Journal of Autoimmunity 133 (2022) 102909

Contents lists available at ScienceDirect

Journal of Autoimmunity

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

# Divergent regulatory T cell responses to high-dose methylprednisolone and tocilizumab in giant cell arteritis

Godehard A. Scholz [a], Michaela Fux [b], Lisa Christ [a], Joseena Iype [c], Yara Banz [d], Peter M. Villiger [e,*]

[a] University Clinic of Rheumatology and Immunology, Inselspital, University Hospital Bern, Switzerland
[b] Institute of Social and Preventive Medicine, University of Bern, Switzerland
[c] University Institute of Clinical Chemistry, Inselspital, University Hospital Bern, Switzerland
[d] Institute of Pathology, University of Bern, Switzerland
[e] University of Bern and Medical Center Monbijou, Switzerland

ARTICLE INFO

*Keywords:*
Giant cell arteritis
Methylprednisolone
Tocilizumab
Regulatory T cells
CD15s
FOXP3 EXON2

## 1. Objective

Giant Cell Arteritis (GCA) is the most prevalent large vessel vasculitis in Western countries. Its pathophysiological hallmark is an imbalance of regulatory T (Treg), T helper (Th) 1 and Th17 cell responses [1]. The current mainstay of treatment includes glucocorticoids (GCs) and the IL-6 receptor antagonist tocilizumab (TCZ) [2]. Despite progress in understanding the pathogenesis of the disease, there is a need to reveal the mechanisms by which GCs and TCZ exert their roles. One might be the mediation of efficient Treg cell responses. CD15s (Sialyl Lewis X), the α-2-3-sialylated form of CD15, characterizes highly immunosuppressive Treg cells [3]. Since CD15s enables leukocyte interaction with the selectin CD62E, it promotes leukocyte migration into the tissue [4]. Another population of highly immunosuppressive Treg cells exhibits the EXON2 of FOXP3, which probably prevents their polarization into Th17 cells [5]. To tackle the question of the restoration of Treg cell responses, we assessed the frequencies and absolute numbers of CD15s+, CD45RA- (activated) and CD25+, FOXP3 EXON2+ Treg cells in the peripheral blood of GCA patients along their treatment course with GCs followed by TCZ. Moreover, we analyzed the presence of CD15s+ Treg cells in the temporal artery vessel wall in relation to the presence of total inflammatory lymphocytes.

## 2. Material and methods

For flow cytometry analysis of Treg cells, we took advantage of full peripheral blood samples from new-onset, previously untreated GCA patients, enrolled in an investigator-initiated, single-arm, single-center, and open-label trial evaluating the safety and efficacy of TCZ after ultra-short GC treatment (*NCT03745586*). Briefly, patients received a course of 500 mg intravenous methylprednisolone (MP) on three consecutive days, followed by an intravenous TCZ application (8 mg/kg body weight) and consecutive weekly subcutaneous TCZ injections (162 mg). Blood samples were taken before MP (day 0), after MP/before intravenous TCZ (day 3) treatment and at weeks 12 and 24. The sequential treatment with MP and TCZ allowed dissection of the cellular effects (day 3 reflecting the effects of MP, week 12 and 24 reflecting the effects of TCZ). For analysis of flow cytometry data, we used DIVA software version 6.1.3. We performed statistical analysis by a paired *t*-test with GraphPad Prism software (version 9.3.1). Temporal artery biopsies

*Abbreviations:* Treg cells, Regulatory T cells; MP, Methylprednisolone; TCZ, Tocilizumab; TABs, Temporal artery biopsies; d, day; w, week.

* Corresponding author.
*E-mail addresses:* godehard.scholz@gmx.ch (G.A. Scholz), michaela.fux@ispm.unibe.ch (M. Fux), lisa.christ@insel.ch (L. Christ), joseenamariam.iype@insel.ch (J. Iype), yara.banz@pathology.unibe.ch (Y. Banz), peter.villiger@hin.ch (P.M. Villiger).

https://doi.org/10.1016/j.jaut.2022.102909
Received 16 June 2022; Received in revised form 28 August 2022; Accepted 31 August 2022
Available online 15 September 2022

