CIRCULATING SOLUBLE TUMOR NECROSIS FACTOR RECEPTORS, INTERLEUKIN-2 RECEPTORS, TUMOR NECROSIS FACTOR α, AND INTERLEUKIN-6 LEVELS IN RHEUMATOID ARTHRITIS

Longitudinal Evaluation During Methotrexate and Azathioprine Therapy

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Objective. **To assess whether circulating concentrations of soluble tumor necrosis factor receptors (sTNFR; p55 and p75), soluble interleukin-2 receptors (sIL-2R), tumor necrosis factor α (TNFα), and interleukin-6 (IL-6) reflect clinical response and whether changes are dependent on the drug used in rheumatoid arthritis (RA) patients taking methotrexate (MTX) or azathioprine (AZA).**

Methods. **These cytokines and soluble receptors were assessed in 20 control subjects and serially for up to 48 weeks in 61 RA patients, by bioassay (IL-6) and immunoassays (sTNFR, sIL-2R, TNFα, and IL-6).**

Results. **Concentrations of p55 and p75, sIL-2R, and TNFα (but not IL-6) were significantly higher in RA patients than in controls. Significant decreases in sIL-2R and p55 concentrations were associated with clinical improvement and were observed in patients treated with MTX, but not AZA. Both treatments induced decreases in IL-6 concentrations, but circulating AZA (or its metabolites) appears to interfere with the measurement of IL-6 bioactivity. TNFα and p75 levels did not show significant changes.**

Conclusion. **Measurement of circulating sIL-2R, p55, and IL-6 may be useful in the evaluation of RA disease activity and response to therapy. Interference by circulating levels of drugs must be ruled out when bioassays are used to evaluate cytokine levels.**

Accumulating evidence indicates that a number of cytokines are crucial in the pathogenesis of rheumatoid synovitis and other autoimmune diseases. Cytokines such as interleukin-1 (IL-1), tumor necrosis factor α (TNFα), and IL-6 have been detected in rheumatoid synovium (1–8) and elevated concentrations have been found in synovial fluid (SF) and serum of rheumatoid arthritis (RA) patients (9,10). Soluble receptor forms of several cytokines have been described. The soluble IL-2 receptor (sIL-2R) is a truncated form of the IL-2 receptor α chain (11). Levels of sIL-2R are markedly elevated in autoimmune diseases

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and are considered to be an activation marker of the immune system (12). Two types of TNF cell receptors have been identified (p55 and p75); both can be shed from the cell surface, yielding soluble fragments (13,14). These soluble receptor forms (sTNFR) function as TNF antagonists in vitro, although they may also prolong TNF activity by protecting it against degradation (15).

In RA, concentrations of IL-6 (16–18), sIL-2R (19), and sTNFR (20) are higher in SF than in serum, suggesting that the inflamed joint is the main production site of these mediators. However, specimens for measurement in the circulation are easily obtained and more suitable for longitudinal studies. The effects of different disease-modifying antirheumatic drugs (DMARDs) on circulating cytokines and their soluble receptors may be helpful in understanding their mechanism of action. So far, these effects have been evaluated mostly in cross-sectional or open studies, but comparative studies with different DMARDs are sparse.

In a recent randomized double-blind trial conducted in our center, methotrexate (MTX) proved to be superior to azathioprine (AZA) in the treatment of active RA (21,22). In the present study, circulating concentrations of sTNFR, sIL-2R, TNFα, and IL-6 were serially measured in patients included in the trial conducted by Jeurissen et al (21). The aim was to assess whether these concentrations (a) reflected differences in clinical response, (b) correlated with each other and with clinical and laboratory parameters of disease activity, and (c) were differentially influenced by the drugs used.

PATIENTS AND METHODS

Patients. The details of the study have been extensively described (21,22). Briefly, 64 patients with active definite or classic RA entered a 48-week, prospective, randomized, double-blind trial of MTX versus AZA.

Active RA was defined by the presence of at least 3 of the following 4 criteria: ≥6 joints tender or painful on motion, ≥3 swollen joints, erythrocyte sedimentation rate (ESR) ≥28 mm/hour, and morning stiffness ≥45 minutes in duration. Patients were randomly assigned to receive either MTX (initial dosage 7.5 mg weekly) or AZA (initial dosage 100 mg daily). Dosages of nonsteroidal antiinflammatory drugs (NSAIDs) and prednisone (≤10 mg/day) were stable for at least 4 weeks before study entry. At study entry 8 patients taking AZA and 2 taking MTX had been on a stable regimen of low-dose prednisone (≤10 mg/day). During the study period, the mean corticosteroid dosage did not change in any patient, but the NSAID dosage was increased in 3 patients (2 taking AZA and 1 taking MTX). Control sera were obtained from 20 healthy laboratory personnel working in our center.

Cytokine assays. Sera had been collected for subsequent analysis, along with concomitantly obtained data from clinical and laboratory evaluations, as part of the trial conducted by Jeurissen et al (21).

Sera were kept frozen at −20°C until assay. Cytokine levels were measured before treatment and at various intervals up to 48 weeks of treatment. To minimize interassay variations, all samples from each patient were measured in a single assay. Levels of sIL-2R were measured using a "sandwich" enzyme-linked immunosorbent assay (ELISA) (Cell-free; T Cell Sciences, Cambridge, MA), according to the manufacturer's instructions. Results are expressed in units/ml relative to a set of standards provided with the test kit. The sensitivity of this assay is 50–100 units/ml.

