Different Effects of Tumor Necrosis Factor and IL-17A Blockades on T Cell Function of Psoriatic Arthritis Patients

Smadar Gertel[1], A Polachek[1], Victoria Furer[1], David Levartovsky[1], Haya Sidis[1], Sara Pel[1], Daphna Paran[1], and Ori Elkayam[1]

[1]Tel Aviv Sourasky Medical Center

June 3, 2020

Abstract

Biologics have revolutionized the treatment of inflammatory arthritis, but their impact on T cell function is unknown. We evaluated the effect of tumor necrosis factor-alpha (TNF-α), interleukin-17A (IL-17A), and IL-6 receptor (IL-6R) blockers on T cell function in psoriatic arthritis (PsA) patients. Peripheral blood mononuclear cells (PBMCs) from PsA patients (n=111) and healthy controls (n=20) were co-cultured with adalimumab (ADA), ixekizumab (IXE), tocilizumab (TCZ), or medium alone for 5 days. T cell activation and proliferation were determined by flow cytometry and cytokines in supernatants were measured by ELISA. Activated $CD4^+CD25^+$ T cells were significantly down-regulated by ADA in naïve, conventional disease-modifying anti-rheumatic drug (cDMARD)- and biologic-treated PsA patients compared to medium ($p < 0.04$, $p < 0.01$, respectively), IXE, and TCZ. In healthy, ADA reduced the activated $CD4^+CD25^+$ T cells proportion but non-significantly as compared to the other groups. Inhibition of PsA patients derived lymphocytes proliferation by the biologics was determined in response to phytohemagglutinin (PHA). The strongest ability to suppress the extent of PHA-induced proliferation was exerted by ADA ($p < 0.01$) compared to IXE and TCZ. IL-1β, IL-17A, and MMP-3 levels were down-regulated by ADA compared to medium ($p < 0.02$, $p < 0.0001$, $p < 0.002$, respectively). IXE reduced IL-17A ($p < 0.0001$) but not IL-1β or MMP-3 levels. TNF and IL-17A blockades are suitable for PsA treatment, but exhibit different activity on T cells. Moreover, the study reveals part of the mechanism exerted by ADA and provides a possible explanation for TCZ inefficacy in PsA.

Different Effects of Tumor Necrosis Factor and IL-17A Blockades on T Cell Function of Psoriatic Arthritis Patients

Smadar Gertel*, Ari Polachek*, Victoria Furer, David Levartovsky, Haya Sidis, Sara Pel, Daphna Paran and Ori Elkayam

From the Department of Rheumatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, affiliated to the Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

* Equal contribution

Address correspondence to: Dr. Smadar Gertel

Department of Rheumatology Tel Aviv Sourasky Medical Center 6 Weizman Street Tel Aviv, 6423906, Israel Tel: 972-52-4543949 E-mail: smadarlg@gmail.com

Summary

Biologics have revolutionized the treatment of inflammatory arthritis, but their impact on T cell function is unknown. We evaluated the effect of tumor necrosis factor-alpha (TNF-α), interleukin-17A (IL-17A), and IL-6 receptor (IL-6R) blockers on T cell function in psoriatic arthritis (PsA) patients. Peripheral blood mononuclear cells (PBMCs) from PsA patients (n=111) and healthy controls (n=20) were co-cultured with

adalimumab (ADA), ixekizumab (IXE), tocilizumab (TCZ), or medium alone for 5 days. T cell activation and proliferation were determined by flow cytometry and cytokines in supernatants were measured by ELISA.

Activated $CD4^+CD25^+$ T cells were significantly down-regulated by ADA in naïve, conventional disease-modifying anti-rheumatic drug (cDMARD)- and biologic-treated PsA patients compared to medium ($p < 0.04$, $p< 0.01$, respectively), IXE, and TCZ. In healthy, ADA reduced the activated $CD4^+CD25^+$ T cells proportion but non-significantly as compared to the other groups. Inhibition of PsA patients derived lymphocytes proliferation by the biologics was determined in response to phytohemagglutinin (PHA). The strongest ability to suppress the extent of PHA-induced proliferation was exerted by ADA ($p < 0.01$) compared to IXE and TCZ. IL-1β, IL-17A, and MMP-3 levels were down-regulated by ADA compared to medium ($p < 0.02$, $p < 0.0001$, $p< 0.002$, respectively). IXE reduced IL-17A ($p< 0.0001$) but not IL-1β or MMP-3 levels. TNF and IL-17A blockades are suitable for PsA treatment, but exhibit different activity on T cells. Moreover, the study reveals part of the mechanism exerted by ADA and provides a possible explanation for TCZ inefficacy in PsA.

Introduction

Psoriatic arthritis (PsA) is a chronic form of arthritis characterized by inflammation at tendon or ligament insertion sites into bone (enthesitis) and by synovitis, eventually leading to joint erosions and new bone formation. Up to 30% of patients with psoriasis develop PsA. The prevalence of PsA in the general population is estimated between 0.5 to 1%. PsA shares genetic and clinical features with other forms of seronegative spondyloarthritis (1, 2). Psoriatic skin and synovial tissue contains activated T cell infiltrates associated with skin and joint damage (3). T cells are major drivers of arthritis mediated through activation of pathogenic cascade that results in production of pro-inflammatory cytokines, such as interleukin 17 (IL-17) and tumor necrosis factor alpha (TNF-α). Type 17 helper (Th17) T cells secrete IL-17, a pro-inflammatory cytokine associated with joint destructive activity. IL-17 stimulates TNF production by macrophages and induces synovial fibroblast activation, osteoclastogenesis, and cartilage degradation (4-6). Both interleukin-1 (IL-1) family and IL-17 cytokines participate in skin inflammation and high expression of IL-1 family members contributes to Th17 development (7). Metalloproteinase-3 (MMP-3) are identified as a biomarker in PsA (8), and IL-17 was reported to induce MMP-3 production by synovial cells of PsA patients (9). Taken together, production of the latter cytokines has a dominant pathogenic role in psoriatic plaques.

Biologic agents have revolutionized the treatment of inflammatory arthritis. Agents targeting pro-inflammatory cytokines are able to reverse the unbalanced inflammatory immune response and alleviate disease symptoms. In PsA, TNF inhibitors are effective in reducing skin lesions and musculoskeletal symptoms (10). Yet, not all PsA patients respond to TNF blockade (11). Newer therapies targeting downstream, disease-specific cytokines associated with the IL-17/IL-23 axis have been developed and demonstrated a significant clinical efficacy in PsA (12). Anti-IL-17 agents have been approved for the treatment of PsA, exhibiting promising results in reducing PsA disease activity (13).

