Pretreatment Cytokine Profiles of Peripheral Blood Mononuclear Cells and Serum from Patients with Rheumatoid Arthritis in Different American College of Rheumatology Response Groups to Methotrexate

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ABSTRACT. ***Objective.*** To determine the role of putative target cytokines for methotrexate (MTX) treatment in patients with rheumatoid arthritis (RA) as predictors for treatment outcome.

Methods. Fifty consecutive patients with RA were characterized according to demographic and disease associated features and followed prospectively before and after 6 months of treatment with MTX. Before starting MTX treatment, serum was obtained from each patient and peripheral blood mononuclear cells (PBMC) were isolated. PBMC were cultured 2 days under resting conditions, and interleukin 1 receptor antagonist (IL-1ra), IL-1ß, soluble tumor necrosis factor receptor p55+75 (sTNFR p55+p75), and TNF-α release into cell culture supernatants and corresponding serum cytokine levels were determined by specific ELISA. Constitutive production and circulating levels of cytokines and cytokine inhibitors were correlated to the clinical response after 6 months of MTX treatment, and patients were categorized into 4 different groups according to the American College of Rheumatology (ACR) response criteria (ACR < 20, 20–50, 50–70, > 70% improvement from baseline).

Results. Good (ACR 50–70) or excellent (ACR > 70) responses to MTX treatment were seen in groups of patients with a higher proportion of males (25 and 43%) associated with a significantly lower ratio of IL-1ra/IL-1ß ($p < 0.00001$) constitutively produced by PBMC (ratio < 100) compared with nonresponding (ACR < 20) patients (males 7.7%; ratio > 100). The ratios in 3 female poor responders (ACR 20–50) were in between. The decreased ratios of IL-1ra/IL-1ß in most good and excellent responders were due to an enhanced constitutive IL-1ß release from PBMC ($p < 0.004$) compared to the groups of non or poor responders. Much less pronounced, there was a slightly significant increase of sTNFR p55 shedding from PBMC and increase of sTNFR p75 serum levels in good and excellent responders (both $p < 0.02$). In contrast, there were no intergroup differences regarding constitutive IL-1ra release, sTNFR p75 shedding, and IL-1ra and sTNFR p55 serum levels and various demographic and disease associated characteristics of patients.

Conclusion. Determination of cellularly produced IL-1ß and even more of the IL-1ra/IL-1ß synthesis in PBMC may be useful to predict the outcome of RA patients undergoing treatment with MTX and may characterize a subset of RA that is more responsive to IL-1 directed therapeutic interventions. (J Rheumatol 2003;30:28–35)

Key Indexing Terms:
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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of unknown origin that is associated with substantial morbidity[1,2] and increased mortality[3-6]. Although the introduction of efficient immunomodulatory agents like methotrexate (MTX) and of biological agents like anti-tumor necrosis factor-α (TNF-α) directed monoclonal antibodies or soluble TNF receptors into the treatment cascade has improved the inflammatory and destructive course of the disease in many cases, a considerable proportion of patients does not respond to a satisfactory extent to either treatment. Predictors of treatment outcome for individual patients have not been defined. Consequently, the choice of second line drugs in early disease, where radiological progression and resulting functional impairment can most probably be halted[7,8], remains very often arbitrary, and is usually based on empirical values of the treating physician, or may be at best determined by the severity of the clinical phenotype of RA. The lack of reliable prognostic markers regarding patients' outcome undergoing individual drug treatment regimens leads

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to the unsatisfactory practice of trial and error in most cases. This circumstance often prevents the initiation of immediate and longterm effective suppression of inflammatory disease activity, and thereby leads to chronic active disease, with the development of joint destruction and deformity. Although there are some indicators of severe disease such as initial rapid polyarticular onset of disease[9,10], high titer of rheumatoid factor (RF) at the beginning[9,11,12], early erosions[8,13], early functional impairment[13], high C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR)[11,14], or the presence of "shared epitopes" in distinct HLA-DR alleles[11,14], the detection of such indicators may indeed lead to early and more aggressive treatment, but will fail to predict the individual patient's reponse to a specific therapy. Because all second line drugs currently used in the treatment of RA have a distinct risk/benefit ratio and because very effective biological agents are far too expensive to treat all RA patients and do not elicit a satisfactory therapeutic response in all individuals, there is urgent need for an individually tailored treatment that suppresses inflammatory disease activity and radiological progression most effectively, has an optimal risk/benefit ratio, and is associated with acceptable socioeconomic costs in an individual patient. We investigated such prognostic factors of treatment outcome in response to a distinct second line drug. Based on our finding[15] that MTX altered the regulation of the IL-1ra/IL-1ß ratio in peripheral blood mononuclear cells (PBMC) we analyzed a cohort of patients with RA followed prospectively during MTX treatment; specifically we examined levels of IL-1ß, TNF-α, and their natural inhibitors for a predominant role in the unbalanced cytokine network of RA[16] in serum and in PBMC cultures before starting treatment; we wished to determine if the pretreatment cytokine profiles might predict the clinical course of disease.