TNFα was assessed by radioimmunoassay (RIA), as previously described (23). This RIA measures total TNFα (both free and complexed to its receptors), as demonstrated by the lack of interference of the addition of up to 5 ng/ml recombinant sTNFR (p55 and p75) to sera containing known amounts of TNFα (Barrera P et al: unpublished observations). The sensitivity of this RIA is between 10 and 100 pg/ml (average 70 pg/ml).

IL-6 was measured by bioassay and ELISA. The IL-6 bioassay was performed as previously described (24). Briefly, 5,000 B9 cells were seeded in Iscove's modified Dulbecco's medium containing 5% fetal calf serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and β-mercaptoethanol ($5 \times 10^{-5} M$) in 96-well, flat-bottom microtiter plates (Costar, Badhoevedorp, The Netherlands) in the presence of serum samples or recombinant IL-6 standard (kind gift of Dr. L. A. Aarden, CLB, Amsterdam, The Netherlands). Cell proliferation was measured by thymidine incorporation after 64 hours (7.4 kBq ^{3}H-thymidine/well). Samples were heat inactivated for 30 minutes at 56°C, and tested in 2-fold dilutions. Half-maximal thymidine incorporation was defined as 1 unit/ml IL-6. The sensitivity of this assay is 0.3 units/ml.

The IL-6 ELISA (materials were a gift from Dr. J. Wijdenes, Innotherapy, Besançon, France) was performed as follows. Flat-bottom microtiter plates were coated for 24 hours at 4°C with anti–IL-6 monoclonal antibody (MAb) BE8 (7 μg/ml in phosphate buffered saline [PBS], 100 μl/well). The plates were washed, and serial dilutions of recombinant IL-6 and samples were added (100 μl/well) and incubated for 1 hour at 37°C. Plates were washed and incubated for 1 hour at room temperature with a second biotinylated anti–IL-6 MAb, BE4 (2 μg/ml in PBS with 0.25% bovine serum albumin [BSA], 100 μl/well). After washing, horseradish peroxidase–streptavidin was added (4 μg/ml in PBS with 0.25% BSA 100 μl/well), and plates were incubated for 45 minutes at room temperature. Plates were washed and developed with a solution of 47% OPD in 14 mM citric acid and 36 mM trisodium citrate (pH 5.2, 100 μl/well; Merck). The reaction was stopped by addition of 2.5M H_2SO_4 (50 μl/well). Plates were read at 492 nm in a Titertek Multiscan reader. The sensitivity of this ELISA is 20 units/ml.

Soluble TNF receptors (p55 and p75) were measured by enzyme-linked binding assay (Hoffmann–La Roche,

Table 1. Course of treatment and clinical outcome in patients treated with azathioprine or methotrexate*

Time of study	Azathioprine	Methotrexate
No. of patients enrolled	33	31
Week 24		
Continued therapy	20	30
No. (%) improved	12 (60)	23 (76)
Switched therapy	13	1
Reason	Side effects	Noncompliance
Week 48		
Continued therapy	12	25
No. (%) improved	9 (75)	21 (84)
Switched therapy	8	5
Reason	No effect	Side effects/no effect

* Improvement determined according to the overall index (see Patients and Methods for details).

Basel, Switzerland). This assay measures total (both free and bound) receptor concentrations, since addition of up to 10 ng/ml recombinant TNFα to serum containing known amounts of sTNFR does not influence the assay. The sensitivity of this assay is 100 pg/ml.

Definition of clinical improvement. The clinical outcome was evaluated by means of an overall index. This index was based on the same parameters used to define active RA for study inclusion: 1) patient's assessment of pain, using a visual analog scale (0 mm = no pain, 100 mm = very severe pain), 2) Ritchie articular index, 3) ESR, and 4) duration of morning stiffness. Improvement was defined as >30% reduction in at least 2 of these 4 values *and* no worsening of the other variables. Stable disease was defined as 0–29% reduction in the 4 variables. Worsening was defined as an increase in the 4 values compared with baseline (21,22). To simplify, we compared patients with improvement versus those with lack of effect (stable disease or worsening).

Statistical analysis. Unless otherwise specified, data are expressed as the median and range. Since the variables studied were not normally distributed, distribution-free methods were applied. Comparisons of sTNFR, sIL-2R, TNFα, and IL-6 concentrations during therapy with pretreatment concentrations were made by Wilcoxon signed rank test for paired samples. To utilize all data gathered during the followup, comparison between both therapies (MTX versus AZA) and between patients with clinical improvement versus those with lack of effect was analyzed by a distribution-free test for curve analysis (25). Comparison of therapy groups included only patients who completed 24 weeks and 48 weeks of treatment with the originally assigned drug. The analysis according to clinical response included all patients randomized into the study, irrespective of therapy. Correlations were evaluated using Spearman's rank correlation coefficient for each data point during the followup.

