Increased Degradation of Tryptophan in Blood of Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Activation of the enzyme indoleamine-(2,3)-dioxygenase (IDO) by interferon (IFN)-γ leads to enhanced tryptophan conversion to kynurenine. In consequence of chronic immune activation, tryptophan availability is reduced, leading to inhibition of cell proliferation as protein synthesis is affected. Tryptophan deprivation due to IDO activation could therefore be effective in abrogating processes with high metabolic turnover, thus modulating cellular immune response.

Methods. Concentrations of tryptophan, kynurenine, and neopterin were measured by HPLC in the sera of 38 patients with rheumatoid arthritis (RA). The kynurenine:tryptophan ratios (kyn/trp) were calculated to estimate IDO activity.

Results. Tryptophan concentrations were lower in patients with RA (median, interquartile range: 44.95 µM, 40.31–49.95 µM) compared to healthy blood donors (62.62 µM, 57.27–74.61 µM; $p < 0.001$). Kynurenine in patients (1.86 µM, 1.54–2.31 µM) did not differ from controls (2.06 µM, 1.58–2.65 µM; NS). The kyn/trp ratio was higher in patients (42.39 mM/M, 37.02–48.60 mM/M) than in controls (31.72 mM/M; 27.95–35.03 mM/M; $p < 0.001$). Kynurenine concentrations ($r_s = 0.611$; $p < 0.001$) and kyn/trp ratios ($r_s = 0.621$; $p < 0.001$) correlated with neopterin concentrations, which indicate stimulated cellular immune response in patients with RA.

Conclusions. The data point to a role of immune activation and Th1-type cytokine INF-γ to induce elevated tryptophan degradation in patients with RA. (J Rheumatol 2003;30:1935–9)

Key Indexing Terms:
RHEUMATOID ARTHRITIS TRYPTOPHAN KYNURENINE
IMMUNE ACTIVATION NEOPTERIN

Rheumatoid arthritis (RA) is a systemic inflammatory disorder with high prevalence. The pathogenesis of RA is unclear, although much insight into possible molecular and cellular pathomechanisms has been gained during the last few years. Immune system activation and the production of cytokines are known to play a crucial role[1]. Pro-inflammatory cytokines like tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and IL-8 regulate the expression of cell adhesion molecules and they also regulate the migration and retention of leukocytes in the inflamed tissue. Moreover they have a costimulatory effect on leukocyte activation and lymphocyte proliferation[2,3]. Pathophysiological studies have shown that T lymphocytes play an important role in the initiation and perpetuation of RA[4]. Increased numbers of activated lymphocytes can be found not only in the synovial fluid but also in the peripheral blood of patients with RA.

Recently it has been shown that serum soluble markers of immune activation, e.g., soluble cytokine receptors like sTNF-R[5-7] and sIL-2R[6-11], or neopterin[12-14], are raised in patients with RA, correlating well with disease activity. Neopterin is produced and secreted in large amounts by human monocyte-derived macrophages preferentially upon stimulation with IFN-γ[15] and measurement of neopterin concentrations in body fluids allows the monitoring of cellular (Th1-type) immune response[16].

In various cells IFN-γ also activates the enzyme indoleamine-(2,3)-dioxygenase (IDO, EC 1.13.11.42), which performs the initial step in the conversion of the essential amino acid tryptophan to kynurenine and further catabolites[17]. The kynurenine:tryptophan ratio (kyn/trp) allows an estimate for IDO-activity and was found to be increased in states of persistent immune activation, i.e., infectious[18,19] and malignant[20] diseases. We examined the degradation of tryptophan in patients with RA.