MATERIALS AND METHODS

Patients. Fifty consecutive patients with definite RA according to American College of Rheumatology (ACR) criteria[17] and active disease who had previously failed to respond to 1–3 conventional disease modifying antirheumatic drugs (DMARD) were given MTX (15 mg, once weekly, injected subcutaneously or intramuscularly) and were followed prospectively over 24 weeks. Active RA was defined by fulfilment of at least 3 of the following 4 criteria: ≥ 6 joints tender or painful on motion, ≥ 3 swollen joints, ESR > 28 mm/h, and morning stiffness > 45 min duration. MTX and dosage of concomitant nonsteroidal antiinflammatory drugs (NSAID) and steroids (< 10 mg prednisone/day) were kept constant during the first 6 months of treatment. During the study, clinical assessment was performed before and after 12 and 24 weeks of MTX treatment. Laboratory assessments before and during treatment included ESR, routine hematology, erythrocyte folinic acid, serum transaminases, alkaline phosphatase, creatinine, and detection of serum autoantibodies such as IgM rheumatoid factors (RF) and antinuclear antibodies (ANA).

After 24 weeks of MTX at a constant weekly dosage of 15 mg the patients were divided into 4 response groups according to the ACR response criteria[18]. Patients showing improvement of > 70% compared to baseline were defined as "excellent responders," those with an improvement of > 50% but < 70% as "good responders" (ACR 50–70), patients with improvement of > 20% but < 50% as "poor responders" (ACR 20–50), and all patients with improvement < 20% compared to baseline as "nonresponders."

Those patients who started MTX but had to be withdrawn from treatment within the study period because of severe or untolerable side effects were excluded from evaluation because the level of response at the standardized time points could not properly be determined. The numbers of dropout patients were equally distributed over the different response groups, except for the small group of 3 patients belonging to ACR 20–50 (ACR < 20: 23%; ACR 20–50: 33%; ACR 50–70: 20%; ACR > 70: 21%).

Cells. Venous blood was drawn from patients before the first injection of MTX and PBMC were isolated by Ficoll-Hypaque fractionation[19]. The cells were washed 3 times with phosphate buffered saline (PBS) and resuspended in medium (10^6 cells/ml). The number of monocytes was determined by differential counting after staining for nonspecific esterase[20]. Monocyte counts in PBMC preparations of patients ranged between 17 and 38% before treatment, and no statistically significant differences were observed between the 4 response groups. Cells (2×10^5) in 0.2 ml RPMI 1640 supplemented with 100 IU/ml penicillin/streptomycin (Gibco, Basel, Switzerland) and 1% pasteurized plasma protein solution (5% PPL, Swiss Red Cross, Berne, Switzerland) were incubated in flat-bottom microtiter plates (Nunc, Roskilde, Denmark) in a humidified atmosphere of 5% CO_2 at 37°C for 48 h without any further stimuli. Cell-free culture supernatants were collected and stored at –70°C until use.

Cytokine assays. IL-1ß was measured by a 2-site directed ELISA with an exclusion limit of 10 pg/ml[21]. IL-1ra was determined by ELISA using a monoclonal antibody and rabbit antiserum, with a lower detection limit of 20 pg/ml[22]. TNF-α was assessed by a specific ELISA with a sensitivity of 20 pg/ml[23]. Soluble TNF receptors (p55 and p75) were measured by an enzyme linked binding assay with a sensitivity of 100 pg/ml[24].

Statistics. Intergroup comparisons were assessed by the extended Wilcoxon test for trends. Results were considered statistically significant at $p < 0.05$.