RESULTS

Clinical results. The clinical and radiographic findings in this study have been published elsewhere (21,22). Comparison between treatment groups showed significantly more improvement in the MTX group, both in clinical and laboratory measurements of disease activity. Patients treated with MTX showed a more rapid clinical improvement and less radiographic progression than did those treated with AZA. Switching to another therapy due to adverse reactions or lack of effect of the initial DMARD was more often necessary in the AZA group. Irrespective of the therapy, a total of 44 and 46 patients experienced clinical improvement after 24 and 48 weeks, respectively. The course of treatment and the clinical outcome are summarized in Table 1.

Pretreatment cytokine concentrations. Data on 3 patients (2 taking MTX and 1 taking AZA), 2 of whom showed clinical improvement, were excluded from analysis because no pretreatment sera were available. Baseline concentrations of sIL-2R ($P < 0.0001$), TNFα ($P < 0.02$), and the soluble TNF receptors p55 ($P = 0.0001$) and p75 ($P < 0.04$) were significantly higher in the RA patients than in the control group. In contrast, IL-6 bioactivity in the patients did not differ significantly from that in the controls (Table 2). TNFα was undetectable in 8 patients (1 MTX; 7 AZA) and in 10

Table 2. Concentrations of sTNFR (p55 and p75), TNFα, sIL-2R, and bioactive IL-6 in rheumatoid arthritis patients at baseline and in healthy controls*

	sTNFR				
Group (n)	p55 (pg/ml)	p75 (pg/ml)	TNFα (pg/ml)	sIL-2R units/ml	IL-6 units/ml
Patients (61)					
Median	3,000†	2,970‡	130‡	1,380†	11.6
Range	1,650–7,900†	0–15,700‡	0–330‡	365–19,800†	0.8–74.7
Controls (20)					
Median	1,310	2,380	55	300	8.5
Range	0–1,970	1,360–3,480	0–260	0–730	7–13.5

* sTNFR = soluble tumor necrosis factor receptor; sIL-2R = soluble interleukin-2 receptor.
† $P < 0.0005$ versus controls.
‡ $P < 0.05$ versus controls.

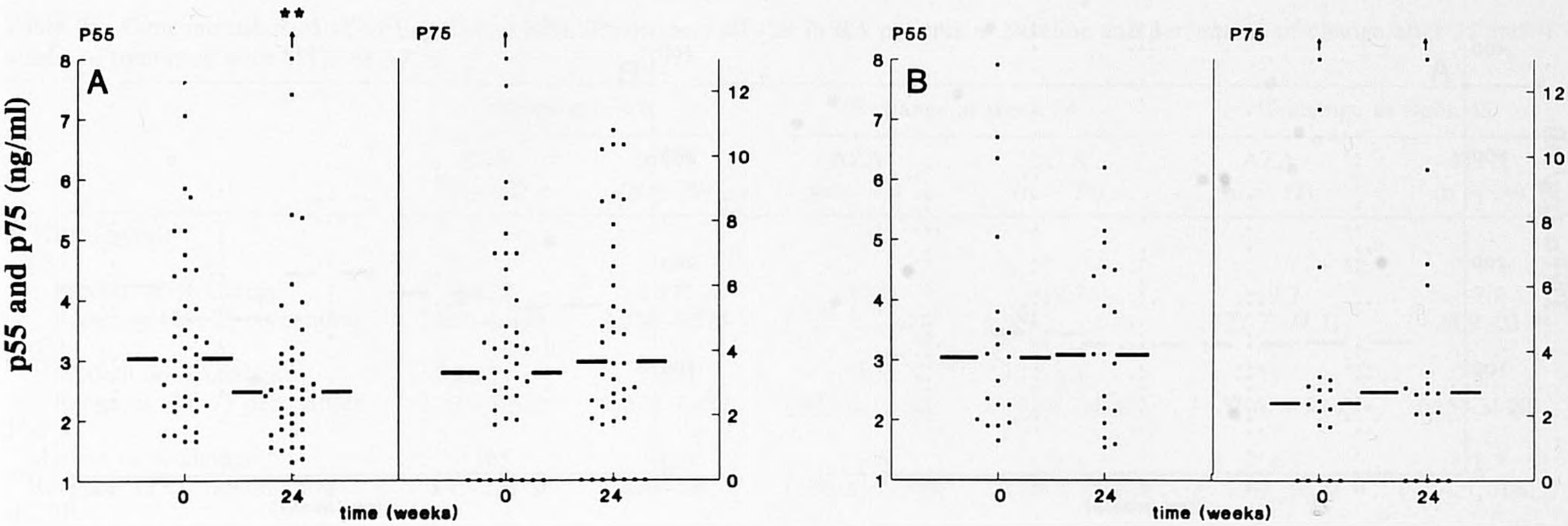

Figure 1. Concentrations of p55 and p75 soluble tumor necrosis factor receptors in the serum of rheumatoid arthritis patients with clinical improvement (**A**) and with lack of response to therapy (**B**) after 24 weeks. Horizontal bars show the median. ** = significant change from baseline ($P < 0.005$); ↑ = concentration above the range of the y axis.

controls; p75 was undetectable in 13 patients (6 MTX; 7 AZA). Pretreatment cytokine concentrations were similar in both treatment groups and in patients who showed clinical improvement or a lack of effect at the end of the study.