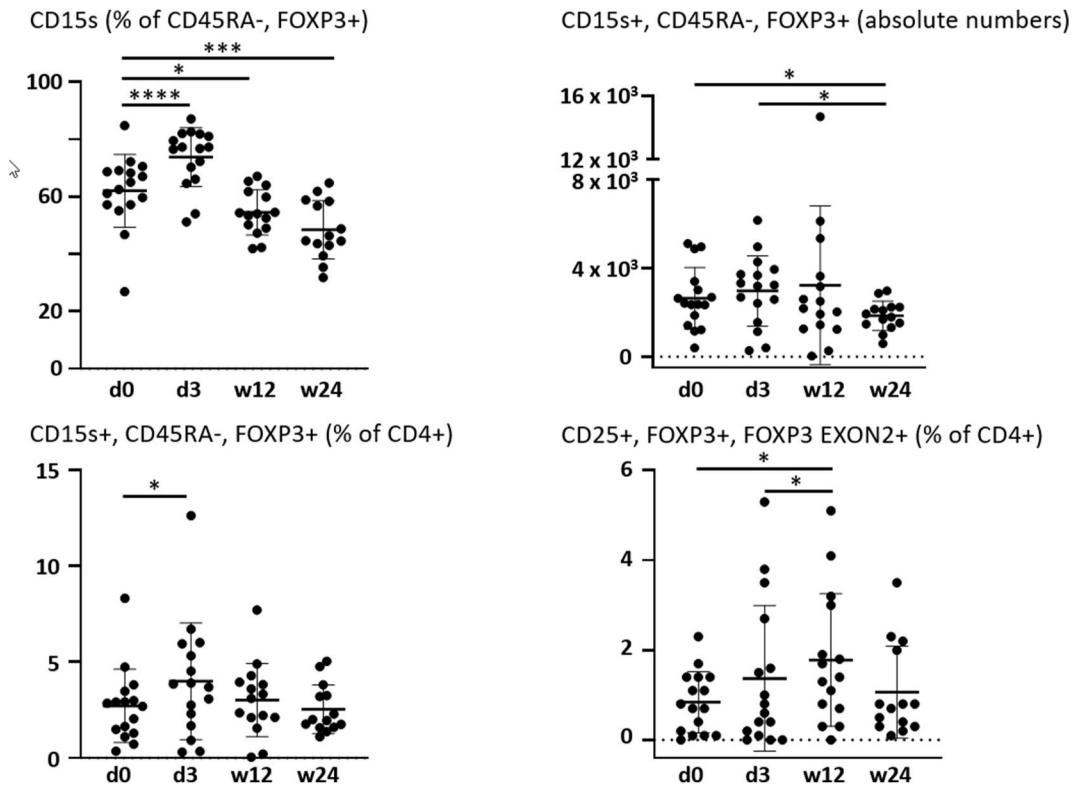


**Fig. 1.** Left, up: Percentage of CD15s+ T cells among activated CD45RA-, FOXP3+ Treg cells in the peripheral blood of GCA patients along the treatment course. In active disease, three days of MP treatment lead to an increase of CD15s positivity among CD45RA-, FOXP3+ Treg cells (day 3), whereas the amount of CD15s+ activated Treg cells declines under TCZ treatment (week 12 and week 24). Left, down: Increased percentages of CD15s+, CD45RA-, FOXP3+ Treg cells among CD4+ T cells reflect MP-triggered CD15s expression (day 3). Under TCZ treatment (weeks 12, 24) the levels of CD15s+, CD45RA-, FOXP3+ Treg cells are restored to baseline levels (day 0). Right, up: Absolute numbers per ml of activated Treg cells decline along the treatment course when clinical remission is achieved (week 24). Right, down: Whereas TCZ does not interfere with the CD15s+ activated Treg cell response, it increases the frequencies of Treg cells exhibiting FOXP3 EXON2 (week 12). *$p < 0.05$, ***$p = 0.0002$, ****$p < 0.0001$.

(TABs) were captured at baseline and double-stained for CD15s+ and FOXP3+ lymphocytes. A focus score was defined to assess cell quantity.

