

High Serum Tryptophan Concentration in Pollinosis Patients Is Associated with Unresponsiveness to Pollen Extract Therapy

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Key Words

Hyposensitization • Specific subcutaneous immunotherapy • Pollinosis • Tryptophan • Indoleamine 2,3-dioxygenase

Abstract

Background/Aims: The immunologic background of allergic asthma and rhinitis includes a preponderance of Th2-type immunity. In parallel, Th1-type immune response is suppressed by Th2-type cytokines. As a consequence, biochemical pathways triggered by Th1-type cytokine interferon- γ , such as tryptophan degradation by indoleamine 2,3-dioxygenase and neopterin production, might be altered. We examined whether they are related to the outcome of hyposensitization therapy in atopic patients. **Methods:** In serum specimens of 44 atopic patients (18 women, 26 men) before any specific immunotherapy, tryptophan and kynurenine concentrations were measured by HPLC, and the kynurenine to tryptophan ratio (kyn/trp) was calculated. Neopterin concentrations were measured by ELISA. Results were compared with concentrations in 38 serum specimens from healthy blood donors and with the outcome of specific subcutaneous immunotherapy in atopics: on clinical grounds, 27 patients were classified as responders, and 17 patients as non-responders. **Results:** Serum tryptophan concentrations were higher in atopics ($84.3 \pm 24.4 \mu\text{M}$) than

in blood donors ($57.9 \pm 7.46 \mu\text{M}$; $p < 0.001$), kynurenine and kyn/trp were not different between the 2 groups. All of the neopterin concentrations measured in patients were $< 8.7 \text{ nM}$, the upper limit of the normal. Non-responders to subcutaneous immunotherapy had significantly higher tryptophan concentrations ($95.7 \pm 27.0 \mu\text{M}$) than responders ($77.1 \pm 19.9 \mu\text{M}$; $p = 0.01$). No other marker concentrations differed between the groups. **Conclusions:** The measurement of serum tryptophan may present an option to predict the outcome of pollen extract therapy. Higher tryptophan levels may result from lower indoleamine 2,3-dioxygenase activity in atopics. However, this possible relationship needs to be confirmed in further studies. Copyright © 2008 S. Karger AG, Basel

Introduction

Allergic asthma and rhinitis seem related to a preponderance of Th2-type immune response. Upon exposure to an allergen, Th2-type cytokines like interleukin (IL)-4, IL-10 and IL-13 are predominantly produced and induce hyperproduction of IgE. In parallel, during an allergic response, Th1-type immune response is suppressed by these cytokines [1, 2]. Thus, also production of Th1-type cytokine interferon (IFN)- γ is probably greatly diminished in allergic diseases.

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IFN- γ induces various very potent biochemical pathways as part of its antimicrobial and antitumoral armature; among them are the induction of the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (EC 1.13.11.42, IDO) [3–5] and of enzyme GTP-cyclohydrolase I (EC 3.5.4.16), which in human monocyte-derived macrophages and dendritic cells gives rise to the production of neopterin [6, 7]. Likewise, IDO activity and neopterin concentrations are enhanced in patients who suffer from various clinical conditions such as infections, allograft rejection, autoimmune syndromes and cancer [8–12]. Such patients often present with increased serum/plasma concentrations of neopterin and kynurenine and with lowered tryptophan concentrations. As a consequence, the kynurenine to tryptophan ratio (kyn/trp) is increased; this ratio allows an estimate of the tryptophan degradation rate [8, 13]. In atopic individuals, lowered kyn/trp has been described recently [14]. Likewise, an inverse relationship was reported between neopterin concentrations and IgE in a population of healthy humans [15].

We were interested to test whether we can confirm a difference between tryptophan, kynurenine and neopterin concentrations in atopics with seasonal rhinoconjunctivitis caused by tree or grass pollen and whether monitoring of these metabolic changes might relate to the outcome of hyposensitization treatment.

