

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 μ M, 40.31–49.95 μ M) compared to healthy blood donors (62.62 μ M, 57.27–74.61 μ M; $p < 0.001$). Kynurenine in patients (1.86 μ M, 1.54–2.31 μ M) did not differ from controls (2.06 μ M, 1.58–2.65 μ M; 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
IMMUNE ACTIVATION

TRYPTOPHAN

KYNURENINE
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 role¹. 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 RA⁴. 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-R⁵⁻⁷ and sIL-2R⁶⁻¹¹, or neopterin¹²⁻¹⁴, 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 response¹⁶.

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 catabolites¹⁷. 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 malignant²⁰ 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 \pm 8.9 yrs) with RA who fulfilled the American College of Rheumatology criteria²¹ were recruited from the University Hospital of Innsbruck. According to the

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Steinbrocker criteria²², 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 described¹⁹. 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 macrophages²³. 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 B6²⁴. 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

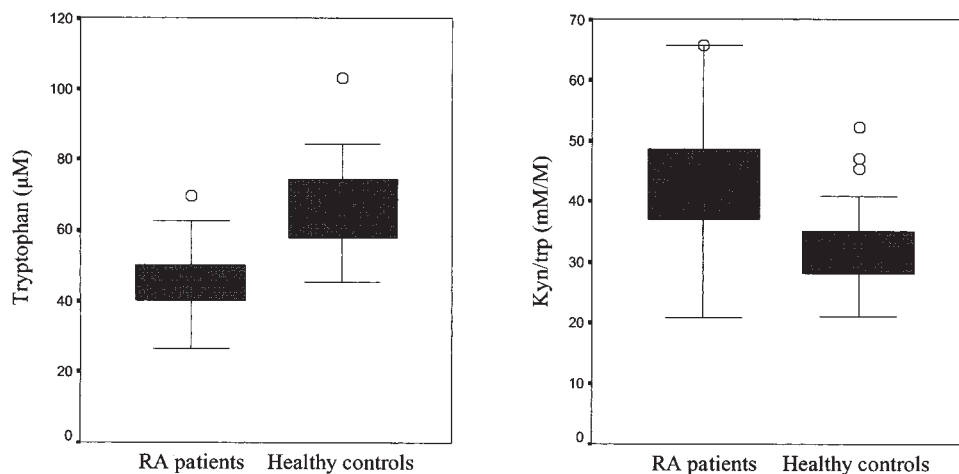


Figure 1. Box plots of serum tryptophan concentration and the kyn/trp ratio in patients with RA compared to blood donors (all comparisons $p < 0.001$). Horizontal lines: medians; boxes: 25th to 75th percentiles; bars: 5th to 95th percentiles; open circles: outliers.

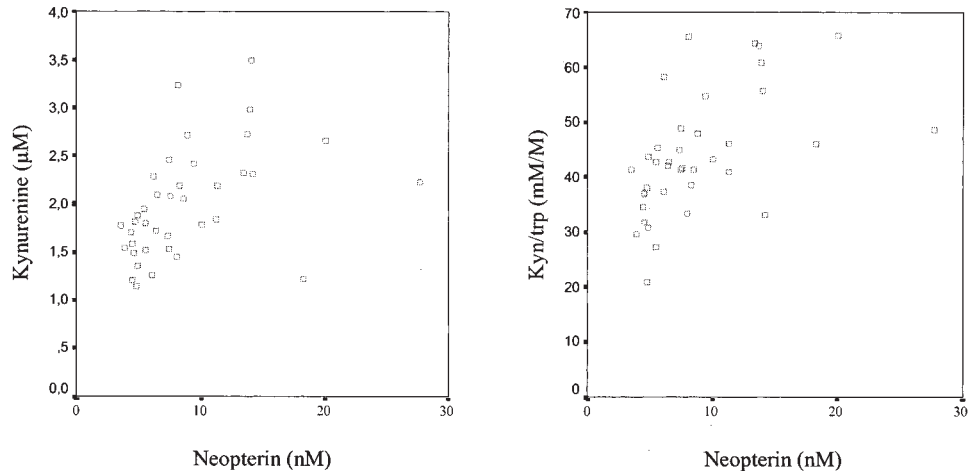


Figure 2. Correlation of serum kynurenine concentrations ($r_s = 0.611$, $p < 0.001$) and of kyn/trp ($r_s = 0.621$, $p < 0.001$) with neopterin concentrations in patients with RA.

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 cytotoxic 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 RA³¹ 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 ago³².

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 patients³³. 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 neuropsychiatry³⁴, 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 infection¹⁸. Similarly in patients with colorectal cancer, lower serum tryptophan concentrations have been associated with reduced quality of life³⁵. Further studies are necessary to test for such a relationship in patients with RA.

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