Cytokine concentrations during followup. The differences between patients with and without clinical improvement were examined. Since analysis after 24 and 48 weeks of treatment yielded similar results, only the data after 24 weeks are presented. At that time, 42 patients showed clinical improvement and 19 showed a lack of response. Significant decreases in p55 concentrations ($P < 0.005$) were observed only in the patients with clinical improvement; in contrast, p75 concentrations did not change significantly in either group (Figure 1). A steady decrease in sIL-2R concentrations ($P < 0.05$ from week 8 onward) occurred in the patients with clinical improvement, but (except for week 16) no significant change was observed in the patients with lack of response to therapy (Figure 2). Although TNFα concentrations did not decrease in either group over the followup period, lower levels were observed in the patients with clinical response compared with those with a lack of effect ($P < 0.02$)

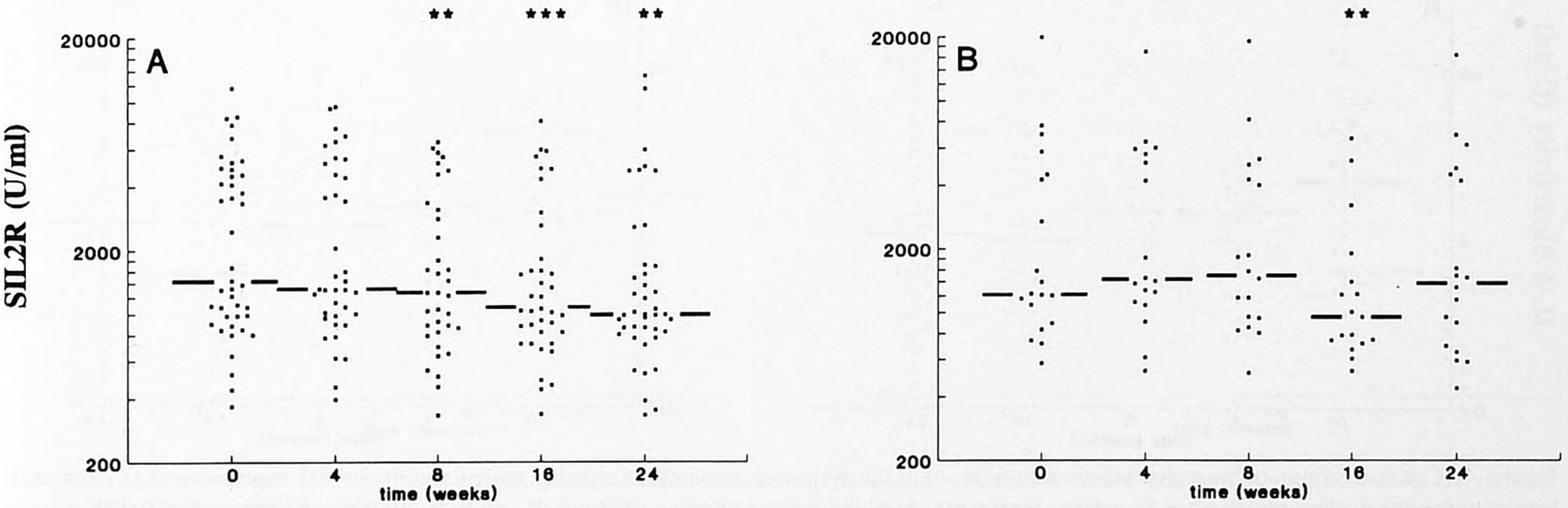

Figure 2. Concentrations of soluble interleukin-2 receptors (sIL2R) in the serum of rheumatoid arthritis patients with clinical improvement (**A**) and with lack of response to therapy (**B**) after 24 weeks. Horizontal bars show the median. ** = $P < 0.005$ and *** = $P < 0.0005$ versus baseline.