Patients and Methods

Study Population

Forty-four atopic patients (18 women, 26 men) were included in this retrospective analysis. Their mean age ($\pm$ SEM) was 27.3 ± 2.43 years, and they were allergic to tree or grass pollen. Stored serum specimens of patients were analyzed, which have been collected before specific immunotherapy with pollen extracts (Alutard SQ, ALK-Abello, Horsholm, Denmark) was initiated in autumn. All specimens used for measurements were thus from the off-pollen season.

Patients were offered specific subcutaneous immunotherapy (SCIT) if they suffered allergic rhinoconjunctivitis (and/or mild asthma) for more than 2 years, more than 6 weeks per season from well-defined pollen allergens (birch and/or grass pollen) and their symptoms were not well controlled with symptomatic treatment. At the time patients started SCIT, no ARIA (Allergic Rhinitis and Its Impact on Asthma) guidelines were available yet. Transcribed to now established ARIA guidelines, all patients with established diagnosis and medium to severe persistent pollinosis were offered specific immunotherapy. Patients who refused SCIT, patients with a reduction in forced expiratory volume in 1 s of $>30\%$ and patients with poor compliance were excluded from this study. Patients who interrupted SCIT during follow-up were also excluded, as well as patients suffering from inflammatory conditions or any

other overt disease. All patients were offered local and oral H₁ antihistamines. If they reported obstructive allergic rhinitis, nasal steroids (mometasone furoate, 1–2 sprays into each nostril once per day) were prescribed. All patients were instructed to use rescue medication early at the beginning of the pollen season.

Tryptophan, kynurenine and neopterin concentrations found in patients were compared with those observed in 38 serum specimens from healthy blood donors. Volunteers for donating blood or blood components were assessed for their health status. Although neither total nor specific IgE antibodies were determined, a questionnaire concerning previous diseases, including allergies, was performed, and individuals taking drugs to suppress acute allergic symptoms, like sneezing, rhinorrhea or obstructive rhinitis and conjunctivitis, e.g., antihistamines or steroids, were not accepted for blood donation. All individuals gave written informed consent that excess amount of serum collected for determination of routine parameters was referred to further scientific testing.

Results of analytical measurements were also compared with the outcome of SCIT, which in a routine setting was performed as follows: during 3 years of SCIT, annual controls were done after the pollen season – usually between October and January. Patients' symptoms were recorded from symptom diary protocols, the number of symptom days, usage of nasal spray, eye drops and/or nasal steroids. A standard prick test procedure was performed, and specific IgE antibodies were measured. At the end of the investigation, each patient was asked to fill in a symptom diary for the next control. However, in reality, most patients did not protocol their symptoms or their drug usage as outlined. Thus, the parameter to continue or stop SCIT after 3 years mostly relied on a subjective feeling of the patient and – if possible – nasal provocation tests. Patients were grouped according to the symptoms recorded as: totally disappeared or much better (successful), unchanged or worse, or new bronchial asthma during SCIT corresponding to the responsible pollen allergen (not successful). Three years after initiation of SCIT, 27 patients (61%; 10 women, 17 men; age 26.8 ± 3.32 years) were classified as responders and 17 (39%; 8 women, 9 men; age 28.0 ± 3.42 years) as non-responders.

Specimen Collection and Assay Methods

Tryptophan and kynurenine concentrations were determined by HPLC using reversed-phase C18 column, 0.015 M acetic acid/sodium acetate buffer and a flow rate of 1 ml/min, as described earlier [13, 16]. Tryptophan was monitored by its native fluorescence at 285 nm excitation and 360 nm emission wavelength, kynurenine was determined by UV absorption detection at 360 nm wavelength, and kyn/trp was calculated. Serum neopterin concentrations were measured by competitive ELISA with a sensitivity of 2 nM (Brahms, Hennigsdorf/Berlin, Germany).

Statistical Analyses

Results are presented as mean values $\pm$ SEM. Because not all the data showed normal distribution, the non-parametric Wilcoxon test was used for comparison of grouped data, and associations between variables were calculated employing Spearman's rank correlation coefficient (r_s). Dichotomized variables were compared using the χ^2 test. The Statistical Package for the Social Sciences (version 14 SPSS, Chicago, Ill., USA) was used. p values <0.05 were considered to indicate significant differences.

