Clinical and Experimental Rheumatology 2018; 36: 140-143.

Ex vivo inhibited cytokine profiling may explain inferior treatment response to golimumab after adalimumab failure in rheumatoid arthritis

L. Tweehuysen[1], K. Schraa[2], M.G. Netea[2], F.H.J. van den Hoogen[1,3], L.A.B Joosten[2], AA. den Broeder[1,3]

[1]Department of Rheumatology, Sint Maartenskliniek, Nijmegen, The Netherlands;
[2]Department of Internal Medicine and Radboud Center for Infectious Diseases,
[3]Department of Rheumatology, Radboud University Medical Center, Nijmegen, The Netherlands.

Lieke Tweehuysen, MD
Kiki Schraa, BASc
Mihai G. Netea, MD, PhD
Frank H.J. van den Hoogen, MD, PhD
Leo A.B. Joosten, PhD
Alfons A. den Broeder, MD, PhD

Please address correspondence to:
Dr Lieke Tweehuysen,
Department of Rheumatology,
Sint Maartenskliniek,
PO Box 9011,
6500 GM Nijmegen, The Netherlands.
E-mail: l.tweehuysen@maartenskliniek.nl

Received on November 28, 2016; accepted in revised form on June 26, 2017.

Key words: rheumatoid arthritis, treatment, adalimumab, golimumab, cytokines

Funding: this study was partly supported by a grant from Oxford Immunotec.

Competing interests: none declared.

ABSTRACT

Objective. *Clinical data suggest that the response of rheumatoid arthritis patients to treatment with golimumab is much lower among those who switched from adalimumab than among those who switched from etanercept. To elucidate the mechanism behind this difference in response to sequential biologic treatment, we examined the effect of TNF inhibitors on ex vivo cytokine production profiling.*

Methods. *In a prospective cohort study, blood samples were obtained from patients before the start of a biologic. Peripheral blood mononuclear cells were pre-incubated for 1 hour with the therapeutic in vivo concentration of adalimumab, etanercept or golimumab and stimulated for 24 hours with heat killed Candida albicans or Pam3Cys. Cytokine concentrations of IL-1β, IL-6 and TNFα were determined by ELISA.*

Results. *Ex vivo cytokine profiling was performed in 71 patients. Golimumab, adalimumab and etanercept significantly ($p<0.01$) decreased Candida albicans-induced IL-1β and IL-6 production and Pam3Cys-induced IL-6 production. In contrast to etanercept, golimumab and adalimumab decreased the concentration of TNFα below the detection limit. Absolute changes in cytokine levels after inhibition by golimumab or adalimumab were all significantly correlated (Spearman rank r_s 0.52–0.99, $p<0.001$). These correlations were much lower or non-significant between etanercept and either golimumab or adalimumab.*

Conclusion. *High similarity between ex vivo inhibited cytokine profiling by golimumab and adalimumab, compared to etanercept, may explain the previously found inferior treatment response to golimumab after adalimumab failure. This suggests that patients who are non-responsive to adalimumab should preferably not switch to golimumab and vice versa.*

Introduction

Treatment of rheumatoid arthritis (RA) consists of the introduction of a biological disease-modifying anti-rheumatic drug (bDMARD) after failure of a conventional DMARD (csDMARD). According to the 2013 update of the EULAR recommendations, no preference of one over another bDMARD should be expressed, because evidence does not suggest any one bDMARD to be better than another one when active disease prevails despite treatment with the initial bDMARD (1). This implies that if a first TNF inhibitor (TNFi) has failed, patients may start with any other TNFi (adalimumab, certolizumab, etanercept, golimumab, infliximab) or a bDMARD with another mode of action [abatacept (CTLA4-Ig fusion protein), rituximab (anti-CD20 monoclonal antibody) or tocilizumab (anti-IL-6 receptor)].

Since the update of the EULAR recommendations, new data on sequential bDMARD treatment have been published. In the randomised controlled trial (RCT) GO-AFTER, the efficacy of golimumab and methotrexate after prior TNFi use was evaluated. Post-hoc analyses showed that among 137 RA patients who had received one prior TNFi (adalimumab, n=33; etanercept, n=47; infliximab, n=57), week 24 ACR20 rates were 30%, 47% and 51% respectively and thus much lower among those who previously failed on adalimumab (2). This finding is relevant for clinical practice, because it seems that RA patients who are non-responsive to adalimumab should preferably not switch to golimumab and perhaps vice versa.