MATERIALS AND METHODS

Patients and tests. Thirty-six women and 2 men (mean age: 56.9 ± 8.9 yrs) with RA who fulfilled the American College of Rheumatology criteria[21] were recruited from the University Hospital of Innsbruck. According to the

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Steinbrocker criteria[22], 18 patients had RA stage 2 (functional capacity adequate to conduct normal activities despite handicap, or discomfort, or limited mobility of one or more joints); the other 20 patients were classified as stage 3 (functional capacity adequate to perform only few or none of the duties of usual occupation or of self-care). All patients were receiving therapy. Table 1 shows treatment regimens as well as C-reactive protein concentrations measured turbidimetrically, and erythrocyte sedimentation rates. Thirty patients were treated with immunosuppressive therapy [methotrexate (MTX), cyclosporine, leflunomide, azathioprine] in combination with conventional therapy (nonsteroidal antiinflammatory drugs, steroids, and opioids). Eight patients were treated with conventional therapy only. Patients were not taking vitamin supplements such as pyridoxine or other B vitamins.

Pregnant women and women in the puerperium, patients with malignant diseases or clinically relevant GI, renal, hepatic, cardiorespiratory, hematological, neurological, or psychiatric diseases were excluded from the study, as well as patients with metabolic disorders or chronic or acute infections.

Within the scope of routine blood examinations, fractions of serum samples of patients were collected and frozen at -20˚C until analysis. Tryptophan and kynurenine concentrations were determined by high performance liquid chromatography as described[19]. To estimate IDO activity, the kyn/trp was calculated. Neopterin concentrations were measured by ELISA (BRAHMS Diagnostica, Berlin, Germany). Results from patients were compared to 20 healthy blood donors of similar age distribution (mean 50.4 ± 7.0 yrs).

Statistical analysis. For statistical comparisons between subgroups of patients, the nonparametric Mann-Whitney U test was employed. Spearman rank correlation analysis was applied to assess correlations. P values < 0.05 were considered to indicate statistical significance.

RESULTS

Concentrations of tryptophan were lower in patients with RA (median, interquartile ranges: 44.95 µM, 40.31-49.95

Table 1. Treatment regimes of 38 patients with RA (0: no treatment; 1: regular treatment; 2: treatment on demand), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

Patient	NSAR	Opioids	Steroids	Methotrexate	Cyclosporine	Azathioprine	Leflunomide	Salazopyrine	Hydroxychloroquine	ESR (mm/h)	CRP (mg/dl)
1	1	1	2	1	0	0	0	0	1	10	0.7
2	0	0	1	0	0	0	1	0	0	54	2.25
3	0	1	1	1	1	0	0	0	1	16	5.96
4	2	0	2	0	0	0	0	1	0	20	1.41
5	1	0	1	0	0	0	0	1	0	24	0.83
6	1	0	0	0	1	0	0	0	0	78	ND
7	2	0	1	1	1	0	0	1	0	22	1.58
8	1	0	1	1	0	0	0	0	0	18	ND
9	0	2	1	0	0	0	1	0	0	72	8.07
10	1	0	0	0	0	0	0	0	0	18	1.07
11	0	0	1	1	1	0	0	0	0	50	5.2
12	0	0	1	1	1	0	0	0	0	22	0.87
13	0	1	1	1	0	0	0	0	0	58	1.25
14	1	0	1	0	1	0	0	0	0	26	5.18
15	2	0	1	0	1	1	0	0	0	16	ND
16	0	0	2	0	0	0	0	0	0	n.a.	n.a.
17	0	0	2	1	1	0	0	0	0	60	2.76
18	0	0	1	1	0	0	0	0	0	18	ND
19	1	0	1	1	0	0	0	0	0	32	3.29
20	2	0	0	0	0	0	0	0	1	8	ND
21	1	0	1	1	0	0	0	0	0	28	ND
22	0	0	1	1	1	0	0	0	0	22	ND
23	2	0	0	0	0	0	0	0	1	6	ND
24	0	0	1	1	0	0	0	0	1	30	0.85
25	0	0	0	0	0	0	0	0	1	54	0.85
26	0	0	1	1	0	0	0	0	0	30	ND
27	1	0	1	1	0	0	0	0	0	52	2.32
28	1	0	0	1	0	0	0	0	0	14	ND
29	0	0	1	1	0	0	0	0	0	18	ND
30	2	0	1	1	0	0	0	0	0	22	1.09
31	1	1	1	1	0	0	0	0	0	12	ND
32	1	0	1	1	1	0	0	0	0	40	2.76
33	1	0	0	1	0	0	1	0	0	26	ND
34	2	0	1	1	1	0	0	0	0	24	ND
35	2	0	1	1	0	0	0	0	0	36	0.85
36	1	1	1	0	0	0	0	1	0	14	0.7
37	1	0	1	0	0	0	1	0	0	30	0.96
38	0	1	1	1	1	0	0	0	0	14	0.82