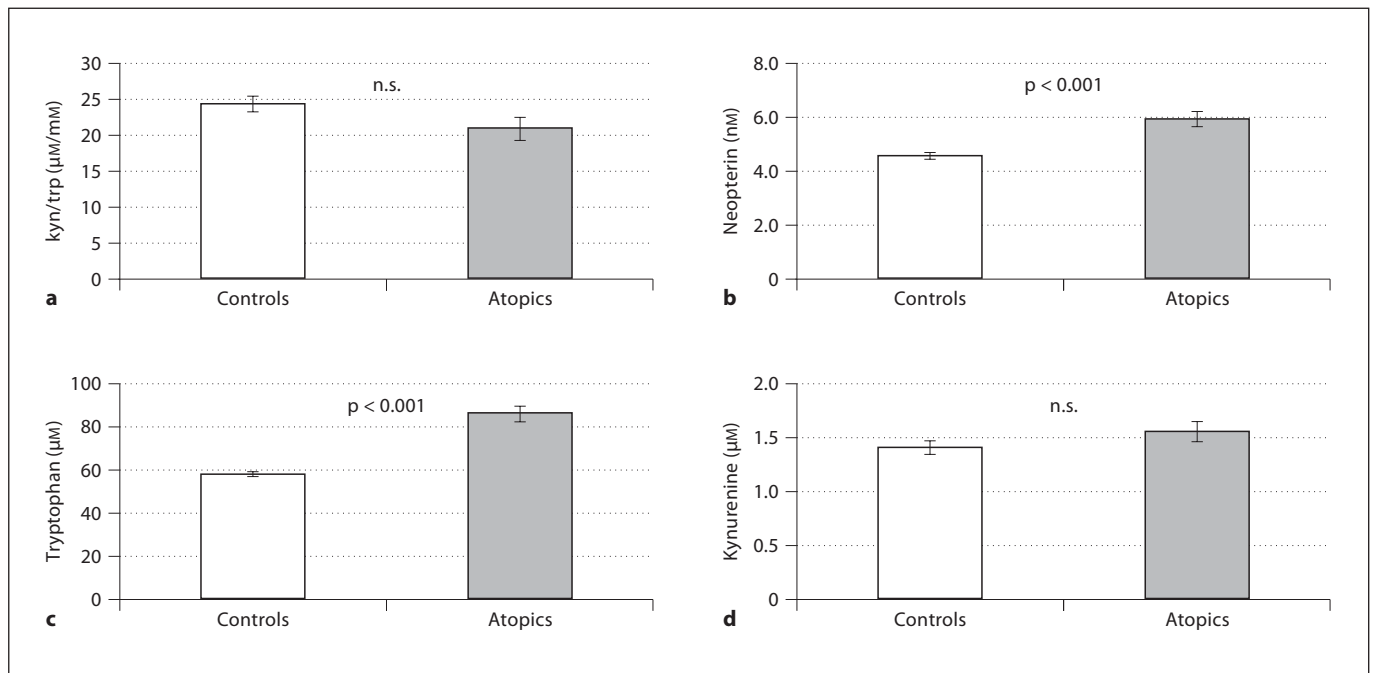


Fig. 1. kyn/tryp (a) and concentrations of neopterin (b), tryptophan (c) and kynurenine (d) in healthy controls (white columns) versus atopic patients (gray columns); means (boxes) $\pm$ SEM (vertical lines) are shown. n.s. = Not significant (Wilcoxon test).