### 2.1. Flow cytometry

For assessment of Treg cells the antibodies as depicted in Supplementary Table 1 were used. Staining was performed in 100 μl EDTA anticoagulated venous patient blood. Samples were incubated (not longer than 24 h after the blood drawing, otherwise samples were not used for analysis) by fluorochrome-labelled antibodies for 15 min at room temperature. After red blood cell lysis, samples were washed using stain buffer consisting of 1xPBS containing 2% heat-inactivated FCS (FCS, Sera Pro, ultra-low endotoxin, Pan Biotech, Aidenbach, Germany) and 0.05% sodium azide (Merck Millipore, Zug, Switzerland). Lymphocytes were then permeabilized by incubation in 50 μl FoxP3 buffer (BD Biosciences) for 30 min at room temperature. After incubation, samples were washed twice with stain buffer and exposed to anti-FoxP3 Alexa Fluor 674 and anti-FoxP3Exon2 Alexa Fluor 488 for 30 min at room temperature. Thereafter, samples were washed with stain buffer and cell pellets were resuspended in 600 μl stain buffer. In order to enable calculation of absolute cell numbers CountBrightTM absolute counting beads (Invitrogen, Basel, Switzerland) were added shortly before data acquisition. Data acquisition was performed with a BD FACSCanto II Flow cytometer (BD Biosciences) with the stopping gates set at $10^5$ lymphocytes (based on CD45 and SSC) or 6 min acquisition time, which ever was reached first. Analysis of flow cytometric data was performed with DIVA software Version 6.1.3. The gating strategy is depicted in Supplementary Figure 1.

## 3. Results

Three days of MP treatment led to an increase in the percentage of CD15s positivity among activated CD45RA-, FOXP3+ Treg cells in the peripheral blood of GCA patients (day 3). In contrast, the percentage of CD15s among activated Treg cells declined during subsequent TCZ treatment (weeks 12, 24). The levels were even lower at week 24 as compared to day 0 (Fig. 1, *left, up*). An increased percentage of CD15s+ activated Treg cells among CD4+ T cells also reflects the expression of CD15s triggered by MP (day 3). During TCZ treatment, the levels of CD15s+ activated Treg cells fell to baseline levels (weeks 12, 24; Fig. 1, *left, down*). In TABs, we detected increased numbers of CD15s+ Treg cells in areas of active inflammation over time (Fig. 2). Thus, migration from the peripheral blood into the inflamed vessel wall may contribute to the decline of numbers of activated Treg cells along the treatment course (week 24; Fig. 1, *right, up*). At the systemic level, TCZ increased frequencies of Treg cells exhibiting FOXP3 EXON2 (week 12, Fig. 1, *right, down*). At the clinical level ESR and CRP rapidly normalized, but signs and symptoms gradually remitted. In the main study, the median time to full remission was 11.1 weeks, 14/18 patients reached remission within 24 weeks [2].

## 4. Discussion

In a longitudinal approach, we present mechanistic insights into the respective roles of GCs and TCZ on Treg cells in GCA. We identified two complementary responses: 1) MP leads to a rapid systemic augmentation of CD15s+ activated Treg cells and thereby promotes their entry into the inflamed vessel wall. This finding is supported by the presence of