RESULTS

Characteristics of patients before MTX treatment. Fifty consecutive and prospectively followed patients with RA were classified as excellent (ACR > 70; n = 14), good (ACR 50–70; n = 20), or poor responders (ACR 20–50; n = 3) or nonresponders (ACR < 20; n = 13) according to the ACR response criteria to second line drugs[18] after 24 weeks of treatment with MTX. Table 1 shows the patients' various demographic and disease associated variables. There were no intergroup differences regarding age, ESR, swollen and tender joint counts, physicians' and patients' global assessment of disease activity, number of prior DMARD treatments, and number of patients using concomitant NSAID therapy. Only the small group of poor responders had no confounding prednisone treatment. However, this group was too small compared to the others to conclude that the percentage of erosions had an inverse correlation with steroid use. Men were more frequent in groups of patients with ACR 50–70 and ACR > 70 response (25 and 43%) than in the ACR < 20 (7.7%) and ACR 20–50 group (0%). Patients with ACR 20–50 or 50–70 response had a slightly longer disease duration than patients belonging to the other groups. The cohort of excellent responders, with ACR > 70, had a lower frequency of RF positive individuals (35.7%) with lower RF titers, and lower frequency of those with erosive disease (28.6%) compared to patients belonging to the groups with ACR 50–70 (55% for RF+ and erosions), ACR 20–50 (66.7% for RF+ and 100% for erosions), or ACR < 20 (53.8% for RF+ and 38.5% for erosions). Antinuclear

Table 1. Characteristics of patients at study entry according to ACR response status at 24 weeks of methotrexate (MTX) treatment.

	ACR < 20, n = 13	ACR 20–50, n = 3	ACR 50–70, n = 20	ACR > 70, n = 14
Age, yrs	50.9 ± 13.8*	52.2 ± 5.7	55.9 ± 13.4	52.9 ± 18.5
Male/female	1/12	0/3	5/15	6/8
Duration of RA, (mo)	46.2 ± 45.6	96.4 ± 50.9	81.6 ± 78.3	47.6 ± 61.1
ESR, (mm/h)	36.9 ± 27.2	27.5 ± 10.6	32.7 ± 29.9	35.3 ± 34.6
Positive RF, (%)	53.8	66.7	55	35.7
Positive ANA, (%)	15.4	0	30	0
Swollen joint count	16.2 ± 8.9	13.5 ± 2.1	15.3 ± 10.3	14.9 ± 8.5
Tender joint count	18.2 ± 10.3	16.6 ± 2.1	17.7 ± 11.4	17.1 ± 9.1
Morning stiffness, (min)	103 ± 95	40 ± 17	77 ± 82	70 ± 74
Patient assessment (0–5)	3.4 ± 1.1	3.1 ± 0.9	3.0 ± 1.2	3.4 ± 1.3
Physician assessment (0–5)	3.1 ± 0.8	3.4 ± 1.2	3.1 ± 1.4	3.0 ± 0.9
Erosive disease, (%)	38.5	100	55	28.6
Prior DMARD, n	1.5 ± 0.7	2 ± 1	1.5 ± 0.6	1.3 ± 0.5
Current NSAID, (%)	100	100	100	100
Taking prednisone, < 10 mg/day, %	38.5	0	25	42.9

* Values are given as the mean ± SD.

antibodies (ANA) were found only in a small subgroup of patients with ACR < 20 (15.4%) and ACR 50–70 (30%). Morning stiffness was less pronounced in patients with ACR 20–50 compared to others. However, these intergroup differences did not reach statistical significance, which is likely related to the small sample size. Additional steroid use of < 10 mg prednisone/day were used in a similar number of patients belonging to the ACR < 20 (38.5%), ACR 50–70 (25%), and ACR > 70 (42.9%) groups, except for those very few patients (n = 3) with ACR 20–50 who did not receive steroids before starting treatment with MTX. This group of patients was the only to have an inverse correlation of the percentage of erosions and steroid use.

Response to MTX treatment according to ACR criteria. In prospectively following patients using MTX therapy, we found a maximal clinical effect in the majority of patients after a treatment period of 24 weeks, in accord with previous studies[25,26]. Therefore this time point served as the cutoff for dividing patients into 4 different groups according to the ACR response criteria. Figure 1 shows the different response rates after 12 and 24 weeks of MTX treatment. After 24 weeks, 68% of patients achieved an ACR 50–70 or ACR > 70 response rate, as compared to 42% after 12 weeks of MTX treatment; 6% were poor responders (ACR 20–50) and nonresponders remained nearly constant during the whole study period.