Determining the *ex vivo* effect of a TNFi on cytokine production ('inhibited cytokine profiling') in blood samples taken before the start of a next TNFi could be a promising way to examine the mechanism of action and possibly chance of response, since it might closely resemble the actual drug effect in RA patients. Furthermore, to our knowledge, TNFi-mediated inhibition of cytokine production has not been investigated before in RA.

Therefore, the aim of our study was to compare the *ex vivo* effect of adalimumab, etanercept and golimumab on multi-cytokine profiles of RA patients to elucidate the potential reduced clinical response to golimumab after being treated with adalimumab.

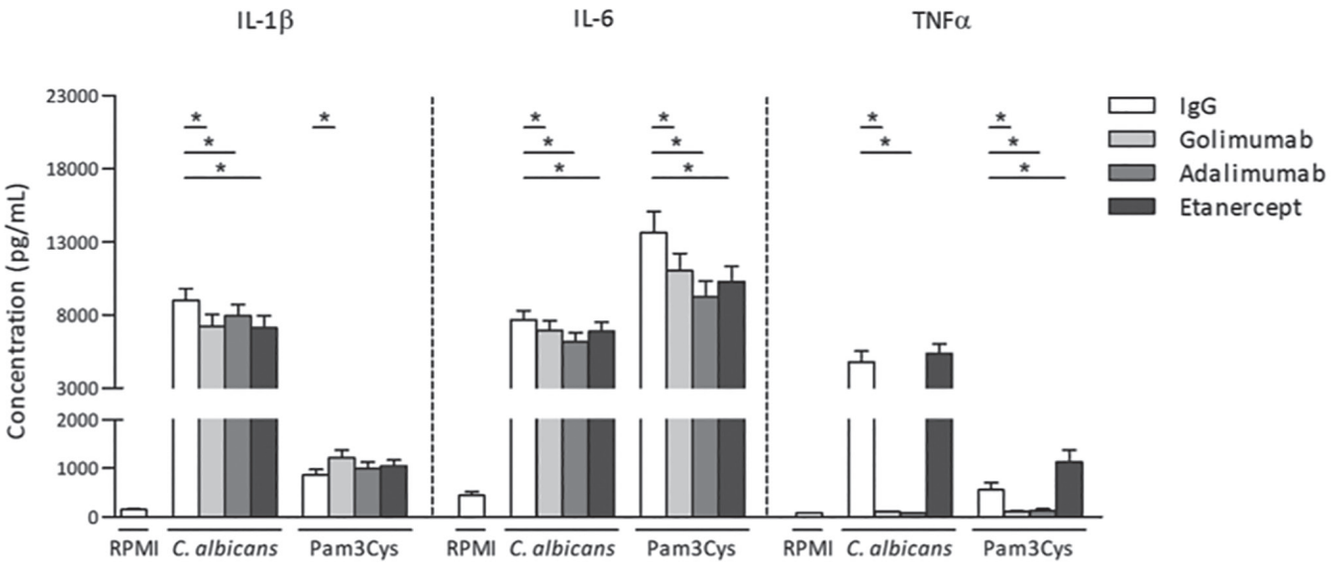

Fig. 1. Effect of golimumab, adalimumab and etanercept on *ex vivo* cytokine production.
Data presented as mean + standard error of the mean (SEM). *p*-values calculated using Wilcoxon signed-rank test. * $p<0.01$.

Materials and methods

Patients

Blood samples of patients included in the prospective longitudinal cohort study BIO-TOP [Biologic Individual Optimized Treatment Outcome Prediction] were used. In this study, RA patients >18 years, treated in the Sint Maartenskliniek (Nijmegen, the Netherlands) who were going to start with (or switch to) a bDMARD were included. The BIO-TOP study was approved by the local ethics committee (CMO region Arnhem-Nijmegen, NL47946.091.14) and a detailed description is available in the Dutch trial register (NTR4647) (3).

Ex vivo cytokine profiling assay

At baseline (before start bDMARD), venous blood was collected into three 10 mL EDTA tubes. Within 24 hours peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation of PBS diluted blood (1:1) over Ficoll-Paque, washed twice with saline and suspended in culture medium (RPMI 1640 supplemented with 2 mM glutamax, 50 μg/mL gentamicin and 1 mM pyruvate). Cells were counted in a Coulter counter. Subsequently, 5×10^5 PBMCs in a volume of 100 μL were pre-incubated in round bottom 96-well plates for one hour at 37 °C with therapeutic *in vivo* concentrations of adalimumab, etanercept, golimumab. Taking into account the different half-life times, dosing and treatment intervals, and therapeutic concentration ranges of the TNFi's, the same concentration of 5 μg/mL was added for all three TNFi (4-7). Nanogam (immunoglobulin (Ig)G; Sanquin, Amsterdam, the Netherlands) was used as negative control. Thereafter, cells were stimulated with RPMI 1640+, Pam3Cys (a TLR2 agonist) or heat killed *Candida albicans* (ATCC MYA-3573 (UC 820)). After 24 hours of stimulation, supernatants were harvested and stored at -20°C until assayed. Cytokine concentrations of IL-1β and TNFα (R&D Systems, Abingdon, UK) and IL-6 (Sanquin, Amsterdam, the Netherlands) were determined by ELISA.

