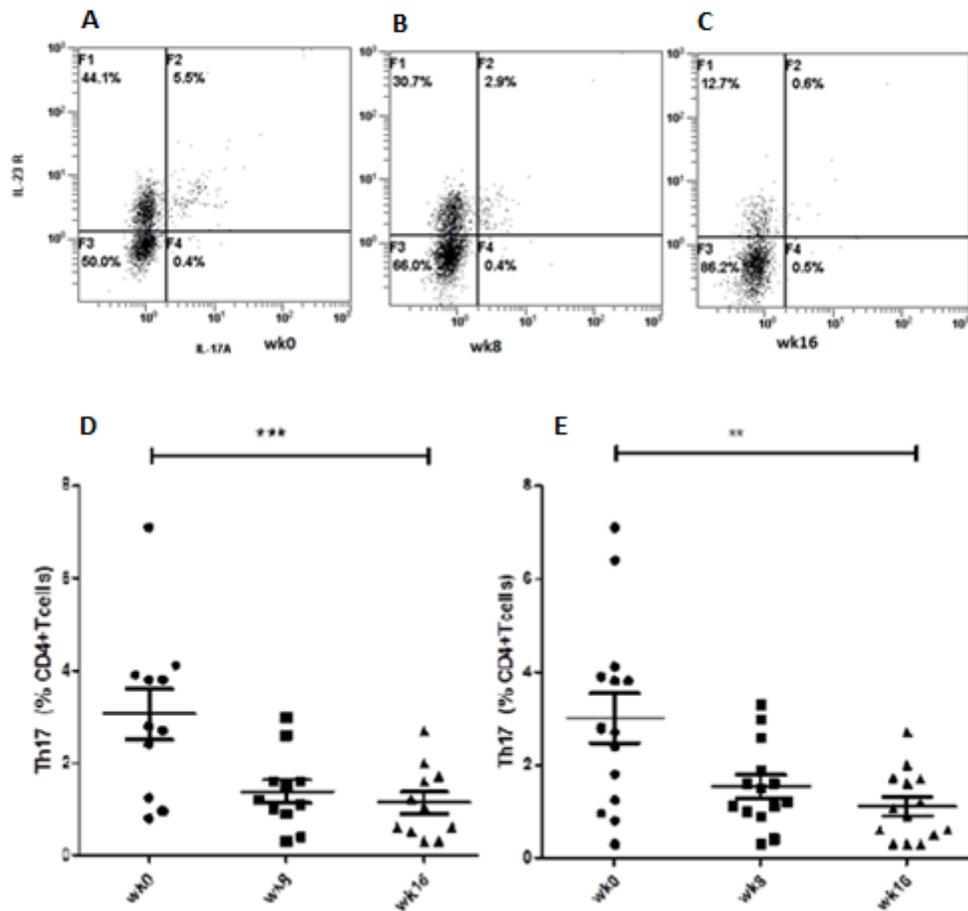

Figure 3

Th17 cells in the circulation of patients with RA following treatment with RTX, analyzed according to ACPA and RF status. Th17 cells from one representative ACPA (+) patient are presented at baseline (week 0) (A), week 8 (B) and week 16 (C) after RTX treatment. The significant decrease of peripheral Th17 cells following RTX administration in the group of ACPA (+) patients with RA (n=11) is depicted in D. The significant decrease of circulating Th17 cells in RF (-) patients with RA treated with RTX (n=8) is shown in E.
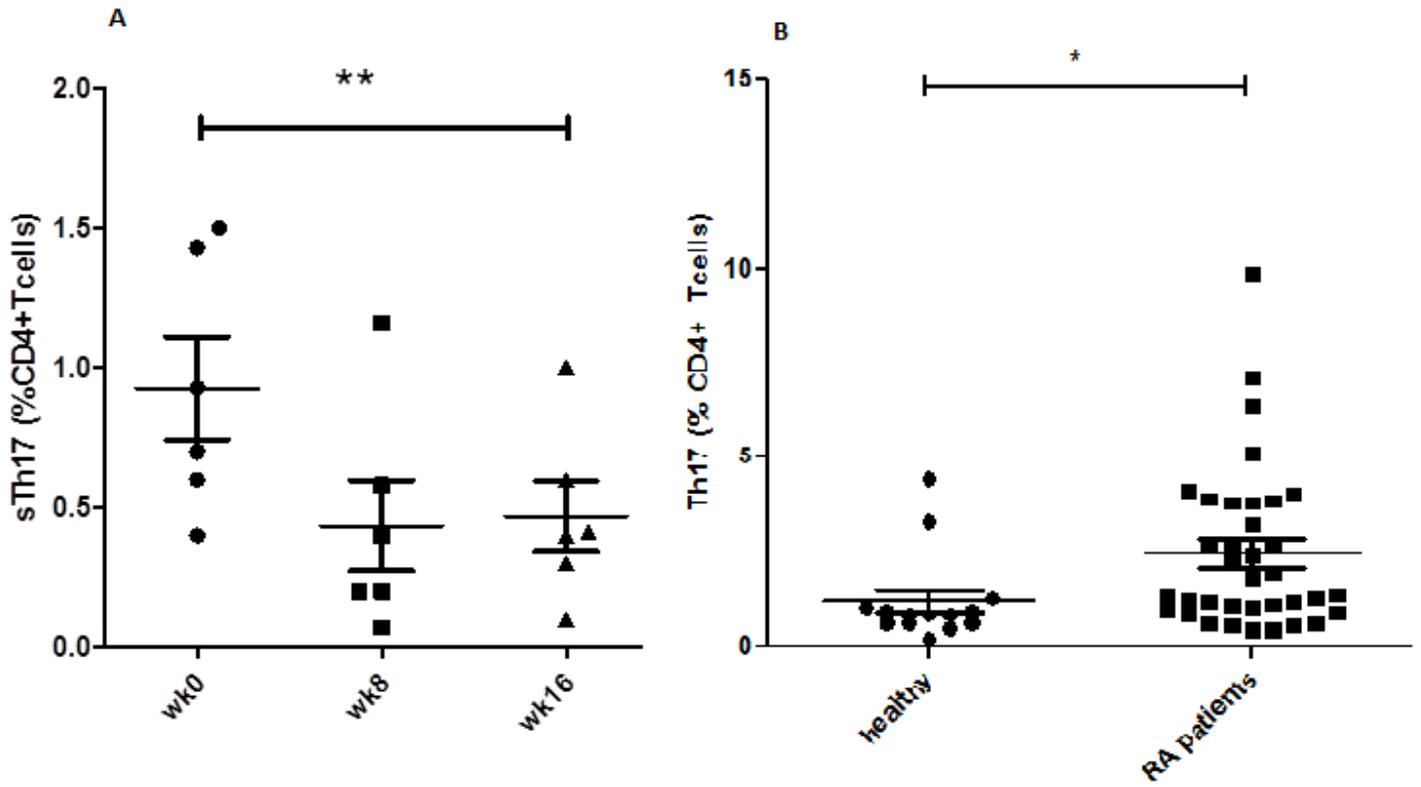
Figure 4

Treatment with RTX significantly reduced circulating sTh17 cells in patients with RA (n=6) (A). Patients with RA (n=36) have a significantly higher percentage of circulating Th17 cells when compared to healthy donors (n=14) (B).