Increased production of IL-6 is one of the pathogenic pathways in both PsA and rheumatoid arthritis (RA) (14). Anti-IL-6 receptor (R) antibodies have been approved for the management of RA (15). However, treatment with anti-IL-6 and anti-IL-6R, such as clazakizumab and tocilizumab (TCZ), has shown disappointing results in PsA (16, 17). Moreover, deficiency of TNF, but not IL-6, suppressed the development of arthritis and skin inflammation (18) in the arthritis and dermatitis IL-1R antagonist–deficient mice model which resembles human psoriasis (19), suggesting that only TNF had a pivotal role in the pathogenesis of PsA in this animal model.

Both TNF and IL-17A inhibitors ameliorate the symptoms and reduce disease activity in PsA. However, their precise mechanisms of action are not completely understood.

Given that pro-inflammatory cytokines promote effector T cell function and that activated T cells are highly involved in PsA pathogenesis (20), biologic agents targeting different immune pathways might differently affect T cell activity. We aimed to study the mechanism of action of biologic agents on functionality of T cells in more details. Therefore, we selected the most prominent T cell activation marker, CD25, the alpha

chain of IL-2R, that is constitutively expressed on lymphocytes, such as regulatory and resting memory T cells. CD25 is upregulated within 24 hours of stimulation of the TCR/CD3 complex and remains elevated for a few days (21).

We evaluated the differential impact of TNF, IL-17A and IL-6R blockade on the immune response by analyzing their inhibitory effect on T cell activation and proliferation and on cytokine secretion in vitro. The activity of the biologics was assessed in peripheral blood mononuclear cells (PBMCs) derived from PsA patients that were classified as Naïve, conventional disease-modifying anti-rheumatic drug (cDMARD)- or biologic-treated PsA patients and in healthy controls.

Material and methods

Study participants. Adult (≥18 yrs) PsA patients diagnosed according the Classification Criteria for Psoriatic Arthritis (CASPAR) criteria (22) attending the Rheumatology Department of Sourasky Medical Center, Tel-Aviv, Israel were eligible to participate in the study. A total of 111 PsA patients either Naive, cDMARD-, or biologic-treated were consecutively enrolled. The control group included 20 healthy subjects. The study was conducted between 2018-2020 according to the guidelines of the Declaration of Helsinki and with the approval of the institute's review board (0182-18-TLV). All patients signed an informed consent upon the enrollment into the study and donated a single blood sample that was used for the various experiments. Clinical and medical information was collected prospectively (Table 1).

In vitro cell culture. PBMCs were isolated from heparinized venous blood by density gradient centrifugation using Lymphoprep (Axis- Shield, Oslo, Norway). Extracted PBMCs were cultured at a density of $1.5x10^6$ cells/ml in a 48-well plate in RPMI 1640 medium containing 10% fetal calf serum supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), 2 mmol/L L-glutamine, and 50 µM 2β-mercaptoethanol. Cells were incubated for 5 days at 37°C with one of the following biologic agents: TNF (adalimumab, ADA), IL-17A (ixekizumab, IXE), and IL-6R (tocilizumab, TCZ) blockers at a concentration of 10 µg/ml (reflecting the biologic agents concentrations in human serum) or medium alone as control.

Analysis of activated $CD4^+CD25^+$ T cells. After 5 days of incubation, the cells were collected and stained with the monoclonal antibodies, anti-CD4-APC-eFluor 780 (eBioscience, 47-0049-42) and anti-CD25-PE (eBioscience, 12-0259-42), for 30 min at room temperature. The cells were acquired by a fluorescence-activated cell sorter (FACS) Canto II flow cytometer (BD Biosciences). A total of 60000 events were collected from each sample. The analysis was performed with FlowJo analysis software, and the lymphocytes were identified on the basis of forward and side-scatter after gating for $CD4^+CD25^+$ T cells had been performed.

Cell proliferation. Cell proliferation was assessed by means of carboxyfluorescein succinimidyl ester (CFSE) dilution after each cell division. Briefly, PBMCs were pretreated with CFSE before the start of the co-culture. The cells were suspended in phosphate buffered solution (PBS) with 5 µM CFSE, incubated for 15 min at 37^0C and then washed twice with PBS-2% fetal calf serum. Cells were resuspended in complete RPMI medium and cultured with phytohemagglutinin (PHA) at a final concentration of 5 µg/ml in the presence of the three biologic agents. Controls consisted of PHA-stimulated PBMCs without biologic agents (representing reference proliferation) and PBMCs without PHA (no proliferation). Cells were harvested after 5 incubation days and analyzed by flow cytometry, a total of 60000 were acquired from each sample.

ELISA. Levels of IL-1β, IL-17A, and MMP-3 were measured in cell culture supernatants with human IL-1β, IL-17A, and MMP-3 ELISA kit DuoSet ELISA (R&D Systems). The relevant standard, capture, and detection antibodies were used according to the manufacturer's instructions. Microwell absorbance was read at 450 nm with an ELISA autoanalyzer (BioTek 800 TS Absorbance Reader, USA). Concentrations of the analytes were determined based on the standard curve included on each plate.

Statistical analysis. Statistical analyses for the effect of biologics on activated $CD4^+CD25^+$ T cells and CFSE-proliferation assays were performed using One-way ANOVA. For analysis of levels of secreted cytokines

by ELISA, 2-tailed Mann-Whitney U test was used. Analyses were undertaken using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA). p values less than or equal to 0.05 were considered as significant.

Results

The effect of TNF, IL-17A and IL-6R blockers on the proportion of activated $CD4^+CD25^+$ T cells in culture of PBMCs from PsA patients and healthy controls.

We evaluated the effect of TNF, IL-17A, and IL-6R blockade on the proportion of activated $CD4^+CD25^+$ T cells in PBMCs of PsA patients. First, we analyzed the effect of the biologic agents in culture of PBMCs derived from Naïve and cDMARD-treated PsA patients on the percentage of activated $CD4^+CD25^+$ T cells (n = 43) (Figure 1). Addition of ADA to the culture significantly reduced the percentage of activated $CD4^+CD25^+$ T cells compared to medium (3.6 ± 0.3 vs 5.0 ± 0.4, $p < 0.04$), representing a 30% decrease compared to medium, while treatment with IXE or TCZ (4.9 ± 0.4 and 4.8±0.4, respectively) did not significantly affect the percentage of activated $CD4^+CD25^+$ T cells (Figure 1B and C). There was also a significant difference between the effect of ADA on the percentage of activated $CD4^+CD25^+$ T cells in comparison with IXE ($p < 0.04$).