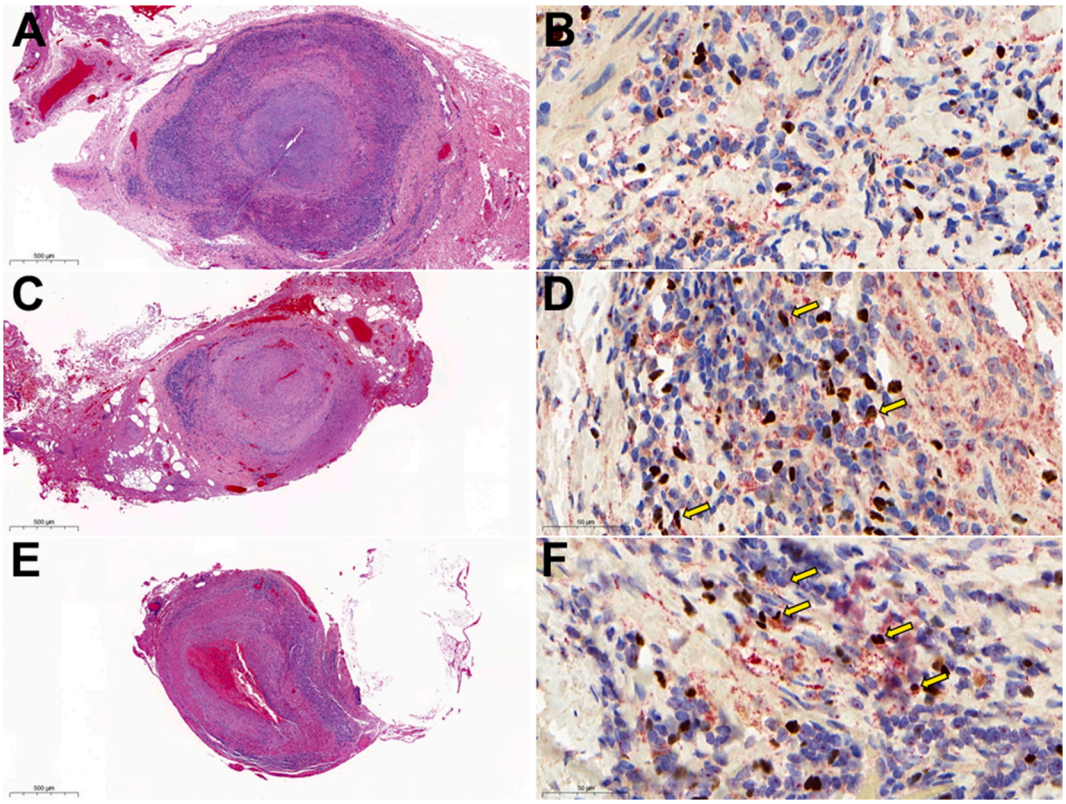


**Fig. 2.** Hematoxylin and Eosin staining images (left panels, 5x) of TABs taken at baseline (A and B), post methylprednisolone treatment (C and D) and post tocilizumab treatment (E and F). Immunohistochemical staining of formalin-fixed, paraffin-embedded samples (right panels, 63x). FOXP3 staining is denoted by a brown nuclear positivity. CD15s stain is shown in red. Double positive cells are highlighted with the yellow arrow.

CD15s+ Treg cells at sites of active inflammation in TABs. Thus, CD15s+ Treg cells appear to be important players when active inflammation needs to be controlled. 2) TCZ does not interfere with CD15s+ Treg cells, but, as recently shown, restores the suppressive function of Treg cells by augmenting the expression of FOXP3 EXON2 [3]. The prevention of Treg cells to polarize into IL-17 producing effector T cells will likely result in a delayed clinical response. Indeed, time kinetics of clinical response to MP and to TCZ, respectively, were different, they perfectly match cellular findings [2]. Collectively, our data might provide the basis for next-generation therapies. A future real life scenario could be the adoptive transfer of previously *ex vivo* sorted or genetically manufactured Treg cells highly expressing CD15s and/or FOXP3 EXON2, thereby mimicking MP and TCZ effects.

## Funding

Research funds of the Department of Rheumatology, Immunology and Allergology, Inselspital, University Hospital of Bern, Switzerland.

## Authors contribution

GAS, MF and PMV designed the T cell study. LC, GAS and PMV were responsible for the clinical study. JI and MF performed data analysis with respect to flow cytometry. YB performed the analyses with respect to TABs. GAS, MF and PMV wrote the manuscript.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2022.102909.

## References

[1] R. Watanabe, E. Hosgur, H. Zhang, Z. Wen, G. Berry, J.J. Goronzy, C.M. Weyand, Pro-inflammatory and anti-inflammatory T cells in giant cell arteritis, Jt Bone Spine 84 (2017) 421–426.
[2] L. Christ, L. Seitz, G. Scholz, A.-C. Sarbu, J. Amsler, L. Bütikofer, et al., Tocilizumab monotherapy after ultra-short glucocorticoid administration in giant cell arteritis: a single-arm, open-label, proof-of-concept study, The Lancet Rheumatology 3 (9) (2021) e619–e626.
[3] M. Miyara, D. Chader, E. Sage, D. Sugiyama, H. Nishikawa, D. Bouvry, L. Claër, R. Hingorani, R. Balderas, J. Rohrer, N. Warner, A. Chapelier, D. Valeyre, R. Kannagi, S. Sakaguchi, Z. Amoura, G. Gorochov, Sialyl Lewis x (CD15s) identifies highly differentiated and most suppressive FOXP3high regulatory T cells in humans, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 7225–7230.
[4] M. Silva, P.A. Videira, R. Sackstein, E-selectin ligands in the human mononuclear phagocyte system: implications for infection, inflammation, and immunotherapy, Front. Immunol. 8 (2018).
[5] C. Miyabe, Y. Miyabe, K. Strle, N.D. Kim, J.H. Stone, A.D. Luster, S. Unizony, An expanded population of pathogenic regulatory T cells in giant cell arteritis is abrogated by IL-6 blockade therapy, Ann. Rheum. Dis. 76 (2017) 898–905.