ND - not detectable; n.a. - not available

µM) compared to controls (62.62 µM, 57.27-74.61 µM; $p < 0.001$; Figure 1), whereas kynurenine concentrations did not differ between groups (1.86 µM; 1.54-2.31 µM vs 2.06 µM; 1.58-2.31 µM). Kyn/trp was higher in patients (42.39 mM/M, 37.02-48.60 mM/M; $p < 0.001$) than in healthy blood donors (31.72 mM/M, 27.95-35.03 mM/M; $p < 0.001$). Concentrations of tryptophan and kynurenine and kyn/trp were similar in patients with RA stage 2 and 3. There were no statistically significant differences between the 2 groups (data not shown). Concentrations of kynurenine and kyn/trp did not differ between patients receiving different treatment regimens; there was a trend towards higher tryptophan and lower kyn/trp in patients receiving steroid therapy ($p = 0.064$), but this was not statistically significant. Lower tryptophan concentrations were found in 4 patients receiving leflunomide (36.55 µM; 41.43-50.27 µM, vs 46.49 µM, 41.43-50.27 µM, $p = 0.01$), but no significant differences in kynurenine and kyn/trp concentrations were observed in comparison to 34 patients receiving other treatment regimens.

Close associations between changes of tryptophan metabolism and neopterin production were seen. Neopterin concentrations were elevated in patients (7.47 nM, 4.90-11.29 nM) when compared to healthy control persons ($p < 0.001$), and neopterin concentrations correlated with kynurenine concentrations ($r_s = 0.611$; $p < 0.001$) and kyn/trp ($r_s = 0.621$; $p < 0.001$; Figure 2).

DISCUSSION

Like tryptophan-(2,3)-dioxygenase (tryptophan pyrrolase, EC 1.13.1.2) in the liver, IDO catalyses the formation of kynurenine in various cells including, e.g., fibroblasts and macrophages[23]. However, unlike tryptophan pyrrolase, enhanced tryptophan degradation to kynurenine by IDO is strongly induced by IFN-γ. Kyn/trp is a suitable estimate for tryptophan degradation catalyzed by activated IDO[18,19], better than the absolute tryptophan concentration in serum which may depend also on variations of dietary intake of this essential amino acid. Reduced dietary intake of tryptophan lowers blood tryptophan together with kynurenine concentrations, but kyn/trp does not change. On the other hand, vitamin deficiency can increase kynurenine levels, since conversion of kynurenine in the liver depends on the availability of vitamin B6[24]. However, immune-mediated changes of tryptophan metabolism are much stronger than dietary influences.

Our results provide evidence for an increased degradation of tryptophan in patients with RA, which is reflected by significantly lower tryptophan concentrations and significantly increased kyn/trp. The data further support the view that immune activation coinciding with increased production of IFN-γ within the scope of an autoimmune process is responsible for the increased conversion of tryptophan. The relatively strong correlations between immune activation marker neopterin and kynurenine ($r_s = 0.611$; $p < 0.001$) and kyn/trp ($r_s = 0.621$; $p < 0.001$) further underline this assumption. Interestingly, kynurenine correlates with neopterin concentrations despite the fact that only neopterin but not kynurenine is found increased in patients. Increased kyn/trp indicates that conversion of tryptophan to kynurenine is increased relative to tryptophan levels. Thus if tryptophan is subnormal, only in cases of very strong immune activation can above-normal kynurenine levels be observed.

Elevated neopterin concentrations in patients with RA compared to controls also indicate immune stimulation, and neopterin has already been shown in previous studies to be a suitable measure of disease activity in RA[12-14,25,26]. Both events, neopterin production and tryptophan degradation, indicate enhanced endogenous formation of IFN-γ in RA.