Pretreatment cytokine production by PBMC in culture. Figure 2 shows the differences of constitutive IL-1ß production by PBMC of patients belonging to all groups. The intergroup comparison reveals a significant increase of spontaneous IL-1ß secretion in cell cultures of patients with ACR 50–70 and with ACR > 70 ($p < 0.004$). As shown in Figure 3, no statistically significant intergroup difference was observed for constitutive IL-1ra production. Strikingly, the strongest association with favorable response to therapy was found for IL-1ra/IL-1ß synthesis ratios of < 100 in the groups of patients with ACR 50–70 and ACR > 70 response ($p < 0.0001$), as shown in Figure 4. This prompted us to analyze predictive values for ratios of > 100 or < 100. Table 2 shows that an IL-1ra/IL-1 ratio of < 100 had a positive predictive value of 94% for good response and a ratio of > 100 a positive predictive value of 82% for bad response. A ratio of < 100 excluded a bad response in 91% of cases and a ratio of > 100 good responses in 88% of cases (negative predictive values).

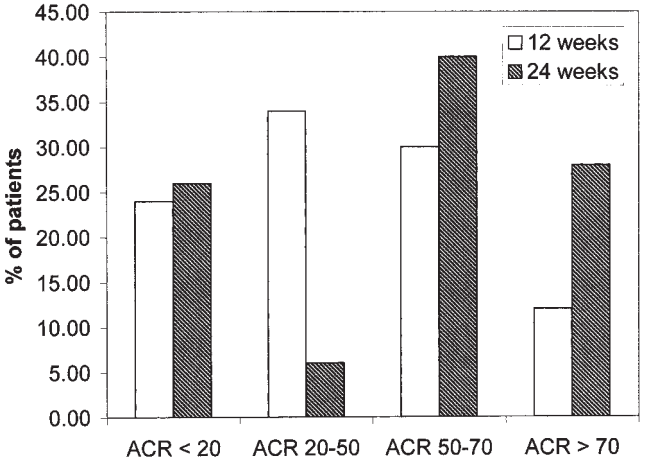

Figure 1. Response of patients with RA to MTX according to ACR criteria. Patients were followed 12 and 24 weeks taking MTX. After 12 and 24 weeks of treatment (15 mg MTX once per week subcutaneously or intramuscularly) patients were categorized into 4 different response groups (ACR < 20, ACR 20–50, ACR 50–70, ACR > 70 improvement from baseline before MTX treatment).

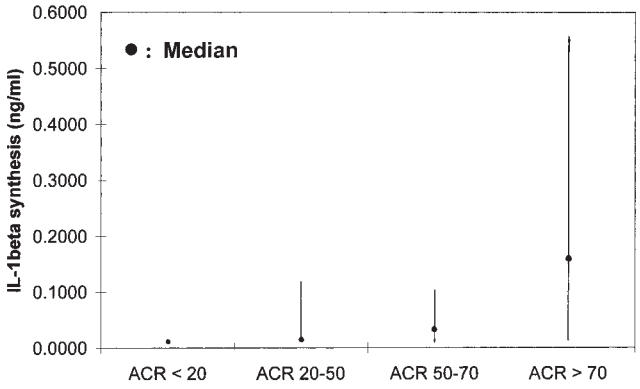

Figure 2. IL-1ß production by PBMC before treatment and ACR response to MTX after 24 weeks. PBMC of patients from each of 4 response groups were cultured for 2 days in RPMI + 1% PPL and constitutive release of IL-1ß was measured by ELISA in cell-free culture supernatants. Values are given as the median with interquartile range from triplicate cell cultures of RA patients with ACR < 20 (n = 13), ACR 20–50 (n = 3), ACR 50–70 (n = 20), and ACR > 70 (n = 14) response. Statistically significant intergroup difference is p = 0.0038.

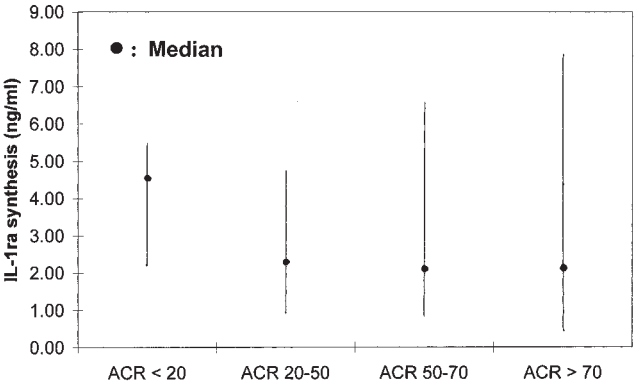

Figure 3. IL-1ra production by PBMC before treatment and ACR response to MTX after 24 weeks. PBMC of patients from each of 4 response groups were cultured for 2 days in RPMI + 1% PPL and constitutive release of IL-1ra in cell culture supernatants. Values are given as the median with interquartile range from triplicate cell cultures of RA patients with ACR < 20 (n = 13), ACR 20–50 (n = 3), ACR 50–70 (n = 20), and ACR > 70 (n = 14) response. Intergroup difference is statistically insignificant (p = 0.24).