Results

Serum tryptophan concentrations were significantly higher in atopics ($84.3 \pm 3.68 \mu\text{M}$) than in blood donors ($57.9 \pm 1.21 \mu\text{M}$; $U = 6.45$, $p < 0.001$) (fig. 1). Kynurenine concentrations (controls 1.40 ± 0.057 vs. atopics $1.56 \pm 0.098 \mu\text{M}$; $U = 1.54$, not significant) and kyn/tryp tended to be lower, but did not differ statistically between the 2 groups (controls 24.5 ± 1.02 vs. atopics $20.9 \pm 1.64 \mu\text{M}/\text{mM}$; $U = 1.12$, not significant). All neopterin concentrations in atopics and controls were < 8.7 nM, which is the upper limit of normal in healthy controls corresponding to the 95th percentile [9]. Still, atopics had higher neopterin concentrations than the controls (atopics 5.92 ± 0.28 vs. controls 4.59 ± 0.16 nM; $U = 4.87$, $p < 0.001$) (fig. 1).

When comparing subgroups according to the outcome of SCIT, non-responders had significantly higher tryptophan concentrations ($95.7 \pm 6.55 \mu\text{M}$) than responders ($77.1 \pm 3.83 \mu\text{M}$; $U = 2.45$, $p = 0.014$) (fig. 2). Again, there were no significant differences in kynurenine concentrations (non-responders 1.53 ± 0.17 vs. responders $1.58 \pm 0.12 \mu\text{M}$; $U = 0.39$, not significant) and kyn/tryp (non-responders 18.3 ± 2.45 vs. responders 22.5

$\pm 2.16 \mu\text{M}/\text{mM}$; $U = 1.37$, not significant). Also, neopterin concentrations did not differ between responders (5.91 ± 0.41 nM) and non-responders (5.96 ± 0.37 nM; $U = 0.37$, not significant). Ten of the patients presented with tryptophan concentrations $> 100 \mu\text{M}$, of whom 7 were non-responders (70%), whereas of 34 patients with tryptophan $< 100 \mu\text{M}$, 10 (29.4%) were non-responders to SCIT (relative risk = 5.60, $\chi^2 = 5.37$, $p = 0.02$).

Whereas kyn/tryp and neopterin concentrations correlated positively in the controls ($r_s = 0.410$, $p < 0.01$), no such association was found in the patients. By contrast, a positive correlation was found between neopterin and tryptophan concentrations in the whole group of patients ($r_s = 0.259$, $p < 0.05$) and in non-responders ($r_s = 0.564$, $p < 0.01$), but not in the group of responders ($r_s = 0.020$, not significant).

Discussion

Compared with healthy blood donors, atopics presented with higher concentrations of essential amino acid tryptophan. The higher tryptophan levels may result from subnormal degradation rate, which could probably