Statistics

All analyses were performed with STATA 13 statistical software. Comparisons between the *ex vivo* cytokine production of stimulated, and stimulated and TNFi inhibited PBMCs were analysed with the Wilcoxon signed-rank test. Statistical significance was considered when $p<0.01$. The absolute changes in cytokine levels after inhibition by each TNFi were calculated and analysed by means of Spearman rank correlations (r_s). They were interpreted according to a commonly used classification: r_s <0.20: very weak, r_s=0.20–0.39: weak, r_s=0.40–0.59: moderate, r_s=0.60–0.79: strong and r_s>0.80: very strong correlation (8).

Results

Patient characteristics

Ex vivo cytokine profiling was performed in 71 patients (66% female, age (mean±SD): 58±11 years, disease duration (median [p25–p75]: 6 [2–14] years). Median number of prior bDMARDs was 1 (p25–p75: 0–2). The bDMARD was started because of active disease, represented by the high DAS28-CRP at baseline (mean±SD): 4.1±1.2.

Ex vivo inhibited cytokine production

The cytokine production of IL-1β, IL-6 and TNFα after inhibition by IgG, golimumab, adalimumab or etanercept and stimulation with heat killed *Candida albicans* or Pam3Cys are depicted in Figure 1. All RPMI values were below detection limit, indicating a comparable baseline quality. Pre-incubation with either golimumab, adalimumab or etanercept significantly ($p<0.01$) decreased *Candida albicans*-induced IL-1β and IL-6 production and Pam3Cys-induced IL-6 production. In contrast to etanercept, golimumab and adalimumab decreased the concentration of TNFα below the detection limit. This can be explained by the specific binding site of golimumab and adalimumab to TNFα, which prevents detection of TNFα with