Constitutive TNF-α production was not detectable in PBMC cultures, and values for sTNFR p55 shedding from the cell surface into the culture medium differed only slightly (p < 0.02; Figure 5) and those of sTNFR p75 did not differ (Figure 6), between the 4 distinct response groups. For comparison, corresponding cytokine and cytokine inhibitor production was previously measured in PBMC cultures of healthy controls, and was found to be different at least from patients with active RA responding to MTX[15].

Pretreatment circulating cytokine levels. Circulating levels of IL-1ß and TNF-α were undetectable in the sera of the majority of patients we studied before they started treatment with MTX. Thus only circulating levels of the cytokine inhibitors

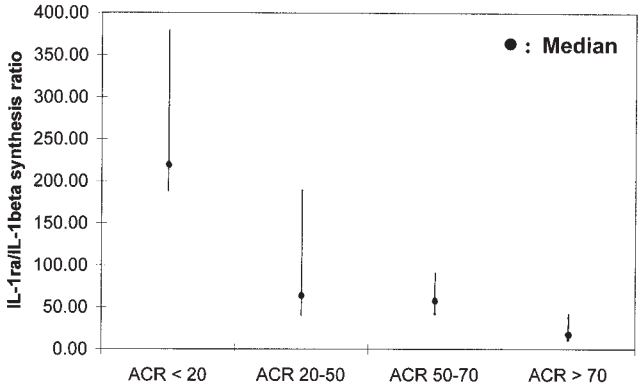

Figure 4. IL-1ra/IL-1ß synthesis ratio by PBMC before treatment and ACR response to MTX after 24 weeks. Corresponding cytokine ratio levels were determined after 24 weeks of MTX treatment in patients from each of 4 response groups. Values are given as the median with interquartile range from triplicates of cell cultures from patients with ACR < 20 (n = 13), ACR 20–50 (n = 3), ACR 50–70 (n = 20), and ACR > 70 (n = 14) response. Statistically significant intergroup difference is p < 0.00001.

Table 2. Predictive values of IL-1ra/IL-1ß synthesis ratios > 100 and < 100 for response and nonresponse of RA patients to MTX treatment.

	IL-1ra/IL-1ß	
	> 100 (Bad Response)*	< 100 (Good Response)**
Positive predictive value, %	82.35	93.94
Negative predictive value, %	87.50	91.18

* Bad response = ACR < 20. ** Good response = ACR 50–70 and ACR > 70.

IL-1ra and sTNFR p55 and p75 are presented in Figures 7, 8, and 9. Finally, comparing the 4 different response groups, a statistically significant trend to increased sTNFR p75 levels was associated with better response to treatment (p < 0.02; Figure 9). In contrast, circulating serum levels of IL-1ra and sTNFR p55 did not differ significantly (Figures 7 and 8).

DISCUSSION

In this study an IL-1ra/IL-1ß synthesis ratio lower than 100 of unstimulated PBMC before treatment was strongly associated with a good or excellent response of patients with RA to MTX. There is some evidence that demographic and disease associated data such as disease duration[27], prior DMARD use[28], disease functional class[10], and disease activity[10,27] have effects on the likelihood of patients' response to treatment. Even some biological/immunological markers may predict and/or rapidly change with clinical response to treatment. These include serum levels of soluble CD30[29] and of matrix metalloproteinase 3[30]. Recently, we have shown that circulating levels of cytokine inhibitors like IL-1ra and sTNFR p55 and p75 as well as the spontaneous production of IL-1ß and

