Regulatory T cell frequencies in patients with rheumatoid arthritis are increased by conventional and biological DMARDs but not by JAK inhibitors

Regulatory T (Treg) cells play an important role in controlling immune responses. Their frequency is decreased in many autoimmune diseases, including rheumatoid arthritis (RA). We read with great interest the article by Rosenzwajg *et al* which presents the results of a clinical trial with low-dose interleukin-2 (ld-IL-2).[1] The authors report that Treg cell frequencies were significantly increased following ld-IL-2 administration in 46 patients with autoimmune diseases. Among them, four patients had RA and received a background therapy with low-dose prednisolone (PRED) or methotrexate (MTX). The study demonstrates that ld-IL-2 administration is a successful strategy to overcome Treg cell deficiency and to increase the ratio between Treg cells and effector T cells in patients with RA and other autoimmune diseases.

Here we want to draw attention to the fact that Treg cell frequencies in patients with RA can be increased by some, but not by all, antirheumatic drugs and that the background treatment can therefore affect the results of clinical trials with IL-2. Using flow cytometry, we analysed the ex vivo frequency of $CD25^{high}CD127^{low}FoxP3^{high}$ $CD4^+$ Treg cells in the peripheral blood of 112 patients with RA and 19 healthy individuals (figure 1A). To confirm the suppressive capacity of the Treg cells, we assessed their ability to suppress effector T cells using a classical Treg suppression assay (figure 1B, C). Our results and previous findings from other groups demonstrate that MTX (≥15 mg/week) and various biological disease-modifying anti-rheumatic drugs (DMARDs) efficiently upregulate Treg cell frequencies to an almost normal level (figure 1D).[2–5] We observed a significant increase in Treg cell frequencies in the peripheral blood of patients treated with MTX, adalimumab, etanercept, golimumab and tocilizumab. Concomitant medication with MTX did not further increase the percentage of Treg cells in patients treated with the biologicals. The RA patients in the study by Rosenzwajg *et al* were either treated with low-dose PRED (<15 mg/day) or with MTX (≤20 mg/week). Interestingly, the fold change from baseline in Treg cells reported by Rosenzwajg *et al* was higher in the low-dose-PRED group compared with the group with MTX background therapy. This could probably be due to higher