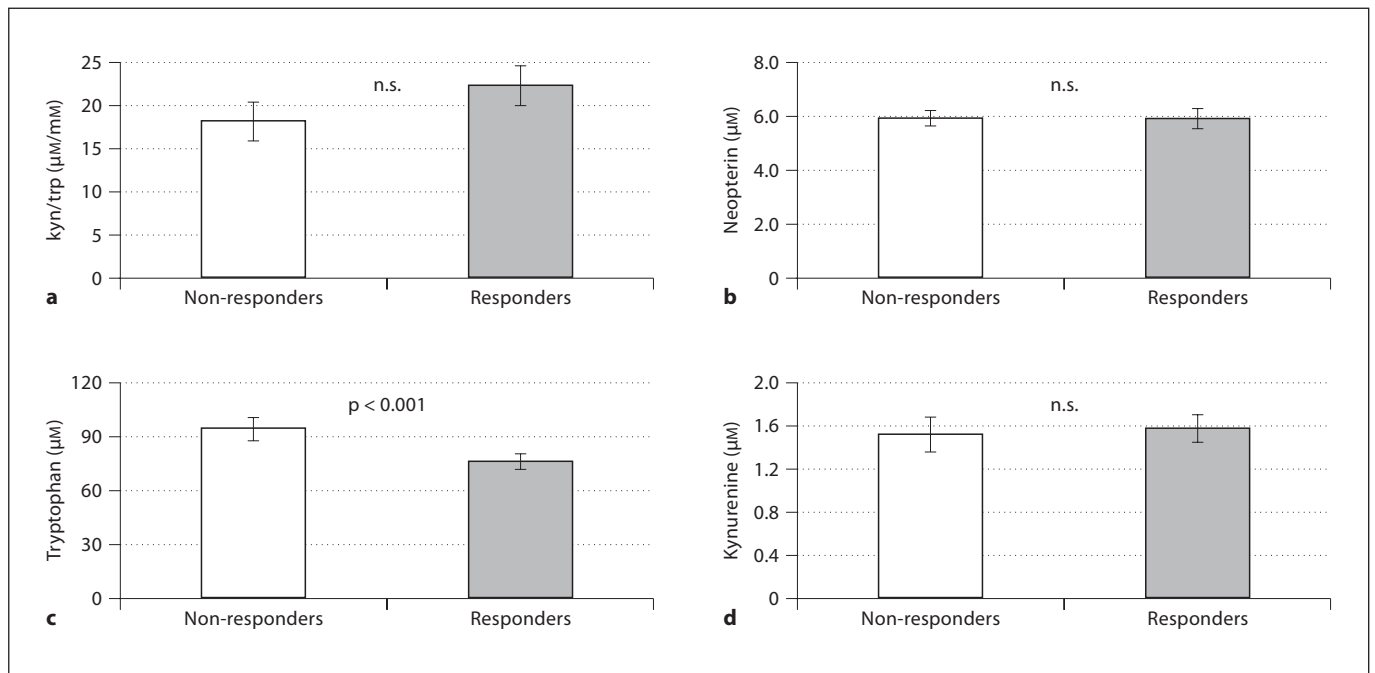


Fig. 2. kyn/tryp (a) and concentrations of neopterin (b), tryptophan (c) and kynurenine (d) in atopic patients split into 2 groups according to the outcome of hyposensitization therapy (white columns: non-responders; gray columns: responders); means (boxes) $\pm$ SEM (vertical lines) are shown. n.s. = Not significant (Wilcoxon test).

be due to suppressed Th1-type immunity and lower IFN- γ and IDO activity in atopics. Such a conclusion would agree well with earlier findings of Raitala et al. [14], who described lower kyn/tryp in atopics versus non-atopics. In our study, we did not find such a significant difference in kyn/tryp between atopics and blood donors. However, our study population was smaller than that of Raitala et al. [14] and probably not large enough to detect a significant difference in kyn/tryp. In fact, the lowering of kyn/tryp in atopics compared with controls was even larger in our study (–15% in atopics compared with controls) than in the study by Raitala et al. [14], in which it was only –8%. Notably, in our study, there was a highly significant difference in tryptophan concentrations between atopics and controls. Since kynurenine concentrations were not different, any change in kyn/tryp thus resulted from a decline in tryptophan. This fact renders an involvement of IDO rather unlikely. Unfortunately, the absolute tryptophan and kynurenine concentrations were not reported in the study by Raitala et al. [14].

Other factors, e.g., alimentation, genetic or environmental factors, could influence tryptophan levels, although the variation in serum/plasma-free tryptophan levels appears to be rather small within normal dietary

habits [17]. During tryptophan loading as well as depletion under experimental conditions, e.g., in rats, a sometimes dramatic influence on tryptophan levels can be achieved [18, 19]. However, this is certainly of limited relevance in our patients. Antioxidant vitamins and antioxidant dietary supplements were found recently to counteract cytokine-induced tryptophan degradation in vitro [20, 21]. If these findings also hold true in vivo, a high intake of such compounds could contribute to the increase in serum tryptophan concentrations [22]. Nevertheless, none of these nutritional aspects was addressed in our study.

An earlier study reported slightly but not significantly higher plasma tryptophan concentrations in symptomatic compared with asymptomatic atopic patients, and this was true only in season but not off season. Although there was no significant difference in kynurenine levels between groups, asymptomatic atopics presented with higher kyn/tryp than symptomatic patients or non-atopic controls [23]. A seasonal influence can be excluded in our study, because all specimens were collected before hyposensitization therapy was initiated in late autumn, and thus, off season. This is particularly true for the comparison of subgroups, namely responders and non-respond-

ers to hyposensitization therapy, when all patients were from the same seasonal window.