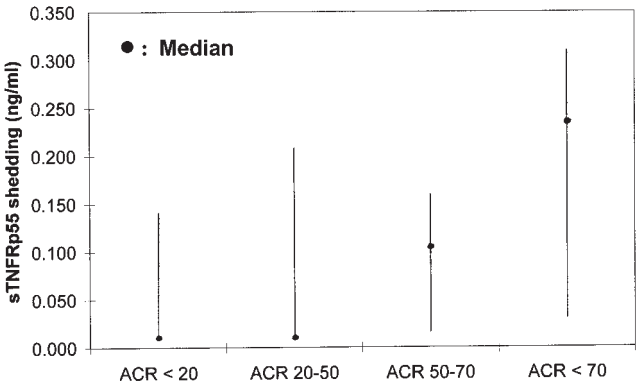

Figure 5. sTNFR p55 shedding from PBMC into cell culture medium before treatment and ACR response to MTX after 24 weeks. PBMC from patients in each response group were cultured for 2 days in RPMI + 1% PPL and constitutive shedding of sTNFR p55 from PBMC into cell culture medium was measured by ELISA. Values given as the median with interquartile range from triplicate cell cultures of RA patients with ACR < 20 (n = 13), ACR 20–50 (n = 3), ACR 50–70 (n = 20), and ACR > 70 (n = 14) response. Statistically significant intergroup difference is p = 0.021.

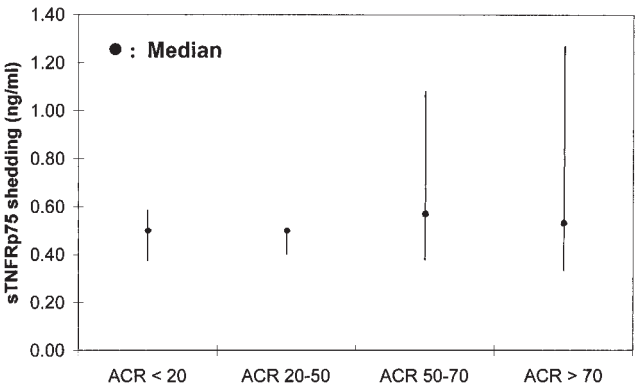

Figure 6. sTNFR p75 shedding from PBMC into cell culture medium before treatment and ACR response to MTX after 24 weeks. PBMC from patients in each response group were cultured for 2 days in RPMI + 1% PPL and constitutive shedding of sTNFR p75 from PBMC into cell culture medium was measured by ELISA. Values are given as the median with interquartile range from triplicate cell cultures of RA patients with ACR < 20 (n = 13), ACR 50–70 (n = 3), ACR 50–70 (n = 20), and ACR > 70 (n = 14) response. Intergroup difference is statistically insignificant (p = 0.573).

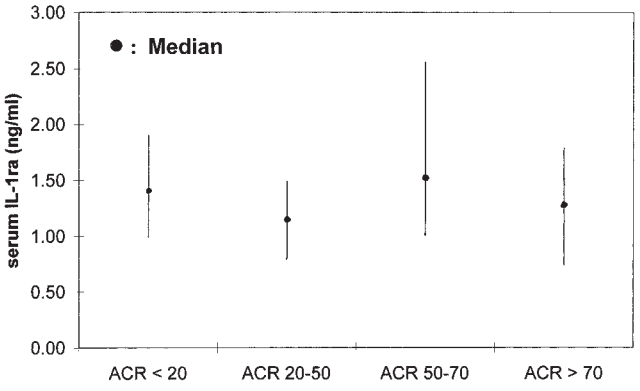

Figure 7. IL-1ra serum levels before treatment and ACR response to MTX after 24 weeks of treatment. IL-1ra serum levels were measured by ELISA in patients from each of 4 response groups. Values are given as the median with interquartile range from triplicates of serum samples of patients with ACR < 20 (n = 13), ACR 20–50 (n = 3), ACR 50–70 (n = 20), and ACR > 70 (n = 14) response. Intergroup difference is statistically insignificant (p = 0.742).

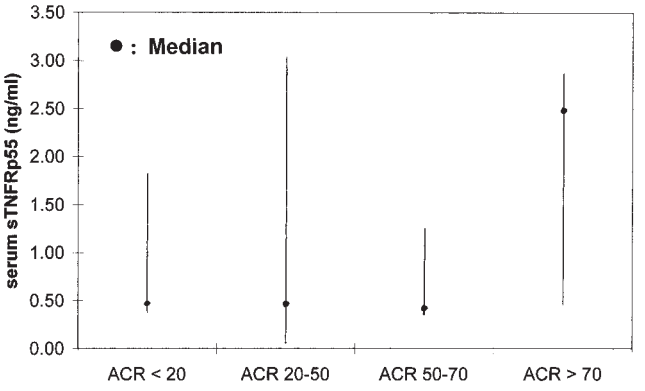

Figure 8. sTNFR p55 serum levels before treatment and ACR response to MTX after 24 weeks. Serum levels of sTNFR p55 were measured by ELISA in patients from each of 4 response groups. Values are given as the median with interquartile range from triplicates of serum samples of patients with ACR < 20 (n = 13), ACR 20–50 (n = 3), ACR 50–70 (n = 20), and ACR > 70 (n = 14) response. Intergroup difference is statistically insignificant (p = 0.19).