We next examined the potential effect of the biologics on the percentage of activated $CD4^+CD25^+$ T cells derived from biologic-treated PsA patients (n=58). As shown in Figure 2B and C, there was a consistently significant reduction in the percentage of activated $CD4^+CD25^+$ T cells in culture in samples of cells incubated with ADA compared to medium (3.50 ± 0.3 vs 5.1 ± 0.4, $p < 0.01$, representing a 32% decrease). In comparison, IXE and TCZ (4.8 ± 0.4 and 4.7 ± 0.4, respectively) did not significantly change the percentage of activated $CD4^+CD25^+$ T cells compared to medium. Furthermore, ADA significantly down-regulated the percentage of activated $CD4^+CD25^+$ T cells compared to IXE ($p < 0.04$). We further evaluated the effect of the tested biologics on the percentages of activated $CD4^+CD25^+$ T cells in culture of PBMCs derived from healthy controls (n=20). Figure 3, summarize the results comparing the effect of the biologics in the three groups (Naïve and cDMARD-, biologic- treated PsA patients and healthy controls). Basal percentages of activated $CD4^+CD25^+$ T cells after incubation with medium alone were not significantly different between Naïve and cDMARD-, biologic-treated PsA patients (4.9 ± 0.4 and 5.1 ± 0.4, respectively) and healthy (5.5 ± 0.5) ($p> 0.05$). As can be seen in the healthy control group, ADA reduced only slightly the percentage of activated $CD4^+CD25^+$ T cells as compared to medium, IXE and TCZ (4.9 ± 0.6 vs 5.5 ± 0.5, 5.5 ± 0.6 and 5.5 ± 0.7, respectively), however the differences were not statistically significant. Furthermore, IXE and TCZ had no significant effect on the percentage of activated $CD4^+CD25^+$ T cells as compared to medium in PBMCs from healthy controls.

Inhibition of lymphocyte proliferation by the different biologic agents.

Analysis of PHA-stimulated proliferation of lymphocyte upon addition of the experimental biologic agents is shown in Figure 4. In culture of PBMCs derived from PsA patients (n=17) after 5 days incubation without PHA, the average extent of proliferation (%) was 5.6 ± 0.7, when PBMCs were stimulated with PHA alone, proliferation rate was 28.5 ± 3.0. In parallel to the effect of ADA on activated $CD4^+CD25^+$ T cells, ADA significantly reduced the PHA-induced proliferation of lymphocytes 16.8 ± 2.0,$p < 0.01$, while IXE 24.9 ± 2.9 and TCZ 22.3 ± 2.4 had no significant impact on PHA-induced proliferation.

Blockade of TNF, IL-17A and IL-6R differentially affects inflammatory mediator release from PBMCs of PsA patients.

The levels of soluble IL-17A, IL-1β, and MMP-3 were measured in culture media of cells incubated with the selected biologic agents. Culture supernatants were collected from the experiments performed to analyse T cell activation in PBMCs derived from PsA patients (n = 14-15) as described above, after 5 days of incubation as shown in Figure 5, the level of IL-17A (Figure 5A) in the culture of cells with medium alone was 19.3 ± 3.2 pg/ml. Both ADA and IXE significantly reduced the IL-17A level compared to the control medium 9.4 ± 0.5 and 8.5 ± 0.6 vs 19.3 ± 3.2 pg/ml, representing decreases of 52% and 56%, respectively,p

< 0.0001). In contrast, TCZ did not reduce IL-17A levels 23.2 ± 7.2 pg/ml compared to the control medium. The IL-1β level was significantly decreased by ADA compared to the control medium 3.9 ± 0.3 pg/ml vs 5.1 ± 0.6 pg/ml, $p < 0.02$, but not by either IXE 5.1 ± 1.0 pg/ml or TCZ 6.6 ± 1.1 pg/ml (Figure 5B). Similarly, MMP-3 levels were reduced by ADA compared to the control medium 26.0 ± 1.0 pg/ml vs 33.4 ± 2.0 pg/ml, $p < 0.002$, whereas neither IXE 30.2 ± 1.8 pg/ml nor TCZ 30.6 ± 2.4 pg/ml could affect significantly MMP-3 levels (Figure 5C). Of note, the mean levels of IL-1β and IL-17A in the supernatants of cells incubated with TCZ were even higher than in the medium control although the difference was not significant.

Discussion

The results of this study on 111 patients with PsA clearly demonstrate that anti-TNF but not anti-IL-17A or anti-IL-6R down-regulated T cell activation and proliferation in vitro. Furthermore, the inhibitory effect of anti-TNF on T cell activation was demonstrated in Naïve/ cDMARD- as well as biologic-treated PsA patients. TNF inhibition markedly suppressed IL-1β, IL-17A, and MMP-3 secretion, whereas IL-17A inhibition suppressed only IL-17A secretion. In contrast, IL-6R blockade failed to inhibit the secretion of any of the inflammatory mediators. Taken together, these results indicate that the therapeutic effect of anti-TNF may be mediated, at least in part, by its direct effects on the function of T cells and their ability to secret pro-inflammatory molecules.

Our data are supported by those of Dahlén*et al* . (23) who demonstrated the ability of infliximab to reduce T cell activation following stimulation with a-CD3 and a-CD28 antibodies, as measured in vitro by reduced percentage of $CD4^+CD25^+$ and $CD8^+CD25^+$ T cells, proliferation and inflammatory cytokine secretion in PBMCs derived from ulcerative colitis patients. Our findings demonstrated that even without external simulation ADA reduced T cell activation in vitro. Moreover, T cell activation was regulated by anti-TNFs as demonstrated both by ADA and infliximab, support the notion that this activity can be promoted by anti-TNFs' blockade in general and not only by ADA.

ADA reduced substantially and significantly the level of activated $CD4^+CD25^+$ T cells in PsA patients, while in healthy controls ADA reduced these cells only slightly and non-significantly. Similarly a study by Coury *et al* . (24) tested in vitro effect of anti-TNFs (mediated by infliximab and etanercept) on T cells apoptosis in PBMCs isolated form RA patients and healthy controls. T cell apoptosis was reduced more in RA patients than in healthy controls. In this respect, in vitro culture conditions to test the effect of biologics were with no additional stimulation to determine solely the biologics activity.

Activated T cells are involved in the pathogenesis of PsA and several reports have shown increased activated T cells with Th1 and Th17 phenotypes as well as an increased proportion of activated $CD8^+$ T cells in the circulation of PsA patients (20, 25, 26). These studies implied that selective inhibition of pro-inflammatory T cell function may be beneficial in PsA.

Only few reports demonstrated T cell subset modulation during long-term anti-TNF therapy, mainly in patients with RA and ankylosing spondylitis (AS). Long-term treatment with ADA consistently down-regulated the $CD4^+CD25^+$ T cells in patients with AS (27). Similarly, Dulic et al. (28) studied RA patients treated with anti-TNF therapy revealing a decrease in activated $CD4^+CD25^+$ T cells. This effect was more evident among anti-TNF responders than in non-responders as compared to naïve untreated RA patients and to healthy controls. On the other hand, IL-6R therapy did not change the activated T cell fraction in this RA cohort.