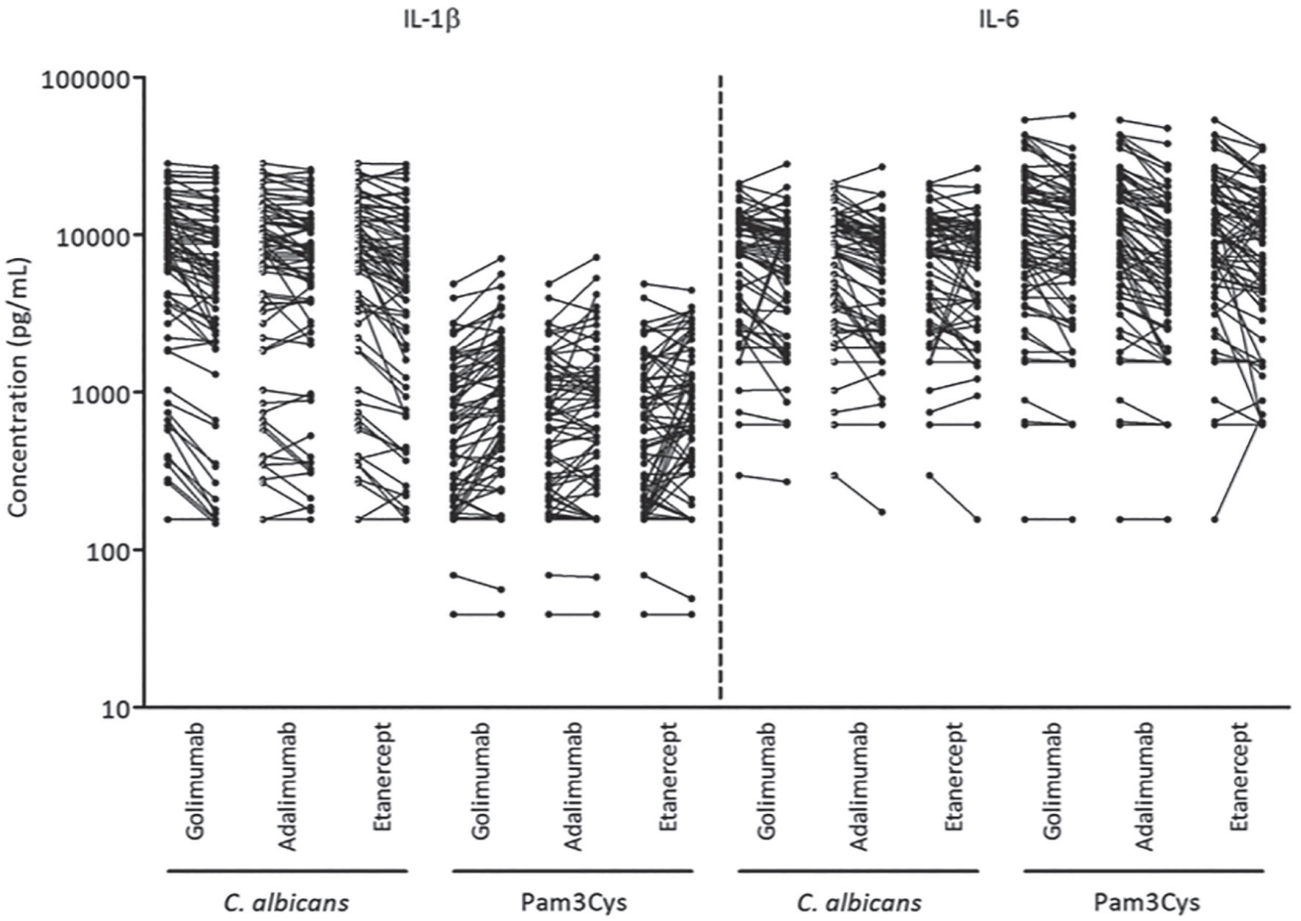

Fig. 2. Absolute changes in cytokine production after inhibition by golimumab, adalimumab or etanercept. Y-axis represents the log transformed values.

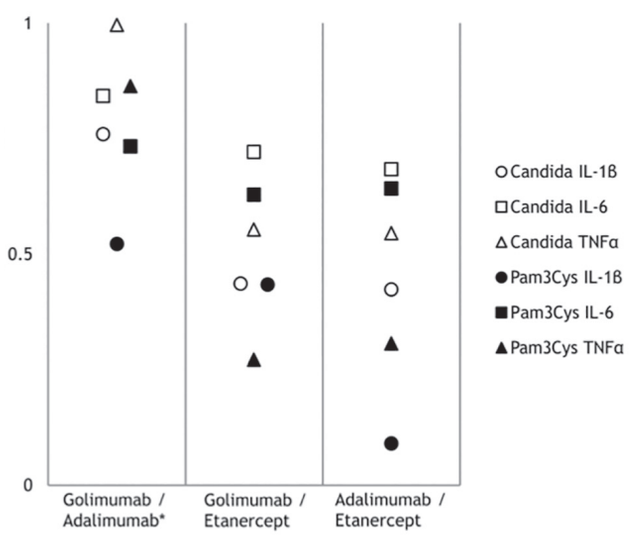

Fig. 3. Spearman rank correlations of cytokine profiles. *all correlations $p<0.001$.

ELISA. The absolute change in cytokine concentration of IL-1β and IL-6 between PBMCs that were only stimulated and PBMCs that were stimulated and inhibited by golimumab, adalimumab or etanercept of each patient is depicted in Figure 2.

Correlations of cytokine profiles

The absolute changes in IL-1β, IL-6 and TNFα levels after inhibition by golimumab were significantly (r_s=0.52–0.99, $p<0.001$) correlated with the absolute changes after inhibition by adalimumab. These correlations were much lower and/or non-significant between etanercept and either golimumab or adalimumab (Fig. 3).

Discussion

To our knowledge, this is the first study in which the *ex vivo* effect of TNFi's on stimulated cytokine production of RA patients has been investigated. We have demonstrated that the cytokine profiles after inhibition by golimumab or adalimumab were moderately to highly correlated with each other, while the correlation with the cytokine profiles after inhibition by etanercept was lower for both. These data suggest similar mechanisms for inhibiting the biological target by golimumab and adalimumab and may serve as an explanation for the previously found inferior treatment response to golimumab after adalimumab failure in RA.

Our findings may represent the pathophysiological link between the known structural resemblance of golimumab

and adalimumab and the observed similarity in clinical response. Both golimumab and adalimumab are fully human IgG1 anti-TNFα monoclonal antibodies and they neutralise soluble and transmembrane TNFα in the same extent (9). It might also be that golimumab and adalimumab bind TNFα at a nearby epitope. In contrast to golimumab and adalimumab, etanercept is a soluble dimeric TNFR2 IgG1-Fc fusion protein which binds both TNF-and TNFβ (8). In addition to our primary finding, our results lend support to the concept of using *ex vivo* inhibited cytokine profiling as a test for *in vivo* efficacy, for example in predicting treatment response. This is currently being investigated in the BIOTOP study.