The concentrations of immune system variables did not

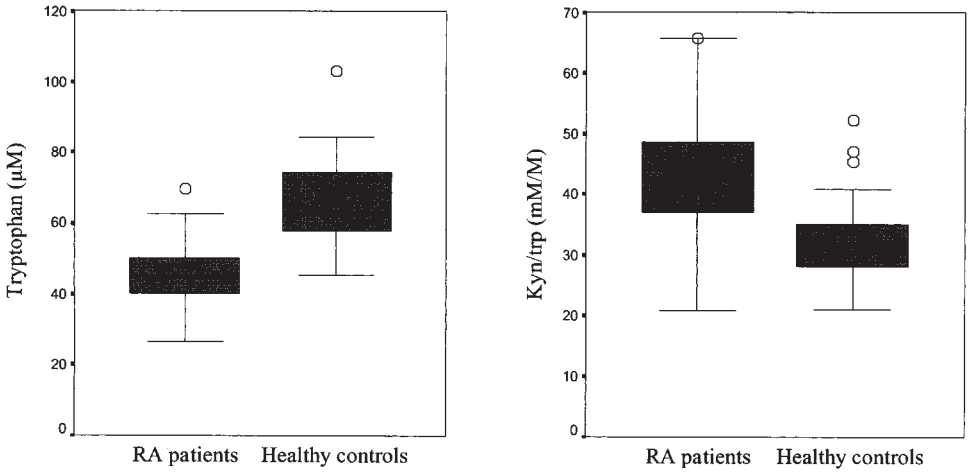

Figure 1. Box plots of serum tryptophan concentration and the kyn/trp ratio in patients with RA compared to blood donors (all comparisons $p < 0.001$). Horizontal lines: medians; boxes: 25th to 75th percentiles; bars: 5th to 95th percentiles; open circles: outliers.

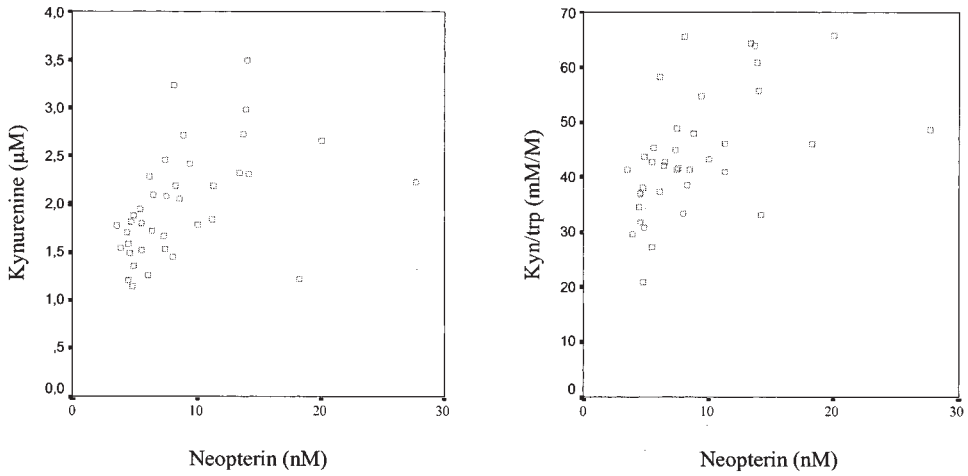

Figure 2. Correlation of serum kynurenine concentrations (r_s = 0.611, p < 0.001) and of kyn/trp (r_s = 0.621, p < 0.001) with neopterin concentrations in patients with RA.

differ between patients with RA stages 2 or 3. However, most patients were receiving treatment when blood specimens were taken, which would have influenced disease activity. In agreement with our results, concentrations of immune activation markers usually correlate much better with disease activity than with stage of RA[9,10,12,13].