An additional activation marker, CD69, identifies a very early activation stage of T cells was found to be a useful predictive marker for response to anti-TNF in RA. Low percentage of $CD4^+CD69^+$ T cells in peripheral blood of RA patients ($< 2.43\%$) predicts a good response to anti-TNF (28). Moreover, the prevalence of $CD4^+CD69^+$ and $CD8^+CD69^+$ T cells was significantly higher in RA patients who had incomplete response to several anti-TNFs compared to those who failed only one anti-TNF inhibitor (28). Those results are consistent with our current findings by demonstrating that in vitro anti-TNF inhibitors acted to reduce T cell activation.

Activated $CD4^+CD25^+$ T cells can be discriminated based on the expression of additional markers, such as transcription factor FoxP3 (29) and CD127, that are used to define T regulatory cells (Tregs). Huang et al. (30) showed that anti-TNF therapy suppressed activated/effector T cells with a concomitant increase in the Treg cell population in treated RA patients. Indeed, restoration of Tregs by anti-TNF treatment in RA was demonstrated in a number of reports (31, 32). Several mechanisms were proposed for the potential of anti-TNF to induce Tregs, one of which was the attenuation of accelerated apoptosis in Tregs following anti-TNF therapy (33). In addition, TNF inhibition was able to induce the expression of anti-inflammatory IL-10 in T cells following in vitro stimulation in a dose- and time-dependent manner, while the blockading of IL-17, IFN-γ, IL-6R, or CD80/CD86-mediated co-stimulation did not significantly regulate IL-10 expression within $CD4^+$ or $CD8^+$ T cell subpopulations (34).

In the present in vitro study, co-culture of PBMCs with a TNF inhibitor reversed aberrant T cell functions and reduced inflammatory cytokine release. Similarly, others have shown that anti-TNF therapy downregulated the production of a large range of inflammatory cytokines/chemokines, including IL-6, IL1β, IL-8, RANTES, and MCP-1 which are associated with decreased joint inflammation (35, 36). Interestingly, although IL-17A blockers, such as secukinumab and ixekizumab (IXE), are efficacious in PsA therapy and achieve similar proportions of American College of Rheumatism response as those of TNF blockers in clinical studies, their effect on T cell proliferation and inflammatory markers differs. Except for a reduction in IL-17A in cell culture supernatants, IL-17A blockade did not alter T cell activation and proliferation and failed to reduce either IL-1β or MMP-3 secretion. Since IXE did not block the latter cytokines, it can by speculated that reduced IL-17A levels in the ELISA test could derived from interaction between residual IXE and soluble IL-17A in supernatants and not by blockade of endogenous IL-17A secretion. Nevertheless, both IL-17A and IL-17F play a role in chronic tissue inflammation in PsA. Dual neutralization of IL-17A and IL-17F by bimekizumab profoundly suppressed joint and skin inflammation in PsA (37) and might differently affect the parameters assessed in our in vitro study.

TCZ showed mixed results in clinical studies and was not approved for PsA treatment (38). In the current study, it was employed as a biologic agent which was not approved for the treatment of PsA and indeed, IL-6R did not alter T cell activation and proliferation nor did it block inflammatory cytokine secretion, thus demonstrating that inhibition of T cell function in PsA is independent of IL-6R blockade. In contrast to PsA, TCZ is an effective agent for RA therapy (39). Treatment with TCZ, both ex vivo and in vitro, resulted in a shift in the Th17/Treg imbalance, favoring a more protective response in the context of RA (40, 41).

Our study has several limitations. First, we determined the effect of the biologic agents on general T cell function and did not look deeper into the modulation of distinct T helper subsets, including Th1, Th17, and Treg. In addition, T cell proliferation was assessed by PHA which assesses general proliferation. Although it is more relevant to determine specific PsA-driven proliferation, the autoantigens in PsA are unknown, therefore determination of the precise ability of biologics to suppress proliferation toward psoriatic-related antigens await their discovery.

In conclusion, this study is the first to evaluate in vitro effect of various biologic agents on T cell function by the use of PBMCs from a large population of PsA patients. The findings reported herein are important for understanding the dissimilarity in the potential effect of anti-TNF and anti-IL-17A therapies on T cell function and inflammatory mediator release in patients with PsA. We now demonstrate the putative activity of the studied biologics to suppress T cells and reduce cytokine production. While one of the mechanisms of action of anti-TNF agents in PsA seems to down-regulate T cell function, this could not be similarly shown for IL-17A blockers.

References

1. Rahman P, Elder JT. Genetic epidemiology of psoriasis and psoriatic arthritis. Ann Rheum Dis 2005;**64** Suppl 2:ii37-9; discussion ii40-1.

2. Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. N Engl J Med 2017;**376** :2095-6.

3. Veale DJ, Ritchlin C, FitzGerald O. Immunopathology of psoriasis and psoriatic arthritis. Ann Rheum Dis 2005;**64** Suppl 2:ii26-9.

4. Cai L, Yin JP, Starovasnik MA, Hogue DA, Hillan KJ, Mort JS, et al. Pathways by which interleukin 17 induces articular cartilage breakdown in vitro and in vivo. Cytokine 2001;**16** :10-21.

5. Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, et al. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J Immunol 1998;**160** :3513-21.

6. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 1999;**103** :1345-52.

7. Lynde CW, Poulin Y, Vender R, Bourcier M, Khalil S. Interleukin 17A: toward a new understanding of psoriasis pathogenesis. J Am Acad Dermatol 2014;**71** :141-50.

8. Chandran V, Cook RJ, Edwin J, Shen H, Pellett FJ, Shanmugarajah S, et al. Soluble biomarkers differentiate patients with psoriatic arthritis from those with psoriasis without arthritis. Rheumatology (Oxford) 2010;**49** :1399-405.

9. Raychaudhuri SP, Raychaudhuri SK, Genovese MC. IL-17 receptor and its functional significance in psoriatic arthritis. Mol Cell Biochem 2012;**359** :419-29.

10. Ritchlin CT, Kavanaugh A, Gladman DD, Mease PJ, Helliwell P, Boehncke WH, et al. Treatment recommendations for psoriatic arthritis. Ann Rheum Dis 2009;**68** :1387-94.

11. Saad AA, Ashcroft DM, Watson KD, Hyrich KL, Noyce PR, Symmons DP, et al. Persistence with anti-tumour necrosis factor therapies in patients with psoriatic arthritis: observational study from the British Society of Rheumatology Biologics Register. Arthritis Res Ther 2009;**11** :R52.

12. Tan WS, Kelly S, Pitzalis C. Targeted therapies: what they teach us about the pathogenesis of psoriasis and psoriatic arthritis. Expert Rev Clin Immunol 2017;**13** :207-22.

13. Mease PJ, Kavanaugh A, Reimold A, Tahir H, Rech J, Hall S, et al. Secukinumab in the treatment of psoriatic arthritis: efficacy and safety results through 3 years from the year 1 extension of the randomised phase III FUTURE 1 trial. RMD Open. 2018;4(2):e000723.

14. van Kuijk AW, Tak PP. Synovitis in psoriatic arthritis: immunohistochemistry, comparisons with rheumatoid arthritis, and effects of therapy. Curr Rheumatol Rep 2011;**13** :353-9.

