Regulation of serum chemokines following infliximab therapy in patients with rheumatoid arthritis

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Abstract

Objective

We studied the effects of the multiple infusions of infliximab, a chimeric anti-tumor necrosis factor alpha (anti-TNF-α) antibody, on the serum chemokines levels in patients with active rheumatoid arthritis (RA).

Methods

RA patients were supposed to receive 9 infusions of infliximab (3mg/kg) at weeks 0, 2, 6, and every 8 weeks thereafter with the same dose. All patients continued treatment with methotrexate (MTX) (7.5-20mg/week). Serum concentrations of interleukin-8 (IL-8), RANTES (regulated upon activation, normal T cell expressed and secreted) and monocyte chemoattractant protein-1 (MCP-1) were assessed by ELISA at weeks 0, 2, 6, 14, 38, prior to infusion, and additionally at week 62.

Results

Initial infusion of infliximab caused reduction in serum IL-8, RANTES and MCP-1 (in all cases $p < 0.001$) levels. Subsequent infliximab administrations also significantly decreased serum chemokines levels, but was less effective. Prior to the first infliximab infusion serum concentrations of studied chemokines correlated with markers of RA activity such as the erythrocyte sedimentation rate (ESR) or CRP levels, number of swollen joints and disease activity score (DAS). Following next drug infusions such associations were far less significant. Infliximab treatment induced a significant reduction in the number of monocytes observed through the whole study (in all cases $p < 0.05$).

Conclusion

Anti-TNF-α antibody therapy accompanied by MTX, beside a rapid clinical improvement, reduced serum chemokines concentrations in RA patients. Subsequent administrations of infliximab sustained chemokines decrease, although to a lesser extent than the first two dose of infliximab.

Key words

Chemokines, IL-8, RANTES, MCP-1, rheumatoid arthritis, infliximab.

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Introduction

Chemokines have an important role in the infiltration of rheumatoid synovium with mononuclear cells, leading to the initiation and progression of rheumatoid arthritis (RA) (1). These proteins act as mediators of the inflammation process by recruiting and activating particular leukocyte populations. In addition, chemokines such as interleukin-8 (IL-8) or monocyte chemoattractant protein-1 (MCP-1) increase inflammatory cell migration into synovium due to the stimulation of neovascularisation of synovial tissue (1, 2). Chemokine synthesis is promoted by cytokines such as IL-1 and tumour necrosis factor α (TNF-α) (3). It was also reported that chemokines may increase production of inflammatory cytokines such as IL-1, IL-6, TNF-α or matrix metalloproteinases involved in the process of joint destruction (2, 4, 5). Single administration of the chimeric anti-TNF-α monoclonal antibody (infliximab), besides the decrease in clinical markers of RA activity (6, 7), was shown to reduce serum levels of IL-8 and MCP-1 (8). The present study was performed to investigate the effects of the repeated infusions of infliximab on serum IL-8, RANTES (regulated upon activation, normal T cell expressed and secreted) and MCP-1 levels in patients with active RA.

Materials and methods

Fifteen patients meeting the ACR 1987 revised criteria for RA (9) were recruited into a study. Patients were excluded if they had previous history of tuberculosis or symptoms of infectious diseases in the previous 3 months. Chest x-rays done prior to the first infliximab administration were normal in all patients. One patient interrupted the infliximab therapy because of tuberculosis, which was diagnosed after third infliximab administration. One patient developed zoster 4 weeks following the sixth infusion, and other one anaphylactic reaction during third infliximab administration. These individuals were eliminated from the study, and received appropriate treatment. One patient discontinued treatment after the fifth infusion because other reasons. The remaining patients completed the study without adverse events.

All patients had active RA, as defined by 6 or more tender joints, 6 or more swollen joints, and two of the following: morning stiffness for more than 45 minutes, C-reactive protein (CRP) level of more than 20 mg/l, erythrocyte sedimentation rate (ESR) of more than 28 mm/h (7). Mean patients age was 44.5 years (range 21-65), and mean disease duration was 10.5 years (range 3-20). One patient was at the 2nd, ten patients were at the 3rd, and four patients were at the 4th radiological stage of the disease according to Steinbrocker's criteria (10). All subjects were receiving methotrexate (MTX) (median 12.5 mg/week, range 7.5-20 mg/week) at a stable dose for at least 2 months and nonsteroidal anti-inflammatory drugs (NSAIDs) at a stable dose for at least 4 weeks before the study. Six patients were receiving prednisone (median 7.5 mg/day, range 5-10mg/day) at a stable dose for at least 4 weeks. MTX, NSAIDs and prednisone were continued through the study.