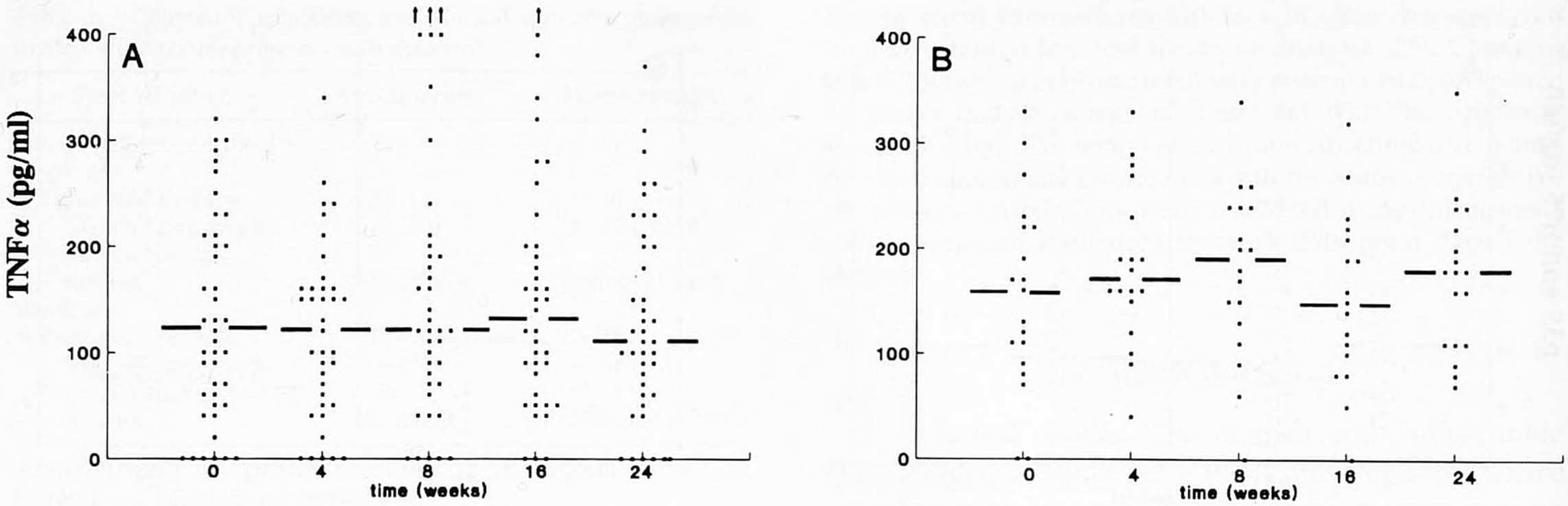

Figure 3. Concentrations of tumor necrosis factor (TNFα) in the serum of rheumatoid arthritis patients with clinical improvement (**A**) and with lack of response to therapy (**B**) after 24 weeks. Horizontal bars show the median. ↑ = concentration above the range of the y axis.

(Figure 3). IL-6 bioactivity decreased after 4 weeks, both in patients who improved ($P < 0.05$) and even more markedly in those with a lack of effect ($P < 0.005$); the differences between these 2 groups, however, were not significant (Figure 4).

When data were analyzed according to therapy, significant decreases in p55 ($P = 0.01$ after 24 weeks) and sIL-2R ($P < 0.005$ from week 8 onward) concentrations were observed only in patients treated with MTX (Table 3). Furthermore, sIL-2R levels in the AZA group were significantly higher than those in the MTX group over the followup period ($P = 0.02$). Concentrations of bioactive IL-6 decreased in both treatment groups. However, in contrast to the results obtained for sIL-2R and p55, this decrease was more marked and occurred earlier in patients treated with AZA ($P < 0.0001$ by week 4) than in those treated with MTX ($P < 0.05$ after 16 weeks of treatment) (Figure 5). Over the followup period, IL-6 bioactivity was significantly lower in the AZA group than in the MTX group ($P < 0.02$). The concentrations of TNFα and p75 did not show significant changes in any of the groups.

Measurement of IL-6 levels by ELISA. In contrast to the superiority of AZA over MTX in decreasing IL-6 bioactivity, MTX seemed slightly more effective at decreasing immunoreactive IL-6 concentrations: After 24 and 48 weeks, immunoreactive IL-6 levels decreased beyond the detection limit in 60% and 55%

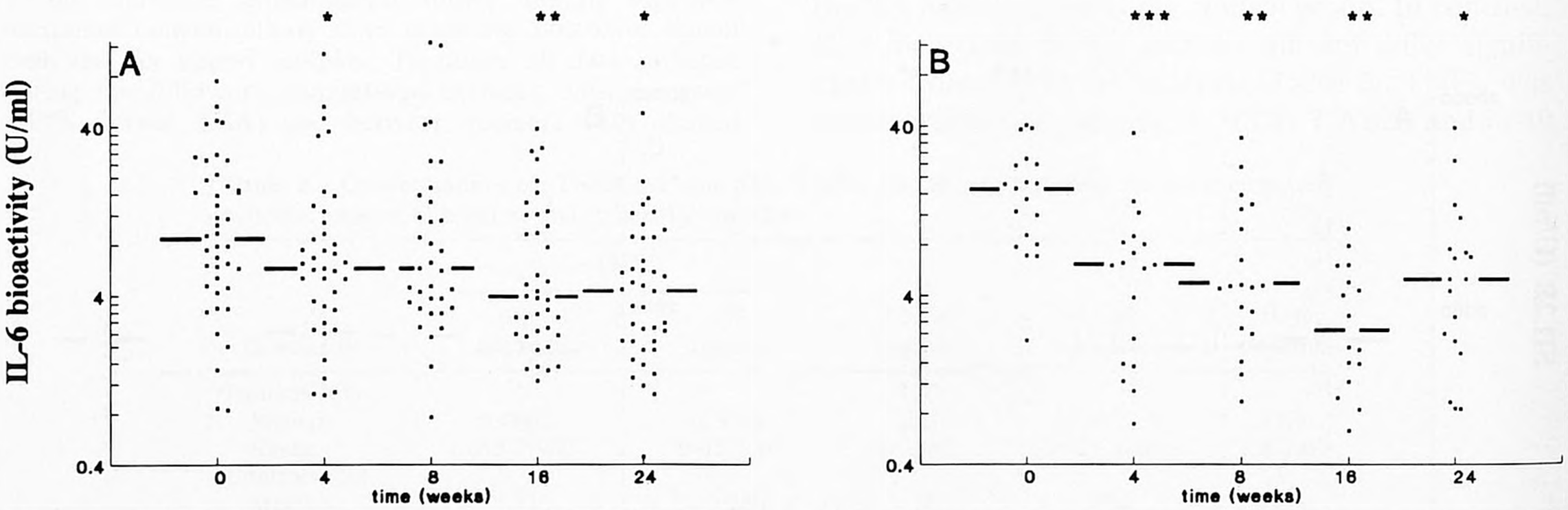

Figure 4. Concentrations of bioactive interleukin-6 (IL-6) in the serum of rheumatoid arthritis patients with clinical improvement (**A**) and with lack of response to therapy (**B**) after 24 weeks. Horizontal bars show the median. * = $P < 0.05$, ** = $P < 0.005$, and *** = $P < 0.0005$ versus baseline.