IL-8 by PBMC are markedly correlated to RA disease activity upon MTX treatment[31]. Finally, reviewing all these biological markers examined so far, most of them correspond well to inflammatory disease activity during the course of treatment, but fail to predict an individual treatment outcome upon a defined therapeutic intervention.

Such predictors, however, are urgently awaited for making specific therapeutic decisions at the very beginning of RA in order to prevent otherwise irreversible structural and/or functional damage. This is particularly important regarding the application of biological agents such as TNF inhibitors and others, which are very effective in many, but not all, patients with RA, and which impose an economic burden that has to be balanced carefully against the individual disease prognosis.

This study was motivated by our previous findings that MTX dampened the inflammatory type of peripheral blood monocyte as indicated by a marked upregulation of the IL-1ra/IL-1ß synthesis ratio in PBMC as a consequence of either IL-1ß downregulation or simultaneous IL-1ra stimulation, or counterregulating effects on both of them during clinical improvement[15,31]. From these observations and others[32] we concluded that MTX might exert coordinated antiinflammatory effects mainly based on counterregulatory effects on pro and antiinflammatory monokines. As a consequence, we hypothesized that a highly inflammatory type of monocyte with a particularly low IL-1ra/IL-1ß synthesis ratio is a pre-

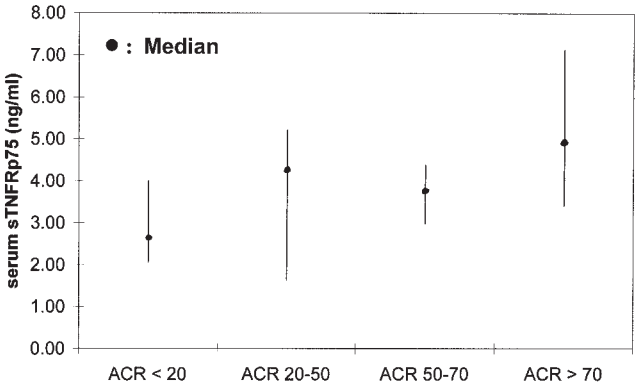

Figure 9. sTNFR p75 serum levels before treatment and ACR response to MTX after 24 weeks. Serum levels of sTNFR p75 were measured by ELISA in patients from each of 4 response groups. Values are given as the median with interquartile range from triplicates of serum samples of patients with ACR < 20 (n = 13), ACR 20–50 (n = 3), ACR 50–70 (n = 20), and ACR > 70 (n = 14) response. Statistically significant intergroup difference is p = 0.015.

requisite for MTX to work efficiently in both pharmacobiological and clinical terms in an individual patient with RA.

Following this hypothesis, we examined if the presence of this inflammatory type of blood monocyte coincided with a particularly high degree of inflammatory disease activity in both clinical and biological terms, because the decision about starting MTX treatment in an individual patient with RA is mostly based on clinical disease activity and/or radiological progression rather than determined by distinct biological and/or immunological factors.

Information about reliable predictors of treatment outcome in RA patients undergoing MTX treatment is very rare. There are indications that either disease duration[33] or IL-4 positive CD4+ T cells at disease onset[34] might be useful prognostic markers.

Considering various demographic and disease associated factors at study entry, we found that the degree of response and the associated IL-1ra/IL-1ß synthesis ratios were independent of such variables as age, disease duration, inflammatory disease activity (swollen and tender joint counts, morning stiffness, ESR, physician and patient global assessment), concurrent drugs, previous DMARD treatment, and immunological markers like RF and ANA, as well as erosiveness. There was only a slight and statistically insignificantly lower number of RF+ patients with less erosive disease in the group of excellent responders to MTX with the lowest ratio of IL-1ra/IL-1ß before starting MTX, which might hint at a better prognosis of this group of patients, being compatible with previous studies[8,9,11-13]. It cannot be ruled out that undetectable differences between the compared groups were due to the relatively small sample sizes. However, among patients' characteristics the most striking demographic feature of the group of good and excellent responders with low pretreatment IL-1ra/IL-1ß ratios of < 100 was the higher proportion of men (25 and 43%, respectively) compared to the groups of poor responders or nonresponders (7.7 and 0%). This gives rise to the assumption that male patients with RA might be particularly promising candidates for successful MTX treatment. This has not yet been reported for MTX or for other DMARD treatment in RA, and certainly deserves more detailed analysis in prospective studies.