Patients were planned to received 9 infusions of infliximab, a chimeric anti-TNF-α antibody, (3mg/kg) at weeks 0, 2, 6, and every 8 weeks thereafter with the same dose. Blood samples drawn before the infusion at weeks 0, 2, 6, 14, 38, and additionally at week 62 (eight weeks after the last drug infusion) were clotted for 30 minutes and next spun for 10 minutes at 1000 x g. Serum was aliquoted and stored at -80°C.

The study was approved by the local ethical committee and all patients gave their written informed consent.

Clinical evaluation included the duration of morning stiffness, Ritchie index (11), the number of tender joints (of 68 joints assessed), the number of swollen joints (of 66 assessed), ESR, DAS (12), CRP concentration and white blood cell counts.

The measurements of serum concentrations of IL-8, RANTES, MCP-1 were assessed by ELISA kits from R&D Systems, Wiesbaden-Nordenstadt, Germany, strictly according to the manufacturer's instructions.

The normally distributed data were analyzed by paired Student t-test. Wilcoxon signed rank test was used to compare the differences between non-normally distributed data. Correlations between variables were assessed by Spearman rank order test. P values lower than 0.05 were considered statistically significant.

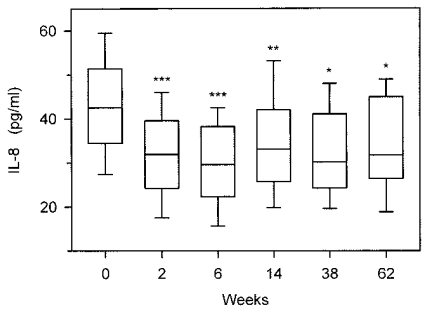

Fig. 1. Serum concentrations of interleukin-8 (IL-8) in RA patients, assessed by ELISA technique. Patients were treated with infliximab (3mg/kg) at weeks 0, 2, 6, 14, 22, 30, 38, 46 and 54. Blood samples were obtained at weeks 0, 2, 6, 14, 38, prior to infusion, and additionally at week 62. Box plots represent median (line), 25th and 75th percentiles (box), and 10th and 90th percentiles (whiskers). Significance of differences between pre-infusion IL-8 values at week 0 and following weeks were expressed as: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

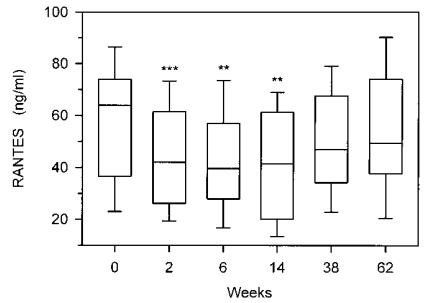

Fig. 2. Serum concentrations of RANTES (regulated upon activation normal T cell expressed and secreted), determined and presented as described in the legend to Figure 1.

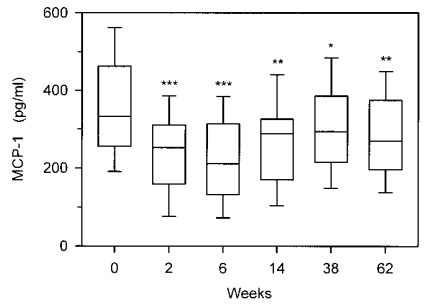

Fig. 3. Serum concentrations of monocyte chemoattractant protein-1 (MCP-1), determined and presented as described in the legend to Figure 1.

Results

Following initial infliximab administration, the concentrations of IL-8, RANTES and MCP-1 in serum of RA patients rapidly decreased (in all cases $p < 0.001$) (Figs. 1-3, Tables I-III). Studied chemokines were especially diminished after second infliximab infusion. Subsequent drug infusions prolonged IL-8, RANTES and MCP-1 suppression, although to a lesser extent than the first two doses of the study drug.

Correlations between serum levels of chemokines and markers of the disease activity are shown in Table IV. Furthermore, we demonstrated correlations between serum levels of IL-8, RANTES and MCP-1. After subsequent infusions such associations were less or not significant. We did not observed any correlations between patient sex, age or disease duration with any serum chemokine concentrations.