15. Maini RN, Taylor PC, Szechinski J, Pavelka K, Broll J, Balint G, et al. Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. Arthritis Rheum 2006;**54** :2817-29.

16. Mease PJ, Gottlieb AB, Berman A, Drescher E, Xing J, Wong R, et al. The Efficacy and Safety of Clazakizumab, an Anti-Interleukin-6 Monoclonal Antibody, in a Phase IIb Study of Adults With Active Psoriatic Arthritis. Arthritis Rheumatol 2016;**68** :2163-73.

17. Ogata A, Umegaki N, Katayama I, Kumanogoh A, Tanaka T. Psoriatic arthritis in two patients with an inadequate response to treatment with tocilizumab. Joint Bone Spine 2012;**79** :85-7.

18. Nakajima A, Matsuki T, Komine M, Asahina A, Horai R, Nakae S, et al. TNF, but not IL-6 and IL-17, is crucial for the development of T cell-independent psoriasis-like dermatitis in Il1rn-/- mice. J Immunol 2010;**185** :1887-93.

19. Horai R, Saijo S, Tanioka H, Nakae S, Sudo K, Okahara A, et al. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. J Exp Med 2000;**191** :313-20.

20. Miyagawa I, Nakayamada S, Nakano K, Kubo S, Iwata S, Miyazaki Y, et al. Precision medicine using different biological DMARDs based on characteristic phenotypes of peripheral T helper cells in psoriatic arthritis. Rheumatology (Oxford) 2019;**58** :336-44.

21. Reddy M, Eirikis E, Davis C, Davis HM, Prabhakar U. Comparative analysis of lymphocyte activation marker expression and cytokine secretion profile in stimulated human peripheral blood mononuclear cell cultures: an in vitro model to monitor cellular immune function. J Immunol Methods 2004;**293** :127-42.

22. Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H, et al. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum 2006;**54** :2665-73.

23. Dahlen R, Strid H, Lundgren A, Isaksson S, Raghavan S, Magnusson MK, et al. Infliximab inhibits activation and effector functions of peripheral blood T cells in vitro from patients with clinically active ulcerative colitis. Scand J Immunol 2013;**78** :275-84.

24. Coury F, Ferraro-Peyret C, Le Cam S, Guerin S, Tebib J, Sibilia J, et al. Peripheral blood lymphocytes from patients with rheumatoid arthritis are differentially sensitive to apoptosis induced by anti-tumour necrosis factor-alpha therapy. Clin Exp Rheumatol 2008;**26** :234-9.

25. Diani M, Casciano F, Marongiu L, Longhi M, Altomare A, Pigatto PD, et al. Increased frequency of activated CD8(+) T cell effectors in patients with psoriatic arthritis. Sci Rep 2019;**9** :10870.

26. Ezeonyeji A, Baldwin H, Vukmanovic-Stejic M, Ehrenstein MR. CD4 T-Cell Dysregulation in Psoriatic Arthritis Reveals a Regulatory Role for IL-22. Front Immunol 2017;**8** :1403.

27. Liao HT, Lin YF, Tsai CY, Chou CT. Regulatory T cells in ankylosing spondylitis and the response after adalimumab treatment. Joint Bone Spine 2015;**82** :423-7.

28. Dulic S, Vasarhelyi Z, Sava F, Berta L, Szalay B, Toldi G, et al. T-Cell Subsets in Rheumatoid Arthritis Patients on Long-Term Anti-TNF or IL-6 Receptor Blocker Therapy. Mediators Inflamm 2017;2017:6894374.

29. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;**299** :1057-61.

30. Huang Z, Yang B, Shi Y, Cai B, Li Y, Feng W, et al. Anti-TNF-alpha therapy improves Treg and suppresses Teff in patients with rheumatoid arthritis. Cell Immunol 2012;**279** :25-9.

31. Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. J Exp Med 2004;**200** :277-85.

32. Nadkarni S, Mauri C, Ehrenstein MR. Anti-TNF-alpha therapy induces a distinct regulatory T cell population in patients with rheumatoid arthritis via TGF-beta. J Exp Med 2007;**204** :33-9.

33. Toubi E, Kessel A, Mahmudov Z, Hallas K, Rozenbaum M, Rosner I. Increased spontaneous apoptosis of CD4+CD25+ T cells in patients with active rheumatoid arthritis is reduced by infliximab. Ann N Y Acad Sci 2005;**1051** :506-14.

34. Roberts CA, Durham LE, Fleskens V, Evans HG, Taams LS. TNF Blockade Maintains an IL-10(+) Phenotype in Human Effector CD4(+) and CD8(+) T Cells. Front Immunol 2017;**8** :157.

35. Charles P, Elliott MJ, Davis D, Potter A, Kalden JR, Antoni C, et al. Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-alpha therapy in rheumatoid arthritis. J Immunol 1999;**163** :1521-8.

36. Taylor PC, Peters AM, Paleolog E, Chapman PT, Elliott MJ, McCloskey R, et al. Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor alpha blockade in patients with rheumatoid arthritis. Arthritis Rheum 2000;**43** :38-47.

37. Glatt S, Baeten D, Baker T, Griffiths M, Ionescu L, Lawson ADG, et al. Dual IL-17A and IL-17F neutralisation by bimekizumab in psoriatic arthritis: evidence from preclinical experiments and a randomised placebo-controlled clinical trial that IL-17F contributes to human chronic tissue inflammation. Ann Rheum Dis 2018;**77** :523-32.

38. Blauvelt A. IL-6 Differs from TNF-alpha: Unpredicted Clinical Effects Caused by IL-6 Blockade in Psoriasis. J Invest Dermatol 2017;**137** :541-2.

39. Dayer JM, Choy E. Therapeutic targets in rheumatoid arthritis: the interleukin-6 receptor. Rheumatology (Oxford) 2010;**49** :15-24.

40. Guggino G, Giardina AR, Raimondo S, Giardina G, Sireci G, Dieli F, et al. Targeting IL-6 signalling in early rheumatoid arthritis is followed by Th1 and Th17 suppression and Th2 expansion. Clin Exp Rheumatol 2014;**32** :77-81.

41. Pesce B, Soto L, Sabugo F, Wurmann P, Cuchacovich M, Lopez MN, et al. Effect of interleukin-6 receptor blockade on the balance between regulatory T cells and T helper type 17 cells in rheumatoid arthritis patients. Clin Exp Immunol. 2013;171(3):237-42.

Figure legends

Figure 1 . The differential effect of biologics on activated $CD4^+CD25^+$ T cells in culture of naïve and cDMARD-treated PBMCs from PsA patients.