Cytokine-induced IDO was at one time considered as a bactericidal and cytocidal strategy of IFN-γ-stimulated cells, limiting availability of tryptophan in infected cells and thereby inhibiting protein synthesis and cell proliferation[27,28]. More recently, activation of IDO was also found to limit T cell responsiveness *in vitro* and *in vivo* and could thus play a role in tolerance induction[29,30]. Tryptophan lowering also may represent a T cell down-regulatory strategy of the immune system in RA although not sufficiently effective. IFN-γ treatment was found to be effective in patients with RA[31] which in some way seems to contrast the proinflammatory nature of this cytokine. Beneficial effects of interferon therapy are probably due to further lowering of tryptophan levels slowing down T cell activation. Notably, beneficial effects of low tryptophan diets were described in RA several decades ago[32].

We observed no significant effects of treatment regimens on tryptophan metabolism with the exception of leflunomide being associated with lower tryptophan concentrations. This is quite interesting, since a decline of tryptophan under leflunomide therapy could relate to the increased risk of weight loss which has been described earlier in treated patients[33]. As the number of patients studied receiving leflunomide was very low (n = 4) further work also including followup therapy will have to confirm this observation.

The interference of immune system activation with tryptophan metabolism may also provide a link between immunology and neuropsychiatry[34], since tryptophan is precursor in the biosynthesis of neurotransmitter serotonin (5-hydroxytryptamine). Reduced tryptophan availability due to enhanced degradation was found to be associated with the development of neuropsychiatric disturbances. For example, a relationship between lower serum tryptophan concentrations and an increased probability for polyneuropathy and dementia existed in patients with HIV-1 infection[18]. Similarly in patients with colorectal cancer, lower serum tryptophan concentrations have been associated with reduced quality of life[35]. Further studies are necessary to test for such a relationship in patients with RA.

REFERENCES

1. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 2001;344:907-16.
2. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994;84:2068-101.
3. Malik AB, Lo SK. Vascular endothelial adhesion molecules and tissue inflammation. Pharmacol Rev 1996;48:213-29.
4. Wilder RL. Rheumatoid arthritis: epidemiology, pathology and pathogenesis. In: Schumacher HR, editor. Primer on rheumatic diseases. Atlanta: Arthritis Foundation; 1993:86-9.
5. Cope AP, Aderka D, Aderka D, et al. Increased levels of soluble tumor necrosis factor receptors in the sera and synovial fluid of patients with rheumatic disease. Arthritis Rheum 1992;35:1160-9.
6. Barrera P, Boerbooms AM, Janssen EM, et al. Circulating soluble tumor necrosis factor receptors, interleukin-2 receptors, tumor necrosis factor-alpha and interleukin-6 levels in rheumatoid arthritis. Arthritis Rheum 1993;36:1070-9.
7. Steiner G, Studnicka-Benke A, Witzmann G, Hofler E, Smolen J. Soluble receptors for tumor necrosis factor and interleukin-2 in serum and synovial fluid of patients with rheumatoid arthritis, reactive arthritis and osteoarthritis. J Rheumatol 1995;22:406-12.
8. Keystone EC, Snow KM, Bombardier C, Chang CH, Nelson DL, Rubin LA. Elevated soluble IL-2 receptor levels in the sera and synovial fluids of patients with rheumatoid arthritis. Arthritis Rheum 1988;31:1358-64.
9. Symons JA, Wood NC, Di Giovine FS, Duff GW. Soluble IL-2 receptor in rheumatoid arthritis: correlation with disease activity, IL-1 and IL-2 inhibition. J Immunol 1988;141:2612-8.
10. Rubin LA, Snow KM, Kurman CC, Nelson DL, Keystone EC. Serial levels of soluble interleukin 2 receptor in the peripheral