Another striking clinical finding in this study was the high percentage (68%) of good or excellent clinical responders to MTX after 24 weeks of treatment. That response rate is obviously higher than observed in other RA cohorts elsewhere. A plausible explanation for this might be that the patients in this study were all treated with parenteral MTX, which allowed 100% bioavailability of the drug, in contrast to oral MTX, which shows a highly and interindividually variable bioavailability[35]. Our patients are started and maintained as long as possible on parenteral MTX, in contrast to common clinical practice and most controlled clinical trials[26,36,37].

Moreover, although this is a post hoc analysis the findings of this study fit well to our previous observation of an upregulation of the IL-1ra/IL-1ß ratio above 100 during treatment in those patients showing an ACR > 50 response to MTX[15]. Based on the results of that study and the present study one could argue that MTX is most effective in RA patients that on the cellular level present blood monocytes with a high degree of inflammatory activity and a disease process driven by IL-1. This argument is supported by the fact that an elevated constitutive IL-1ß release from PBMC essentially contributed to the lowered IL-1ra/Il-1ß synthesis ratio and was associated with good or excellent response to MTX treatment. These data may also suggest that IL-1 receptor antagonist treatment will work less well in patients not responding to MTX and showing an IL-1ra/IL-1ß synthesis ratio > 100.

Because constitutive TNF-α release from PBMC was not detectable in our patients, we have no additional information about what the sTNFR p55 and p75/TNF-α synthesis ratio could have contributed to the prediction of MTX treatment outcome. The levels of sTNFR p55 and p75 detected in RA PBMC cultures or patients' sera most likely did not or only slightly corresponded to patients' therapeutic responses. These findings lead us to the assumption that TNF-α is not a very likely candidate to be targeted by MTX in treatment of RA.

We do not think that our findings are specific for MTX but could also be applicable to other DMARD or biologics targeting IL-1ß, such as gold compounds, sulfasalazine, or IL-1ra. To prove this it would be necessary to evaluate the predictive value of the IL-1ra/IL-1ß ratio for response to other treatments in a large cohort of patients, which was not feasible in our study.

Considering the phenotypic and evolutional heterogeneity of RA as well as the wide variability of responses to defined DMARD therapy and biologics such as anti-TNF and anti-IL-1 in individual patients, in addition to the prediction of treatment outcome, the data generated in this study allow the fol-

lowing conclusion: a good or excellent therapeutic response to MTX characterizes a subgroup of RA patients in which the pathogenesis of the disease is probably driven by IL-1. This conclusion has to be verified by other features of the disease process like joint destruction, which seems mainly mediated by IL-1, at least in animal models of chronic arthritis[38,39]. Our study does not confirm this because the group with the best therapeutic response of ACR > 70 and the proposed IL-1 driven disease process had the lowest number of patients with erosive disease. Obviously, there must also be some other determinants of erosiveness in RA not related to IL-1.

In summary, the data presented here suggest that the cellular IL-1ra/IL-1ß synthesis ratio (1) should be determined before starting MTX treatment in an individual patient with RA; (2) does not coincide with conventional clinical and laboratory measures of inflammatory disease activity in RA, but represents an independent biological predictor of individual treatment outcome with MTX; (3) is indicative (at levels lower than 100) of an IL-1 driven disease pathogenesis in individual patients, allowing characterization of a particular RA subgroup more responsive to IL-1 directed therapeutic interventions; and (4) gives a further rationale for combination therapy with anti-TNF agents acting in a complementary fashion. Although the results of this study require confirmation in a larger prospective cohort of RA patients undergoing MTX treatment, it would also be of interest to look systematically for independent biological/immunological predictors of treatment outcome on the cellular level in other DMARD and/or biologics. To make this search feasible for clinical routine we would need to rely more on pharmacogenomic studies evaluating individual pathogenetically relevant factors, i.e., specific cytokine and cytokine promotor gene polymorphisms, before starting treatment with specific DMARD, biologics, or suitable combinations in individual patients with RA.

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