Concise report

Interleukin-1β measurement in stimulated whole blood cultures is useful to predict response to anti-TNF therapies in rheumatoid arthritis

Ken Kayakabe1, Takashi Kuroiwa2, Noriyuki Sakurai1, Hidekazu Ikeuchi1, Anastasie Tshilela Kadiombo1, Toru Sakairi1, Yoriaki Kaneko1, Akito Maeshima1, Keiju Hiromura1 and Yoshihisa Nojima1

Abstract

Objective. In RA, response to TNF blockers may be associated with a profile of cytokine production unique to each patient. This study sought to predict the response to biologic agents by examining pro-inflammatory cytokine synthesis in stimulated whole blood cultures (WBCs).

Methods. We measured the concentration of TNF-α, IL-1β and IL-6 in supernatants of lipopolysaccharide (LPS)-stimulated WBCs obtained from RA patients (n = 41) before anti-TNF therapy (infliximab, 13; etanercept, 26; and adalimumab, 2) and from healthy controls (n = 12). At 24 weeks after biologics, whole bloods were again drawn from 14 of 41 patients. Response was defined by the European League Against Rheumatism response criteria after 24 weeks of therapy.

Results. Among 41 patients, 32 were responders (good 14/moderate 18), while 9 were non-responders. All cytokines measured were significantly lower in RA patients than in controls. In RA, IL-1β production was lower in non-responders than in responders [median (interquartile range): 3.5 (1.5–9.4) vs 10.0 (5.1–93.1) pg/ml, P = 0.048]. The area under the curve from a receiver operating characteristic curve analysis for the prediction of response using IL-1β was 0.717 (95% CI 0.520, 0.914). The sensitivity and specificity of IL-1β (cut-off value 4.84 pg/ml) was 78.1 and 77.8%, respectively. All cytokines were significantly higher 6 months later compared with their respective baseline.

Conclusion. IL-1β measurement in LPS-stimulated WBC is useful to predict responsiveness to anti-TNF agents. Cytokine production capacities in LPS-stimulated WBCs are up-regulated by biologics.

Key words: rheumatoid arthritis, cytokines, anti-TNF therapies, IL-1b, predictor, whole blood culture.

Introduction

RA is an immune-mediated synovitis with pannus formation ultimately leading to irreversible joint destruction and disability. Pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 play critical roles in provoking and perpetuating synovitis. Biologic agents blocking TNF-α have been developed and have proved to be highly effective. Unfortunately, however, some patients do not respond to these drugs. As biologics are both expensive and may be associated with severe side effects, it is important to identify patients who may not respond to anti-TNF therapies before treatment.

Previous studies have identified several factors that may predict responsiveness to biologics, including single-nucleotide polymorphisms in IL-10, lymphOTOXIN-2 and TNFRSF1A [1–3], clinical demographics such as smoking status and MTX usage [4], serological markers such as cartilage oligomeric matrix protein and type I IFN [5, 6], and histological findings of synovium such as large
lymphocyte aggregates, TNF-α expression, and mononuclear cell infiltration [7, 8]. To date, however, it is not certain whether these markers are clinically useful.

The pattern and amount of cytokine production are undoubtedly variable in individual RA patients [9]. The possibility exists that response to biologics is associated with each patient’s profile of pro-inflammatory cytokine production. One possibility is that higher TNF-α production in an individual may result in poorer response, simply due to a subtherapeutic drug dose.

Lipopolysaccharide (LPS)-stimulated whole blood cultures (WBCs) are useful as a simple method to study monocytic cytokine production [10, 11]. By using this method, several groups have reported that the levels of TNF-α, IL-1β and IL-6 are lower in RA patients than in healthy controls [12, 13]. However, no study has yet compared profiles of cytokine production by WBCs between responders and non-responders. In this study we sought to predict response by examining pro-inflammatory cytokine synthesis in LPS-stimulated WBCs.

Patients and methods

Patients and evaluation of clinical response

Forty-four biologic-naïve RA patients who fulfilled the 1987 ACR revised criteria were enrolled. All patients required treatment with TNF-α blocking agents based on the decision of each attending physician. Three patients were excluded from analysis because they discontinued TNF inhibitors before completion of a 6-month observation period (two due to infections and one due to injection site reaction). Two patients, who stopped anti-TNF therapy because of insufficient efficacy during the observation period, were included in the analysis as non-responders. Thus, 41 patients were enrolled, utilizing three different biologic agents: infliximab (n = 13), etanercept (n = 26) and adalimumab (n = 2). DAS in 28 joints by using CRP at baseline and at 24 weeks was calculated, and response was determined according to the European League Against Rheumatism response criteria [14]. Patients with good or moderate response were termed responders, while those with no response were termed non-responders.