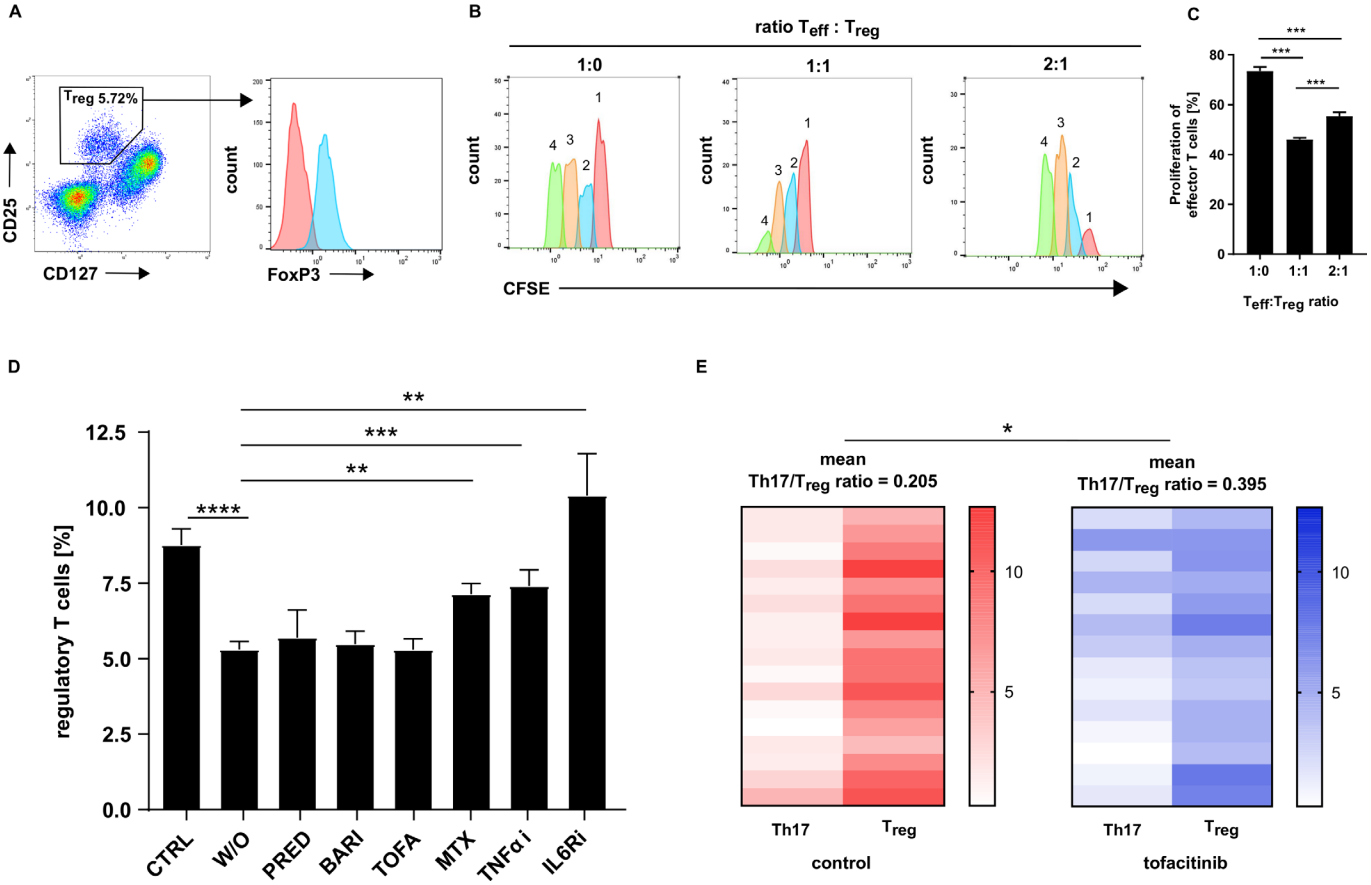

Figure 1 Frequency of regulatory T (Treg) cells in the peripheral blood of patients with rheumatoid arthritis. (A) CD4 +T cells were analysed ex vivo by flow cytometry. A representative example of the gaiting strategy is shown. (B,C) A representative example of a classical Treg suppression assay is shown. The assay was performed as described previously.[7] (D) Percentage of $CD25^{high}CD127^{low}FoxP3^{high}$ Treg cells in patients treated with low-dose prednisolone (<15 mg/day) (PRED, n=10), baricitinib (BARI, n=29), tofacitinib (TOFA, n=19), methotrexate (≥15 mg/week) (MTX, n=24), tumor necrosis factor-α inhibitors (TNF-αi, n=10: adalimumab (n=4), etanercept (n=4) and golimumab (n=2)) or interleukin (IL)-6R inhibitors (IL-6Ri, tocilizumab, n=10). Healthy individuals (CTRL, n=19) and untreated patients (W/O, n=10: first diagnosis n=8 and untreated for ≥6 weeks n=2) served as controls. (E) Th17/Treg cell ratio in healthy controls (red heat map) and patients treated with tofacitinib (blue heat map); *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; data are presented as mean±SEM; significant differences were determined using the Kruskal-Wallis test and the unpaired Mann-Whitney test. CFSE, carboxyfluorescein succinimidyl ester.

baseline Treg cell levels in the MTX group. In striking contrast to MTX and biological DMARDs, we observed no increase in Treg cell frequencies in patients treated with the Janus kinase (JAK)1/2 inhibitor baricitinib or the JAK1/3 inhibitor tofacitinib (figure 1D). Additional treatment with MTX had no influence on the results.