PBMCs were cultured in RPMI medium supplemented with either ADA, IXE, TCZ (10μg/ml) or medium alone for 5 days, then cells were analyzed by flow cytometry to determine the frequencies of activated $CD4^+CD25^+$ T cells. (A) Representative dot plot of acquisition of in vitro cultured PBMCs gated based on side-scattered light (SSC) versus forward-scattered light (FSC). (B) Gated events were plotted against CD4 versus CD25. Positive staining is presented in the upper right quadrant of each dot plot with the percentage indicated. (C) Percent of $CD4^+CD25^+$ T cells determined by flow cytometry (n=43). The comparisons were determined by one-way ANOVA, data are shown as mean values ± SEM, $*p < 0.04$.

Figure 2 . The differential effect of biologics on activated $CD4^+CD25^+$ T cells in culture of biologic-treated PBMCs from PsA patients.

PBMCs were cultured in RPMI medium supplemented with either ADA, IXE, TCZ (10μg/ml) or medium alone for 5 days, then cells were analyzed by flow cytometry to determine the frequencies of activated $CD4^+CD25^+$ T cells. (A) Representative dot plot of acquisition of in vitro cultured PBMCs gated based on side-scattered light (SSC) versus forward-scattered light (FSC). (B) Gated events were plotted against CD4 versus CD25. Positive staining is presented in the upper right quadrant of each dot plot with the percentage indicated. (C) Percent of $CD4^+CD25^+$ T cells determined by flow cytometry (n=58). The comparisons were determined by one-way ANOVA, data are shown as mean values ± SEM, $*p < 0.04$, $**p< 0.01$.

Figure 3 . In vitro effect of biologics on percentages of activated $CD4^+CD25^+$ T cells in PBMCs derived from naïve and cDMARD-, biologic- treated PsA patients and healthy controls.

Percentage of activated $CD4^+CD25^+$ T cells were determined by flow cytometry analysis after in vitro incubation with biologics as described above in PBMCs isolated from patients with PsA that are either: naïve and cDMARD- (n=43), biologic-treated (n=58) and healthy controls (n=20). The comparisons were determined by one-way ANOVA, data are presented as mean values ± SEM, p values indicate difference of ADA as compare to medium, $*p< 0.04$, $**p < 0.01$.

Figure 4 . Reduction of PHA-induced proliferation in PBMCs from PsA patients in vitro by ADA.

CFSE-labeled PBMCs from PsA patients (n=17) were incubated without PHA or with PHA (5μg/ml), or with PHA in the presence of the biologics. The cells were harvested and analyzed after 5 days. (A) Representative plots showing examples of CFSE dilution assay with PBMCs cultured without PHA or incubation with PHA alone or PHA with the biologics. Percentages indicate % of proliferating cells. (B)

Summary of data showing % proliferation. The comparisons were determined by one-way ANOVA, data are presented as mean values ± SEM, $*p < 0.01$.

Figure 5. Cytokine levels determined in culture supernatants of PBMCs from PsA patients. Cytokine were evaluated by ELISA assay. The analysis was performed on supernatants that were collected at day 5 of culture (n=14-15). Data are expressed as mean concentration (pg/ml) ± SEM and statistical analyses were performed by Mann-Whitney U analysis, $*p < 0.02$, $**p < 0.002$ and $***p< 0.0001$.