blood of patients with rheumatoid arthritis: correlations with disease activity. J Rheumatol 1990:17:597-602.
11. Tebib JG, Letroublon MC, Bienvenu J, Bouvier M. sIL-2R levels in rheumatoid arthritis: poor correlation with clinical activity is due to part to disease duration. Br J Rheumatol 1995;34:1037-40.
12. Reibnegger G, Egg D, Fuchs D, et al. Urinary neopterin reflects clinical activity in patients with rheumatoid arthritis. Arthritis Rheum 1986;29:1063-70.
13. Hannonen P, Tikanoja S, Hakola M, Mottonen T, Viinikka L, Oka M. Urinary neopterin index as a measure of rheumatoid activity. Scand J Rheumatol 1986;15:148-52.
14. Nassonov EL, Samsonov MY, Tilz GP, et al. Soluble adhesion molecules in rheumatoid arthritis. Rheumatology 2000;39: 808-10.
15. Huber C, Batchelor JR, Fuchs D, et al. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon? J Exp Med 1984;160:310-6.
16. Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP, Wachter H. Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. Immunol Today 1988;9:150-5.
17. Carlin JM, Ozaki Y, Byrne GI, Brown RR, Borden EC. Interferons and indoleamine 2,3-dioxygenase: role in antimicrobial and antitumor effects. Experientia 1989;45:535-41.
18. Fuchs D, Möller AA, Reibnegger G, Stöckle E, Werner ER, Wachter H. Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symmptoms. J Acquir Immune Def Syndr 1990;3:873-6.
19. Widner B, Werner ER, Schennach H, Wachter H, Fuchs D. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. Clin Chem 1997;43:2424-6.
20. Denz H, Orth B, Weiss G, et al. Weight loss in patients with hematological neoplasias is associated with immune system stimulation. Clin Investig 1993;71:37-41.
21. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. 1988;31:315-24.
22. Steinbrocker O, Traeger CH, Battermann RC. Therapeutic criteria in rheumatoid arthritis, JAMA 1949;140:659-62.
23. Werner ER, Werner-Felmayer G, Fuchs D, Hausen A, Reibnegger G, Wachter H. Parallel induction of tetrahydrobiopterin biosynthesis and indoleamine 2,3-dioxygenase activity in human cells and cell lines by interferon-gamma. Biochem J 1989;262:861-6.
24. Houpt JB. Tryptophan: new questions for an old amino acid. J Rheumatol 1990;17:1431-4.
25. Maerker-Alzer G, Diemer O, Strumper R, Rohe M. Neopterin production in inflamed knee joints: high levels in synovial fluids. Rheumatol Int 1986;6:151-4.
26. Altindag ZZ, Sahin G, Inaninci F, Hascelik Z. Urinary neopterin excretion and dihydropteridine reductase activity in rheumatoid arthritis. Rheumatol Int 1998;18:107-11.
27. Pfefferkorn ER. Interferon gamma blocks the growth of Toxoplasma gondii in human fibroblasts by inducing the host cells to degrade tryptophan. Proc Natl Acad Sci USA 1984;81:908-12.
28. Burke F, Knowles RG, East N, Balkwill FR. The role of indoleamine 2,3-dioxygenase in the anti-tumour activity of human interferon-gamma in vivo. Int J Cancer 1995;60:115-22.
29. Munn, DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T-cell proliferation by macrophage tryptophan catabolism. J Exp Med 1999;189:1663-72.
30. Mellor AL, Munn DH. Tryptophan catabolism and T-cell tolerance: Immunosuppression by starvation? Immunol Today 1999;20:469-73.
31. Lemmel EM, Franke M, Gaus W, et al. Results of a phase-II clinical trial on treatment of rheumatoid arthritis with recombinant interferon-gamma. Rheumatol Int 1987;7:127-32.
32. Houpt JB, Ogryzlo MA, Hunt M. Tryptophan metabolism in man (with special reference to rheumatoid arthritis and scleroderma). Semin Arthritis Rheum 1973;2:333-53.
33. Coblyn JS, Shadick N, Helfgott S. Leflunomide-associated weight loss in rheumatoid arthritis. Arthritis Rheum 2001;44:1048-51.
34. Widner B, Laich A, Sperner-Unterweger B, Ledochowski M, Fuchs D. Neopterin production, tryptophan degradation, and mental depression. What is the link? Brain Behav Immun 2002;16:580-5.
35. Huang A, Fuchs D, Widner B, Glover C, Henderson DC, Allen-Mersh TG. Tryptophan decrease in advanced colorectal cancer correlates with immune activation and impaired quality of life. Br J Cancer 2002;86:1691-6.