The peripheral blood of patients with RA is characterised by a higher percentage of Th17 cells and the balance between Th17 cells and Treg cells is shifted.[6] In RA patients, the Th17/Treg cell balance is not recovered by JAK inhibitors, although the percentage of Th17 cells is significantly suppressed (figure 1E). Even though JAK inhibition is an efficient treatment in RA, an increase in Treg cells could be beneficial for the patients. The influence of combined treatment with ld-IL-2 and JAK inhibitors on Treg cells has not been investigated yet. However, it is likely to be very low as JAK1 and JAK3 are located downstream of the IL-2 receptor. Taken together, our data confirm that MTX and biological DMARDs increase Treg cell frequencies. Moreover, they reveal that the percentage of Treg cells is not modified by JAK inhibitors. We suggest that the ability of background therapy to increase Treg cell frequencies is a critical factor that should be taken into account when planning future clinical studies on ld-IL-2 treatment.

Anja Meyer, Paula S Wittekind, Konstantin Kotschenreuther, Joanna Schiller, Julia von Tresckow, Thomas H Haak, David M Kofler

Department I of Internal Medicine, University of Cologne, Cologne, Germany

Correspondence to Dr David M Kofler, Department I of Internal Medicine, University of Cologne, Cologne 50937, Germany; david.kofler@uk-koeln.de

Contributors AM, PSW, and DMK made substantial contributions to the study concept and design. AM, PSW, KK, JS, JvT and THH made substantial contributions to the acquisition of the data. AM, PSW, KK, JS, JvT, THH and DMK drafted the article or revised it critically for important intellectual content. All authors reviewed the draft and approved the submission of the manuscript.

Funding This work was supported by a grant from the Fritz Thyssen foundation (10.17.2.019MN to DMK), the Köln Fortune Program of the Faculty of Medicine of the University of Cologne (PSW and DMK) and the foundation 'Exzellenz initiieren' - Stiftung Kölner Krebsforschung (DMK). AM was supported by a fellowship from the German Federal Ministry of Education and Research (BMBF): Professorinnenprogramm II, Förderung der Regelprofessur im Fach Archäoinformatik - 01FP14039G, Projekt-Nr.: 4600/8116/01).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was approved by the Ethics Committee of the University Hospital Cologne (no. 13-091).

Provenance and peer review Not commissioned; internally peer reviewed.

AM and PSW contributed equally.

To cite Meyer A, Wittekind PS, Kotschenreuther K, *et al*. *Ann Rheum Dis* 2021;**80**:e196.

Received 1 November 2019
Accepted 5 November 2019
Published Online First 19 November 2019

► http://dx.doi.org/10.1136/annrheumdis-2019-216598

Ann Rheum Dis 2021;**80**:e196. doi:10.1136/annrheumdis-2019-216576

ORCID iD
David M Kofler http://orcid.org/0000-0001-6164-3980

REFERENCES

1 Rosenzwajg M, Lorenzon R, Cacoub P, *et al*. Immunological and clinical effects of low-dose interleukin-2 across 11 autoimmune diseases in a single, open clinical trial. *Ann Rheum Dis* 2019;78:209–17.
2 Karagiannidis C, Akdis M, Holopainen P, *et al*. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 2004;114:1425–33.
3 Cribbs AP, Kennedy A, Penn H, *et al*. Methotrexate restores regulatory T cell function through demethylation of the FOXP3 upstream enhancer in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:1182–92.
4 Nguyen DX, Ehrenstein MR. Anti-Tnf drives regulatory T cell expansion by paradoxically promoting membrane TNF–TNF-RII binding in rheumatoid arthritis. *J Exp Med* 2016;213:1241–53.
5 Thiolat A, Semerano L, Pers YM, *et al*. Interleukin-6 receptor blockade enhances CD39+ regulatory T cell development in rheumatoid arthritis and in experimental arthritis. *Arthritis Rheumatol* 2014;66:273–83.
6 Klasen C, Meyer A, Wittekind PS, *et al*. Prostaglandin receptor EP4 expression by Th17 cells is associated with high disease activity in ankylosing spondylitis. *Arthritis Res Ther* 2019;21.
7 Kofler DM, Chmielewski M, Rappl G, *et al*. Cd28 costimulation impairs the efficacy of a redirected T-cell antitumor attack in the presence of regulatory T cells which can be overcome by preventing Lck activation. *Molecular Therapy* 2011;19:760–7.