Figures

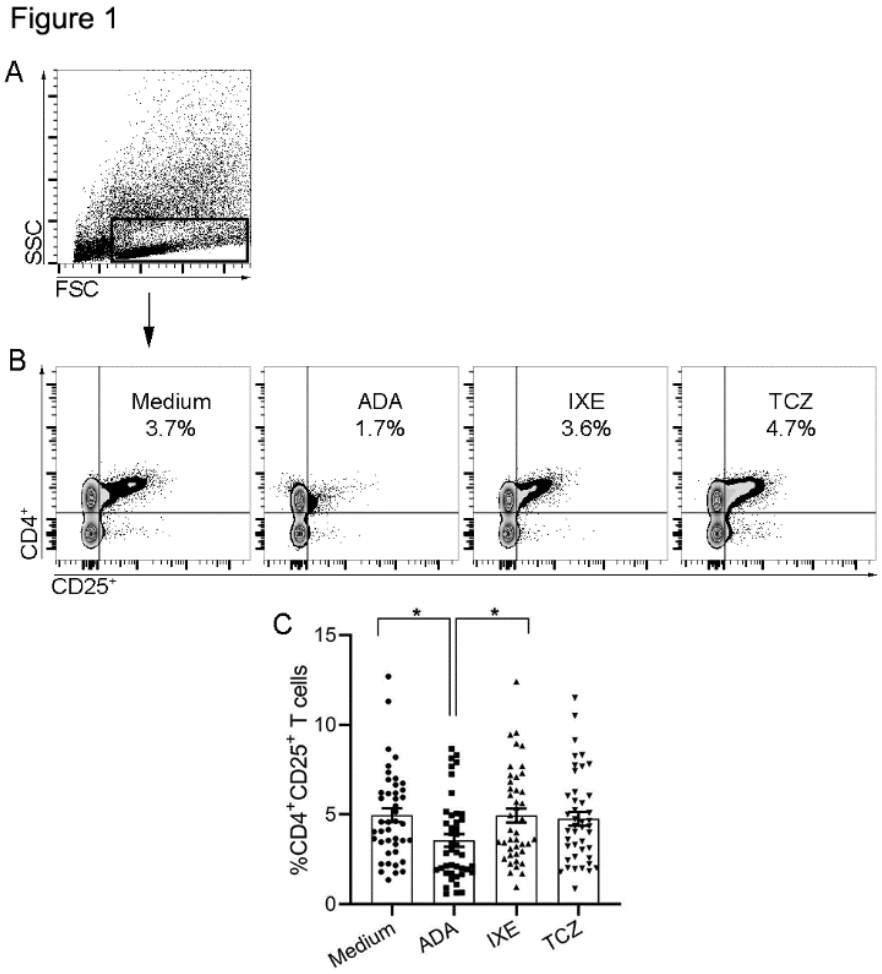


Figure 2

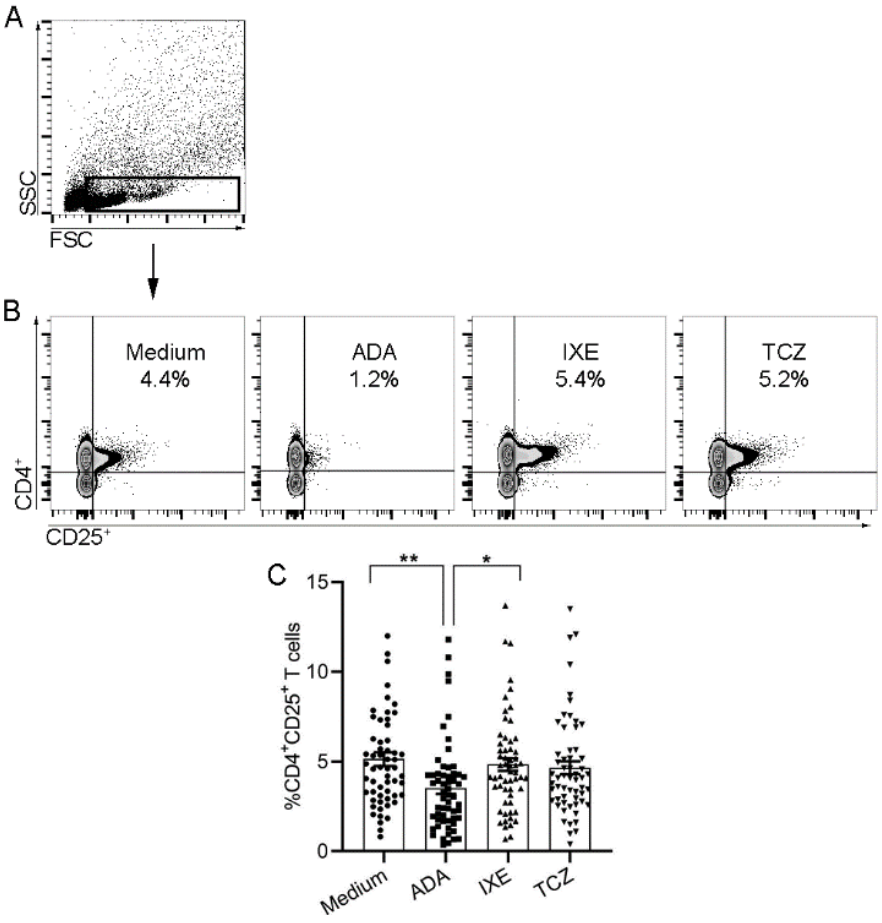


Figure 3

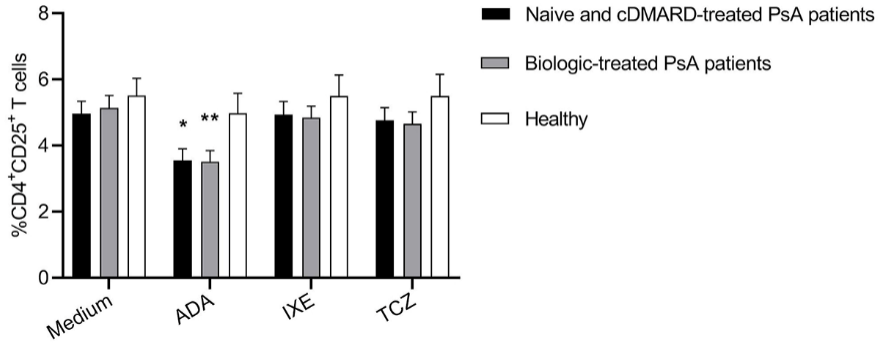


Figure 4

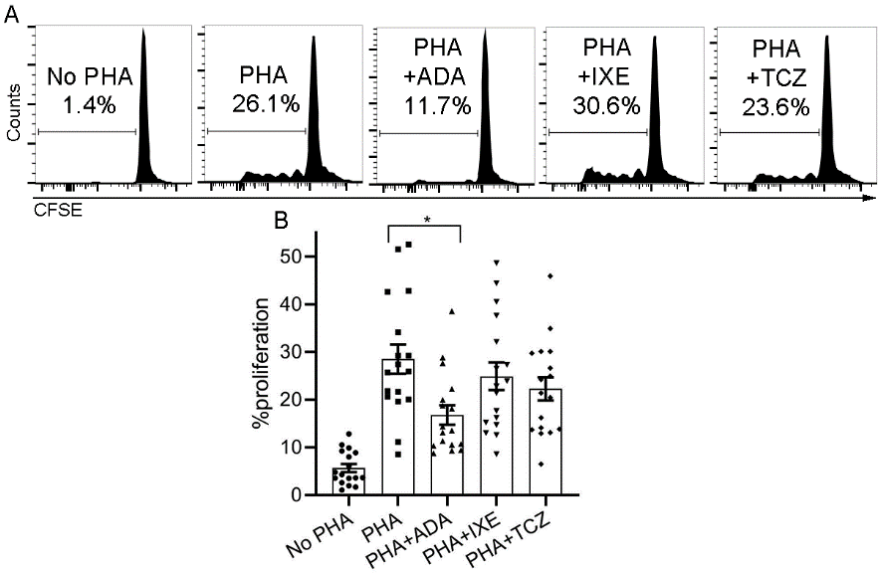
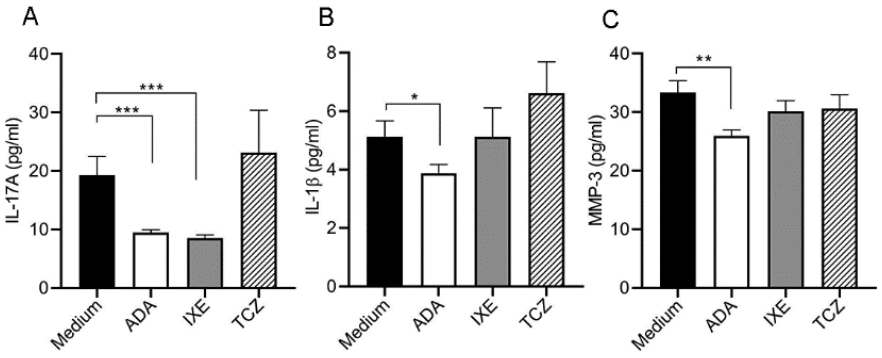


Figure 5



Hosted file

Table 1_2_6_20.docx available at https://authorea.com/users/329449/articles/456444-different-effects-of-tumor-necrosis-factor-and-il-17a-blockades-on-t-cell-function-of-psoriatic-arthritis-patients