Suppressed Th1-type immunity and lowered IFN- γ production in atopics might cause a decline in IDO activity. However, it should also slow down neopterin concentrations. However, the opposite was the case: atopics presented with slightly higher neopterin concentrations than controls, although all the individual concentrations measured in our atopic patients were within the normal range. In fact, the mean value measured in our control population was somewhat below the one found in larger populations of blood donors and healthy controls, i.e. 5.3 ± 2.7 nM [24], but the neopterin concentrations found in atopics were still higher. The changes in neopterin concentrations were unrelated to the outcome of SCIT, and moreover, did not correlate with kyn/trp. Usually, kyn/trp and neopterin production were induced in parallel and found to correlate strongly in patients with, e.g., infections, autoimmune syndromes or malignancy [8, 11, 12]. Notably, in our study, a correlation between kyn/trp and neopterin was found in the healthy controls but not in the atopic patients. Thus, a role of IDO in the abnormal tryptophan levels in atopics appears to be rather unlikely, even if it would fit nicely with the common understanding of atopy to be driven by Th2-type immune response [25]. Still, there is no good explanation for the divergent development of neopterin and kyn/trp in our population of atopics.

Although the background of high tryptophan concentrations in the non-responders to SCIT compared with responders remains unexplained, the increased tryptophan availability may, on the one hand, influence T cell function, and, on the other hand, represent a consequence of IDO expression in dendritic cells and the induction of specific regulatory T cells, which are discussed to be strongly involved in the pathogenesis of allergic disease [26]. An earlier study by von Bubnoff et al. [23] suggested that in allergen-sensitized individuals, a higher IDO activity may inhibit allergic reaction. However, specimens of all our patients were collected off season, and therefore, our results cannot be used to support or contradict this conclusion. Given that the elevated tryptophan concentrations found in our study might be involved in the pathogenesis of the allergic response, an increased IDO activity would rather slow down allergic reactions. During Th1-type immune response, IDO is induced preferentially in monocytic and dendritic cells, and like IL-10, it may represent a kind of feed-back control to slow down T cell activation and proliferation via the induction of regulatory T cells and Th2-type polarization [26]. The

finding of a correlation between kyn/trp and IL-10 concentrations solely in the protected patients would support such a conclusion [23].

Other mechanisms than IDO could underlie the increase in tryptophan in atopics. Recently, analysis of the IFN- γ +874(T/A) genotypes revealed that tryptophan catabolism is genetically controlled by the IFN- γ gene [27]. Thus, polymorphisms of the IFN- γ gene could be relevant in disease conditions which are associated with elevated or subnormal IDO activity. However, one would expect that this genetic polymorphism should affect neopterin production in the same way as IDO activity. Alternatively, or in addition, lower activity of tryptophan pyrrolase (EC 1.13.11.11), an isoenzyme of IDO, which is preferentially expressed in hepatic cells but almost insensitive to IFN- γ , could relate to subnormal catabolism of tryptophan and thus be responsible for an increase in tryptophan levels. Importantly, enhanced tryptophan pyrrolase activity would be independent from any influence on neopterin concentrations.

Atopics with higher tryptophan concentrations were found to be less likely to respond to SCIT compared with those with lower tryptophan. These higher tryptophan levels could be due to lower IFN- γ and IDO activity, especially in atopics, who fail to respond to SCIT [1, 25]. Data point to stronger Th2-type immunity in non-responders [28], which results in higher tryptophan concentrations due to a lower rate of tryptophan degradation. Although there was a considerable overlap of tryptophan concentrations in these 2 subgroups of patients, the measurement of tryptophan concentrations may present an option to predict unresponsiveness to such therapy. Clearly, a greater patient population is required to confirm the value of tryptophan measurements before hyposensitization to predict its outcome.

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