An important benefit of *ex vivo* testing could be the optimal response of freshly isolated PBMCs, as it has been demonstrated that freezing affects PBMC proliferation and cytokine secretion (10). On the other hand, a possible limitation of *ex vivo* testing is the required logistics to execute each test within 24 hours after blood collection. Furthermore, a possible limitation of the test itself as currently used is the requirement of stimuli to reduce the risk of floor effects in the detection of cytokine levels, as this deviates from the *in vivo* pathophysiology.

The finding that all TNFi's are equal, but some are more equal than others, has interesting implications (11). Recently, the ROC trial and the SWITCH-RA study have demonstrated higher efficacy of a non-TNFi in comparison to a second TNFi in patients with insufficient response to the first TNFi (12, 13). Based on our results, the inferiority of a second TNFi might be due to inferior responses when switching from adalimumab to golimumab and vice versa. In contrast to the GO-AFTER trial (2), these two studies have not made a distinction between the sort of first and second TNFi and golimumab was not included in the ROC trial. Therefore, the hypothesis of different chances of good response to combinations of first and second TNFi's needs confirmation.

In conclusion, the high similarity between *ex vivo* inhibition of cytokine production by golimumab and adalimumab may explain the previously found inferior treatment response to golimumab after adalimumab failure in RA. This suggests that RA patients who are non-responsive to adalimumab should preferably not switch to golimumab and vice versa. Further research is needed to validate if *ex vivo* inhibited cytokine profiling correlates with clinical response to TNFi's in RA patients.

Acknowledgements

We would like to thank the rheumatologists in the Sint Maartenskliniek for participation in patient recruitment and all the patients who were willing to participate. Furthermore, we would like to thank H. Lemmers and H. Dijkstra for their help in conducting the laboratory measurements and L.M. Nieboer for her contribution to the data collection.

References

1. SMOLEN JS, LANDEWÉ R, BREEDVELD FC *et al*.: EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann Rheum Dis* 2014; 73: 492-509.
2. SMOLEN JS, KAY J, MATTESON EL *et al*.: Insights into the efficacy of golimumab plus methotrexate in patients with active rheumatoid arthritis who discontinued prior anti-tumour necrosis factor therapy: post-hoc analyses from the GO-AFTER study. *Ann Rheum Dis* 2014; 73: 1811-8.
3. Dutch trial register website. www.trialregister.nl. Accessed: 10-4-2017.
4. EPAR adalimumab website. www.ema.europa.eu. Accessed: 10-4-2017.
5. EPAR etanercept website. www.ema.europa.eu. Accessed: 10-4-2017.
6. EPAR golimumab website. www.ema.europa.eu. Accessed: 10-4-2017.
7. POUW MF, KRIECKAERT CL, NURMOHAMED MT *et al*.: Key findings towards optimising adalimumab treatment: the concentration-effect curve. *Ann Rheum Dis* 2015; 74: 513-8.
8. CAMPBELL MJ, SWINSCOW TDV: Statistics at Square One. 11th ed., Chichester, Wiley-Blackwell, 2009: 119-32.
9. TAYLOR PC: Tumor necrosis factor-blocking therapies. *In* HOCHBERG MC, SILMAN AJ, SMOLEN JS, WEINBLATT ME, WEISMAN MH (Eds.): Rheumatology. Philadelphia, Elsevier Mosby, 2015: 492-500.
10. MALLONE R, MANNERING SI, BROOKS-WORRELL BM *et al*.: Isolation and preservation of peripheral blood mononuclear cells for analysis of islet antigen-reactive T cell responses: position statement of the T-Cell Workshop Committee of the Immunology of Diabetes Society. *Clin Exp Immunol* 2011; 163: 33-49.
11. ORWELL G: Animal farm: a fairy story. 1st ed., London, Secker and Warburg, 1945.
12. GOTTENBERG JE, BROCQ O, PERDRIGER A *et al*.: Non-TNF-targeted biologic vs a second anti-TNF drug to treat rheumatoid arthritis in patients with insufficient response to a first anti-TNF drug: A randomized clinical trial. *JAMA* 2016; 316: 1172-80.
13. EMERY P, GOTTENBERG JE, RUBBERT-ROTH A *et al*.: Rituximab versus an alternative TNF inhibitor in patients with rheumatoid arthritis who failed to respond to a single previous TNF inhibitor: SWITCH-RA, a global, observational, comparative effectiveness study. *Ann Rheum Dis* 2015; 74: 979-84.