Figure 1

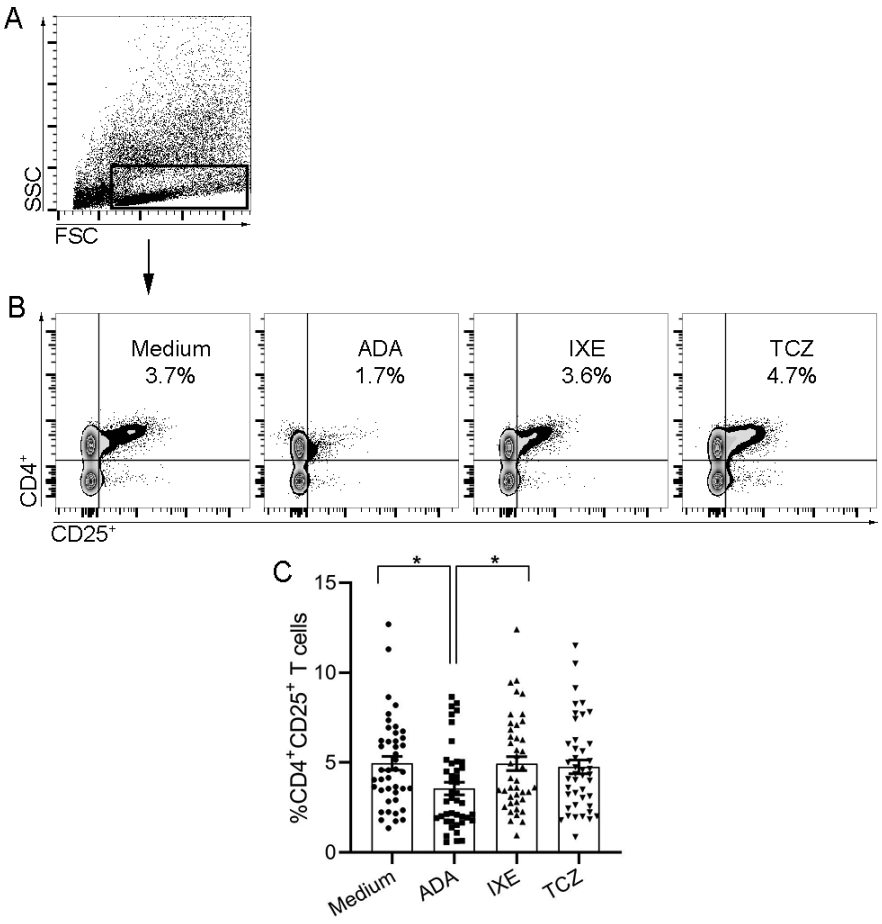


Figure 2

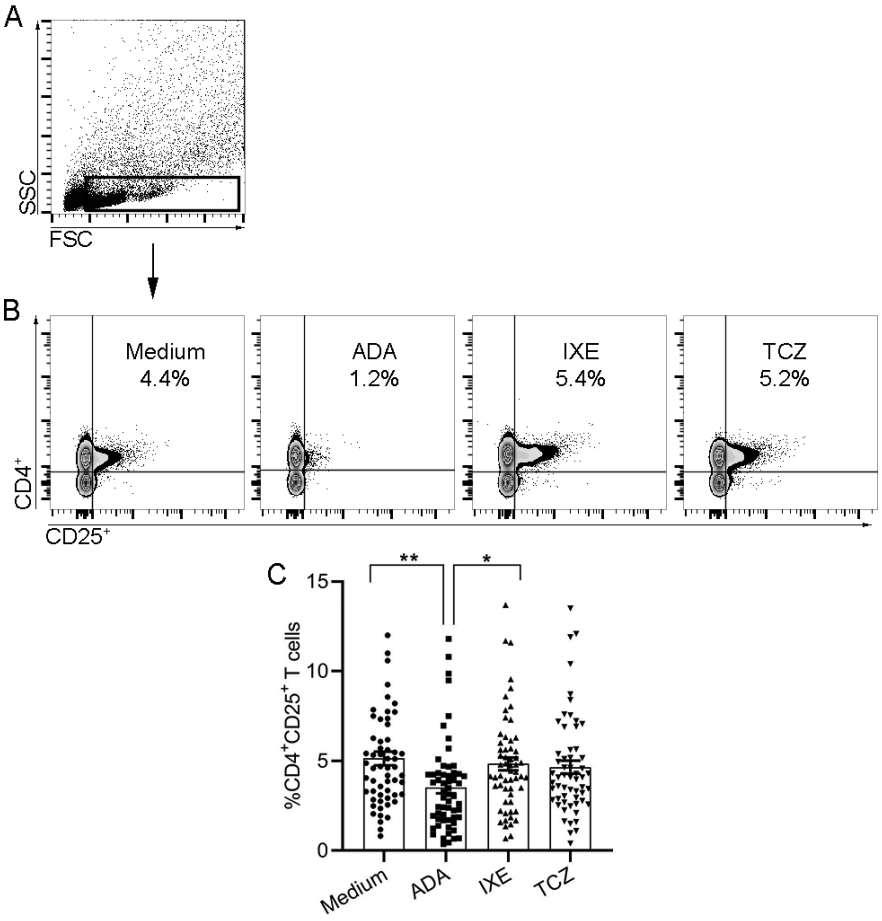


Figure 3

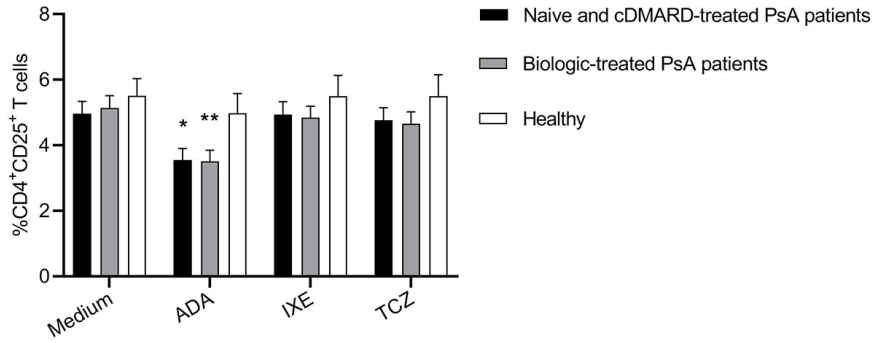


Figure 4

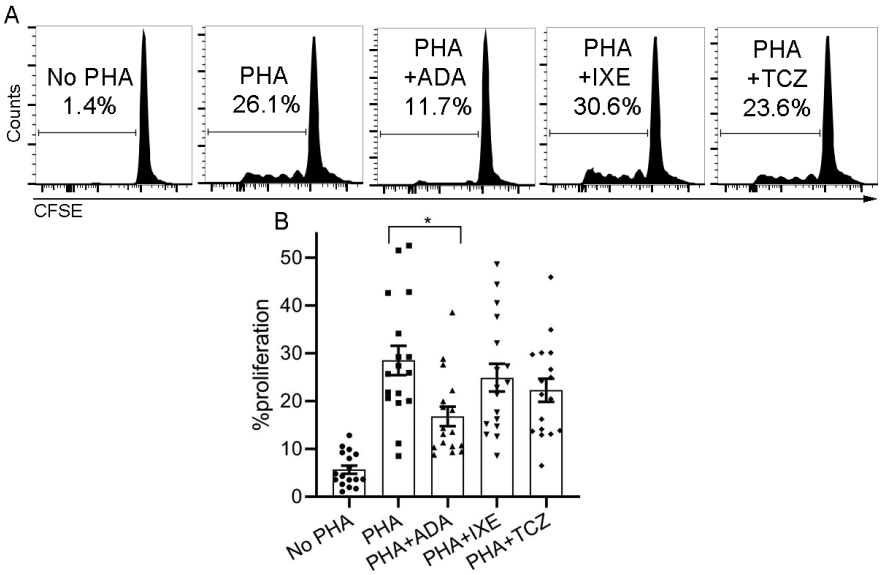


Figure 5