WBCs

Heparinized venous blood samples (0.2 ml) were diluted 10 times with Roswell Park Memorial Institute 1640 medium (Wako, Osaka, Japan) and stimulated with LPS from Escherichia coli 026:B6 (Sigma, St Louis, MO, USA) at 1 ng/ml for 24 h. WBCs were performed using one well of a 24-well plate for each patient’s sample. After collecting supernatants, each cytokine concentration was determined using ELISA (R&D Systems, Minneapolis, MN, USA), where data were presented as the average of duplicated results, based on the manufacturer’s instructions. The data acquired by this method represent the sole cytokine data point for each patient and were used for statistical analysis. LPS stimulation of WBC was also performed in 14 patients (7 receiving infliximab and 7 receiving etanercept) at 24 weeks after starting drug therapy. In healthy controls, the mean (s.d.) age was 37 (12) years and 58.3% were women. Written informed consent was obtained from each patient. This study was approved by the Gunma University ethical committee.

Statistical analysis

Comparisons between the variables were performed using either Student’s t-test or the Mann-Whitney U-test. Pearson’s χ²-test was used to compare the percentages of parameters. One-way analysis of variance (ANOVA) was employed to compare variance. The Wilcoxon signed-rank test was used for comparison of time points. A receiver operating characteristic (ROC) curve was generated by plotting the sensitivity against one specificity, and the area under the curve (AUC) with 95% CIs was calculated. SPSS Statistics 17.0 (Chicago, IL, USA) was used for statistical analysis. Bonferroni correction for multiple testing (three tests) was applied, and P-values < 0.017 (0.05/3) were regarded as significant.

Results

Patient characteristics

Characteristics of RA patients at baseline are shown in supplementary data Table S1 (available at Rheumatology Online). Briefly, the mean (s.d.) age was 56 (12) years and 87.8% of the patients were women. Median [interquartile range (IQR)] disease duration was 44 (15–120) months and mean (s.d.) DAS-28-CRP at baseline was 4.5 (0.9). Steinbrocker class and stage were 2.0 (0.5) and 2.4 (1.1), respectively. RF and ACPAs were positive in 69.2 and 84.2%, respectively. Patients had taken a mean number of 1.1 DMARDs at baseline, and in principle continued after initiation of TNF blockers. Among patients, 32 were responders (14 were good and 18 were moderate), while 9 were non-responders. Baseline characteristics were not significantly different between the two groups, except for more frequent MTX use (90.6 vs 33.3%, P < 0.001) and lower corticosteroid dose [5.3 (2.6) vs 7.8 (3.2) mg/day, P = 0.032] in responders.

Pro-inflammatory cytokine production in LPS-stimulated WBCs is lower in RA than in controls

Previous studies have shown that pro-inflammatory cytokine production in LPS-stimulated WBCs is suppressed in RA patients. Before comparing responders and non-responders, we examined the difference between RA and healthy controls in our system. As shown in Fig. 1A–C, all cytokines examined in this study were lower in RA patients than in controls (RA vs controls: TNF-α, 30.4 vs 256 pg/ml, P = 0.002; IL-1β, 6.9 vs 256 pg/ml, P < 0.001; IL-6, 358 vs 1933 pg/ml, P = 0.004). Although age and gender were not matched between the RA and control groups, cytokine production was not affected by gender or age in healthy controls (supplementary Fig. S1, available as supplementary data at Rheumatology Online).
IL-1β measurement in LPS-stimulated WBCs is useful to predict the response to anti-TNF therapy

We next examined whether cytokine production in LPS-stimulated WBCs is different between responders and non-responders. As shown in Fig. 1D–F, IL-1β was lower in non-responders than in responders, although not reaching statistical significance [median (IQR): 3.5 (1.5–9.4) vs 10.0 (5.1–93.1) pg/ml, P = 0.048]. TNF-α
and IL-6 were also lower in non-responders, but again statistical significance was not observed [TNF-α 23.0 (2.2–66.5) vs 31.5 (11.0–224) pg/ml, P = 0.393; IL-6 185 (45.3–150) vs 31.5 (11.0–224) pg/ml, P = 0.546].