Table 3. Concentrations of sTNFR (p55 and p75), TNFα, and sIL-2R in RA patients at baseline and percentage of change after 24 and 48 weeks of treatment with MTX or AZA*

	Baseline levels		% change at week 24		% change at week 48	
	AZA (n = 32)	MTX (n = 29)	AZA (n = 19)	MTX (n = 28)	AZA (n = 12)	MTX (n = 24)
sTNFR (pg/ml)						
p55						
Median or % change	3,075	2,875	−6.5	−19.7†	−9.2	−7.2
Range or (25, 75 percentile)	2,250–4,150	2,250–3,575	(−22.2, 22.8)	(−29.7, −6.4)	(−21.7, 24.1)	(−28.9, 21.8)
p75						
Median or % change	3,175	2,400	−31.1	−4.2	−35	−18
Range or (25, 75 percentile)	2,375–5,525	1,675–4,450	(−72.8, 52.3)	(−53.5, 24.2)	(−53.6, −5.6)	(−53.3, 20)
TNFα						
Median or % change	105	130	7.6	0	4.5	1.7
Range or (25, 75 percentile)	45–220	100–200	(−16.6, 17.6)	(−23.3, 45.4)	(−12.4, 26.7)	(−14.3, 106.3)
sIL-2R						
Median or % change	1,480	1,300	−22.6	−24.1†	−27.9	−28.2‡
Range or (25, 75 percentile)	987–4,700	890–4,250	(−42, 3.2)	(−51.1, 6.5)	(−37.7, 12.1)	(−55, −5.6)

* All rheumatoid arthritis (RA) patients whose data are included had continued to take the assigned study drug. MTX = methotrexate; AZA = azathioprine. See Table 2 for other abbreviations.
† $P < 0.05$ versus baseline.
‡ $P < 0.0005$ versus baseline.

of the patients treated with MTX and in 47% and 50% of those treated with AZA, respectively (Figure 6). Subsequent experiments showed that serum from patients treated with AZA (but not MTX) exerted an inhibitory effect on the IL-6 bioassay (Barrera P et al: unpublished observations).

Interrelationships between cytokines and soluble receptors and correlation with disease activity parameters. In the entire group of 61 patients, a significant correlation between sIL-2R and p55 ($r \geq 0.50$, $P < 0.0005$) was observed during followup. In contrast, the correlation between sIL-2R and p75 was weaker and present only at baseline ($r = 0.37$, $P < 0.005$). Both sTNFR correlated significantly with each other ($r \geq 0.50$, $P < 0.0005$). TNFα concentrations showed no significant correlations with either sTNFR or any of the parameters studied. The positive correlations between pretreatment IL-6 bioactivity and sIL-2R and sTNFR in the RA patients tended to decrease during the followup (data not shown); however, this was not the case when only the patients treated with MTX were analyzed (correlation coefficients between IL-6