ESR, CRP levels, duration of morning

Table I. Serum concentrations of IL-8 (pg/ml) in RA patients treated with infliximab (3mg/kg) on weeks 0, 2, 6, 14, 22, 30, 38, 46 and 54.

	Week 0	Week 2	Week 6	Week 14	Week 38	Week 62
Patient 1	34.2	28.1	26.9	33.1	39.4	36.1
Patient 2	38.2	31.9	32.4	31.6	33.4	25.8
Patient 3	59.5	39.1	21.5	20.6	26.4	28.2
Patient 4	46.8	30.6	24.9	29.2	30.1	31.7
Patient 5	57.6	23.4	27.6	26.7	23.5	29.3
Patient 6	31.3	19.4	16.7	22.8	18.7	20.4
Patient 7	41.8	33.2	35.3	35.8	27.8	39.2
Patient 8	52.9	47.6	45.7	52.5	46.2	51.6
Patient 9	45.1	39.7	40.8	39.3	41.6	47.1
Patient 10	27.4	13.4	15.7	16.9	20.2	16.5
Patient 11	42.5	37.4	33.7	40.4	50.6	46.8
Patient 12	43.7	41.1	39.2	46.9		
Patient 13	61.3	46.0	42.5	55.6		
Patient 14	35.1	26.8	29.6			
Patient 15	26.5	17.5	15.4			

RA: rheumatoid arthritis; IL-8: interleukin-8. Serum samples were obtained at weeks 0, 2, 6, 14, 38, prior to infusion, and at week 62. Significances of the differences between weeks are shown on Fig. 1.

Table II. Serum concentrations of RANTES (ng/ml)in RA patients treated with infliximab (3mg/kg) on weeks 0, 2, 6, 14, 22, 30, 38, 46 and 54.

	Week 0	Week 2	Week 6	Week 14	Week 38	Week 62
Patient 1	26.4	29.2	39.6	16.1	36.3	47.4
Patient 2	16.6	19.4	11.6	14.1	19.2	16.5
Patient 3	66.8	57.4	26.8	46.6	38.5	67.1
Patient 4	63.9	32.1	33.4	31.6	33.5	23.1
Patient 5	86.5	61.8	43.1	64.3	71.8	95.6
Patient 6	40.7	25.1	31.2	10.8	25.3	35.8
Patient 7	96.1	91.6	80.5	60.2	46.9	56.4
Patient 8	68.0	52.5	47.9	21.5	52.4	43.2
Patient 9	72.2	73.3	68.3	67.0	89.7	76.3
Patient 10	49.5	42.0	73.5	76.2	69.0	86.6
Patient 11	60.2	38.7	36.1	41.4	63.1	49.4
Patient 12	35.3	23.0	16.7	25.3		
Patient 13	80.1	60.5	57.6	54.1		
Patient 14	74.4	66.1	55.2			
Patient 15	23.0	15.9	20.4			

RA: rheumatoid arthritis; RANTES: regulated upon activation normal T cell expressed and secreted. Serum samples were obtained at weeks 0, 2, 6, 14, 38, prior to infusion, and at week 62. Significances of the differences between weeks are shown on Fig. 2.

Table III. Serum concentrations of MCP-1 (pg/ml) in RA patients treated with infliximab (3mg/kg) at weeks 0, 2, 6, 14, 22, 30, 38, 46 and 54.

	Week 0	Week 2	Week 6	Week 14	Week 38	Week 62
Patient 1	191	45	73	109	175	220
Patient 2	376	252	126	231	253	279
Patient 3	315	317	211	295	322	240
Patient 4	619	386	282	288	361	391
Patient 5	503	291	323	382	443	416
Patient 6	251	76	30	84	109	126
Patient 7	562	417	413	439	547	499
Patient 8	354	238	337	451	394	326
Patient 9	332	262	285	298	294	188
Patient 10	230	154	162	134	277	269
Patient 11	448	175	151	182	203	145
Patient 12	298	210	190	250		
Patient 13	467	355	384	307		
Patient 14	268	283	241			
Patient 15	159	107	88			

RA: rheumatoid arthritis; MCP-1: monocyte chemoattractant protein-1. Serum samples were obtained at weeks 0, 2, 6, 14, 38, prior to infusion, and at week 62. Significances of the differences between weeks are shown in Fig. 3.

Table IV. Correlations between serum concentrations of studied chemokines and clinical parameters of RA patients.