To evaluate the accuracy of IL-1β production as a predictor of response to anti-TNF therapies, ROC curve analysis was employed. AUC for IL-1β production was 0.72 (95% CI 0.52, 0.91). When the cut-off value was set at 4.84 pg/ml, sensitivity and specificity for predicting response were 78.1 and 77.8%, respectively (Fig. 1G).

When patients were limited to MTX users (n = 32), IL-1β production was also lower in non-responders than in responders (P = 0.033) (Fig. 1H). The AUC from ROC analysis using IL-1β was 0.879 (95% CI 0.728, 1.000), and the sensitivity and specificity (cut-off value 4.50 pg/ml) were 82.8 and 100%, respectively (Fig. 1).

Cytokine production in LPS-stimulated WBCs increased after anti-TNF therapy

Cytokine production in LPS-stimulated WBCs was generally suppressed in RA patients as compared with healthy controls, and the extent of suppression was more pronounced in non-responders. We further investigated whether cytokine production in stimulated WBCs could be reversed after anti-TNF treatments. In the analysis of 14 patients (7 treated with infliximab, 7 with etanercept), all cytokine production significantly increased after therapy [median (IQR) of TNF-α 16.8 (7.5–24.8) vs 142 (38.0–431) pg/ml, P = 0.002; IL-1β 5.9 (1.0–10.6) vs 30.9 (7.1–84.5) pg/ml, P < 0.001; IL-6 122 (50.6–275) vs 1061 (385–2286) pg/ml, P = 0.004] (Fig. 2). A significant difference in the trends was not detected between infliximab and etanercept.

Discussion

In RA patients, response to TNF blockers may be associated with a profile of cytokine production unique to each individual. Using LPS-stimulated WBCs, we found that IL-1β production was lower in non-responders than in responders (P = 0.048). The sensitivity and specificity of IL-1β (cut-off value 4.84 pg/ml) as a predictor for response to biologics was 78.1 and 77.8%, respectively. Thus we conclude that IL-1β measurement in LPS-stimulated WBCs is useful in predicting response to anti-TNF therapies.

It is unclear why IL-1β was more suppressed in non-responders. In this study, IL-1β synthesis was lower in RA patients than in healthy controls, largely consistent with previous reports [12, 13]. Of note, IL-1β production significantly increased after anti-TNF therapies. This suggests that immune dysregulation associated with inadequate production of IL-1β may be more pronounced in non-responders. Several possibilities might explain this dysregulation. First, cellular pathways causing IL-1β production in circulating monocytes might be suppressed by negative feedback after long-term exposure to large amounts of serum inflammatory cytokines, presumably produced from inflamed joints. Secondly, monocytes preferentially producing IL-1β may have been depleted from the circulation after migration to inflamed joints. Thirdly, there may be as yet unidentified factors directly inhibiting the capacity of monocytes to produce IL-1β.

In this study, IL-1β production significantly increased after anti-TNF therapies. Popa et al. [13] also previously examined IL-1β and IL-6 production in LPS-stimulated WBCs after 3 months of anti-TNF therapy. However, in contrast to our results, cytokine production did not change significantly. Although it is difficult to explain this...
discordance, it might have resulted in part from methodological differences; for example, dose or quality of LPS.

Regarding the use of MTX and corticosteroids, baseline use of MTX was significantly infrequent in non-responders. We analysed the effect of MTX on cytokine production in patients in this study. All cytokine production was comparable between patients on \( n = 32 \) and off \( n = 9 \) MTX (supplementary Fig. 2, available at Rheumatology Online). MTX has also been reported to slightly reduce monocytic cytokine production \([15, 16]\). Taken together, it is unlikely that this baseline difference resulted in lower IL-1 synthesis in non-responders. It is difficult to assess the effect of corticosteroids, as the average dose was significantly higher, but use was less frequent in non-responders.

In conclusion, this study suggests that IL-1\( \beta \) measurement in LPS-stimulated WBCs is useful to predict the response to anti-TNF therapy. As limitations, response to TNF inhibitors was defined after a relatively shorter duration of therapy. To examine the real efficacy, other assessments, such as functional and imaging studies of longer observation are warranted. In this context, although patients were followed up prospectively, this study has a cross-sectional aspect. In addition, the number of included patients was small and non-responders were only nine. To confirm the result observed in this study, longitudinal studies with a larger number of patients need to be examined.

### Rheumatology key messages

- IL-1\( \beta \) measurement in LPS-stimulated WBC is useful in predicting responsiveness to anti-TNF agents.
- Cytokine production capacity in LPS-stimulated WBC is up-regulated by biologics.

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### Supplementary data

Supplementary data are available at Rheumatology Online.

### References