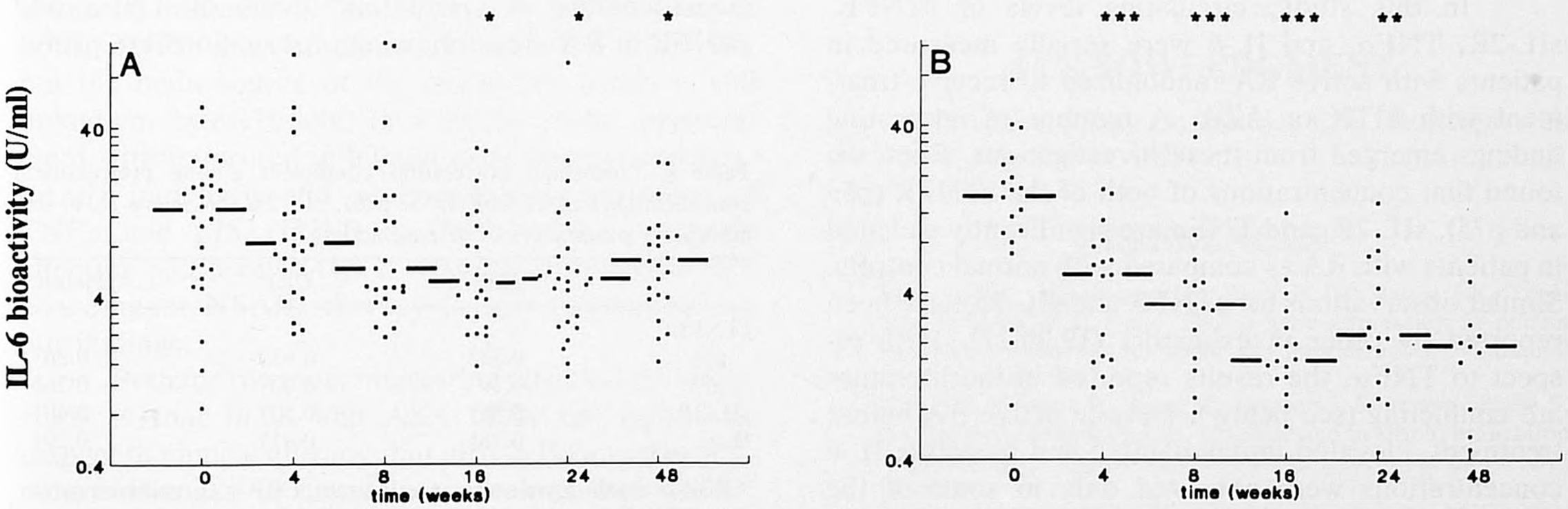

Figure 5. Concentrations of bioactive interleukin-6 (IL-6) in the serum of rheumatoid arthritis patients treated with methotrexate (**A**) or with azathioprine (**B**) for 24 weeks (n = 28 and n = 19) and 48 weeks (n = 23 and n = 11). Horizontal bars show the median. * = $P < 0.05$, ** = $P < 0.005$, and *** = $P < 0.0005$ versus baseline.

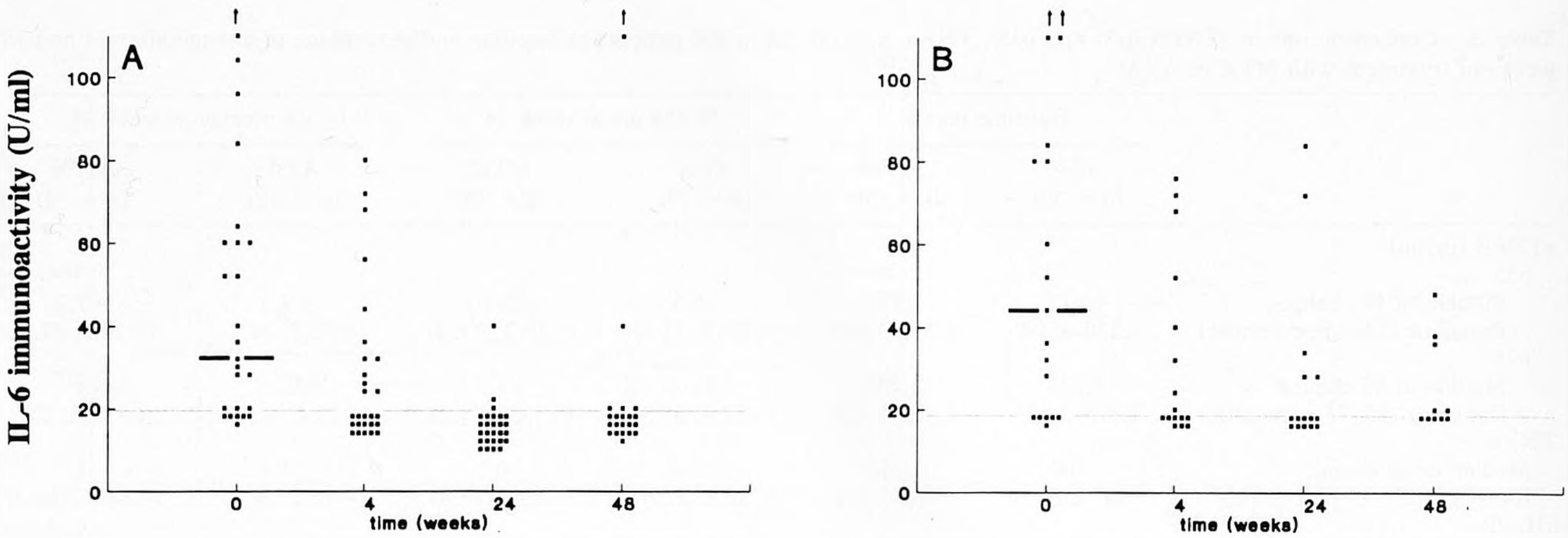

Figure 6. Concentrations of immunoactive interleukin-6 (IL-6) in the serum of rheumatoid arthritis patients treated with methotrexate (**A**) or with azathioprine (**B**) for 24 weeks (n = 28 and n = 19) and 48 weeks (n = 23 and n = 11). Horizontal bars show the median. Faint line near 20 units/ml represents the detection limit of the enzyme-linked immunosorbent assay. ↑ = concentration above the range of the y axis.

and sIL-2R, p55, and p75, respectively, were 0.65, 0.34, and 0.44).

The parameters measured in this study showed no significant correlation with clinical (disease duration, Ritchie articular index, number of tender joints, duration of morning stiffness, and disease activity score [26]) or with radiologic (erosion score and total score [21]) measurements. On the other hand, positive correlations were found with some laboratory parameters of disease activity, such as the C-reactive protein (CRP) level, the ESR, and the platelet count (Table 4).