	RANTES	MCP-1	ESR	CRP	No. of tender joints	No. of swollen joints	DAS
IL-8	0.589*	0.686**	0.540*	0.429	0.635*	0.731**	0.664**
RANTES		0.593*	0.569*	0.775***	0.481	0.697**	0.564*
MCP-1			0.442	0.539*	0.488	0.726**	0.596*

RA: rheumatoid arthritis; IL-8: interleukin-8; RANTES: regulated upon activation normal T cell expressed and secreted; MCP-1: monocyte chemoattractant protein-1; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS: disease activity score.
Data expressed as r values (correlation coefficient) according to Spearman rank correlation: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
Clinical evaluations were performed, and blood samples were obtained at week 0, prior to first infliximab infusion.

stiffness, Ritchie index, the number of tender and swollen joints, and DAS decreased after the first infusion of infliximab (in all cases $p < 0.001$), and their values remained stable until the end of study. White blood counts revealed a not significant tendency to increase following infliximab administrations, the number of lymphocytes increased after the initial infusion ($p < 0.05$). However, infliximab treatment caused a significant reduction in the number of monocytes, observed through the whole study ($p < 0.05$). The number of platelets diminished significantly only after the first drug administration ($p < 0.05$) (data not shown).

Discussion

Chemokines play an important role in the pathogenesis of RA. Together with adhesion molecules they regulate the migration of mononuclear cells into synovium, leading to the initiation and progression of the disease. Chemokine production is stimulated by cytokines such as IL-1 and TNF-α (1, 3). It was reported that single administration of the chimeric anti-TNF-α monoclonal antibody (infliximab), besides the decrease in clinical markers of RA activity (6, 7), reduce serum levels of IL-8 and MCP-1 (8). The present study was performed to investigate the effects of repeated infusions of infliximab on serum IL-8, RANTES and MCP-1 levels in RA patients.

IL-8 was one of first chemokines shown to be involved in leucocyte chemotaxis (2, 3). Here we confirmed that after infliximab infusion, the levels of IL-8 in serum of RA patients rapidly decrease. Furthermore, we showed that IL-8 was especially diminished after the second infliximab infusion. After subsequent drug administrations IL-8 concentrations were characterized by the tendency to increase. However, they remained below the values at week 0. RANTES is engaged in monocyte and T lymphocyte migration (1, 2) and was shown to be predictive of radiological erosions (13). We demonstrated that serum RANTES is downregulated following infliximab therapy. However, further drug administrations were followed by the tendency to an increase of serum RANTES concentrations towards the baseline values. MCP-1 is not only monocyte specific chemoattractant, but has also been shown to attract T cells, natural killer cells and basophils. It also plays a role in T cell differentiation and angiogenesis (2). Others showed the tendency toward a reduction in serum MCP-1 concentration in RA patients following infliximab infusion (8). In our study, serum MCP-1 levels decreased rapidly following infliximab administration, especially after the second infusion. Subsequent drug administrations maintained MCP-1 suppression, however to a lesser extent than the first two doses of infliximab. Similarly to other studies (6, 7), parameters of disease activity such as ESR and CRP level, duration of morning stiffness, Ritchie index, the number of tender and swollen joints, and DAS decreased after the initial infusion of infliximab and their values remained stable until the end of study. Prior to infliximab treatment, serum chemokines correlated with clinical markers of the disease activity. Following further infliximab administrations these associations were less or not significant.

Total white blood count revealed the insignificant tendency to increase after infliximab administration. Only the number of lymphocytes remarkably increased after infliximab infusion. However, this effect was transient and did not last until the end of the study. It is possible that the noted increase in the number of circulating lymphocytes in RA patients might be the result of limited migration of lymphocytes into

rheumatoid synovium following anti-TNF-α treatment (14). The diminished number of monocytes might be the effect of the antibody and complement dependent cytotoxity induced by the interaction between infliximab and TNF-α on the monocyte membrane. Such reactions can contribute to the infections reported in patients treated with TNF-α blockers (14, 15).

In conclusion, anti-TNF-α antibody treatment together with MTX, besides a rapid clinical improvement, reduced serum IL-8, RANTES and MCP-1 concentrations in RA patients. Subsequent administrations of infliximab prolonged suppression of studied chemokines, however were less effective compared to the first two infusions of infliximab. Furthermore, we demonstrated that serum concentrations of IL-8, RANTES and MCP-1 correlated with markers of disease activity such as ESR or CRP levels, number of tender and swollen joints, and DAS prior to the first infliximab infusion and, to a lesser extent, following further drug administrations.

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