DISCUSSION

In this study, circulating levels of sTNFR, sIL-2R, TNFα, and IL-6 were serially measured in patients with active RA randomized to receive treatment with MTX or AZA. A number of interesting findings emerged from these investigations. First, we found that concentrations of both of the sTNFR (p55 and p75), sIL-2R, and TNFα are significantly elevated in patients with RA as compared with normal controls. Similar observations for sTNFR and sIL-2R have been reported by other investigators (19,20,27). With respect to TNFα, the results reported in the literature are conflicting (see below). Despite active RA before treatment, elevated immunoactive and bioactive IL-6 concentrations were observed only in some of the patients, resulting in mean IL-6 concentrations that were not significantly higher than in controls. The relatively low serum IL-6 concentrations in this and other studies (16–18) and the thousandfold higher levels found in synovial fluid suggest a predominant intraarticular production or a rapid clearance of the circulating IL-6.

A second important finding in our study is that clinical improvement is accompanied by significant reductions in the concentrations of p55 and sIL-2R, the parameters which best correlated with laboratory indices of RA activity such as CRP, ESR, and platelet counts. Decreases in sIL-2R concentrations, together with clinical improvement, have been observed in RA (28–30), and associations between sTNFR levels and disease severity were recently reported in a cross-sectional study (27). However, to our knowledge, this is the first report of a long-term study of concomitant measurements of circulating levels of TNFα and sTNFR in RA. The following observations are proba-

Table 4. Spearman correlation coefficient among pretreatment concentrations of sTNFR (p55, p75), sIL-2R, bioactive IL-6, and laboratory parameters of disease activity*

	ESR	CRP	Platelets
sTNFR			
p55	0.38†	0.63‡	0.36†
p75	0.19	0.40†	0.26§
sIL-2R	0.38†	0.50‡	0.40†
IL-6	0.28§	0.41†	0.29§

* ESR = erythrocyte sedimentation rate; CRP = C-reactive protein. See Table 2 for other abbreviations.
† $P < 0.005$.
‡ $P < 0.0005$.
§ $P < 0.05$.

bly relevant: (a) The concentrations of each type of sTNFR individually exceeded by 20–30-fold those of TNFα, the p75 sTNFR levels being higher than those of p55; (b) The TNFα concentrations throughout the study period were higher in patients with lack of response to therapy than in those who improved; and (c) A decrease in p55 sTNFR concentrations occurred in patients with clinical improvement, but no significant changes in TNFα or p75 concentrations were observed, irrespective of response to therapy or the DMARD used.

Elevated TNFα levels and correlations with disease severity have been found in various infectious and noninfectious diseases (31–33). Similar observations in RA (34–36) have not been confirmed in other studies which failed to detect circulating TNFα (27). Some of these discrepancies are probably due to the presence of sTNFR in biological fluids, which may interfere to a different extent in TNF bioassays and immunoassays (37). With the assays used in this study, such interference could be excluded; however, the question is whether circulating TNFα retains its biological activity. Recently, van Zee et al (38) showed that a great excess of sTNFR is required to block the bioactivity of high concentrations of TNFα, whereas lower TNFα concentrations (approximately 4 times higher than those in our study) can be efficiently inhibited by an 8–9-fold excess of p55. The ratio of sTNFR to TNFα in this study, together with the unchanged TNFα concentrations even in patients with clinical improvement, suggest that circulating TNFα may not be biologically active.

The different course of the p55 versus the p75 sTNFR and TNFα in the patients with clinical response is interesting. The expression and shedding of both sTNFR may be distinctly regulated (27,39,40), but the main source of the circulating forms is still unknown. Interestingly, in a recent study, pretreatment with ibuprofen in human experimental endotoxinemia increased and prolonged concentrations of TNFα and p75 sTNFR in the circulation without affecting p55 levels (41). It is therefore possible that concomitant NSAID therapy is also responsible for our findings.

Perhaps the most interesting observation in this study is that MTX and AZA differ not only with respect to clinical efficacy, but also in their capacity to induce changes in circulating cytokine and soluble receptor concentrations. Corroborating the clinical results, the decreases in concentrations of sIL-2R and p55 were significant in the patients treated with MTX (but not with AZA), and MTX was slightly more effective in decreasing immunoactive IL-6 levels. In view of the better clinical response obtained with MTX, it is possible that these differences are due to a more effective suppression of the inflammation by this drug, rather than to a specific effect of MTX. The early and pronounced decrease in IL-6 bioactivity in patients treated with AZA has to be explained by the inhibitory effects of circulating AZA or its metabolites in the bioassay (Barrera P et al: unpublished observations). This bioassay is therefore not suitable for evaluating IL-6 concentrations in patients treated with this drug.

Decreases in sIL-2R concentrations in RA have been observed during treatment with amiprilose HCl (Therafectin; Greenwich Pharmaceuticals, Fort Washington, PA) (28) and with corticosteroids (30), but not with intramuscular gold (42). Decreases in IL-6 concentrations have been reported with intramuscular (43) and oral gold (44), while for sulfasalazine the results are contradictory (44,45). Neither MTX nor AZA altered TNFα concentrations in this study, but decreases have been observed during therapy with corticosteroids (36) and sulfasalazine (45).

Taken together, these results indicate that diverse DMARDs distinctly affect circulating cytokines and soluble receptors. The differences in cytokine patterns between patients with improvement versus those without, suggest that measurements of sIL-2R, p55, and IL-6 may be useful in the evaluation of therapeutic response. Further studies are needed to determine whether these effects are causally related to disease-modifying activity or merely reflect epiphenomena.

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