

Specific Immunotherapy Normalizes Tryptophan Concentrations in Patients with Allergic Rhinitis

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Key Words

Pollinosis · Specific immunotherapy · Tryptophan · Indoleamine 2,3-dioxygenase

Abstract

Background/Aims: An immune shift towards Th2-type immunity seems to be critical in the pathogenesis of allergic asthma and rhinitis. In a previous study, we found higher serum tryptophan concentrations in patients with seasonal tree or grass pollen rhinoconjunctivitis who underwent specific immunotherapy (SCIT) than in controls, and those with the highest levels at baseline responded less well to SCIT. In the present study, we examined whether 'booster immunotherapy' after cessation of SCIT had any influence on tryptophan metabolism during follow-up. **Methods:** Serum concentrations of tryptophan, kynurenine and neopterin were assayed in 19 patients (mean age: 26.2 years; 6 females) allergic to grass and/or tree pollen before and after they had received a booster immunotherapy with 4 injections of an allergoid vaccine (Pollinex Quattro; Bencard Vienna, Austria) over 8 ± 3 months outside the pollen season. **Results:** Serum tryptophan and kynurenine concentrations decreased after booster immunotherapy (mean $\pm$ SD, before immunotherapy: 81.1 ± 14.2 μ mol/l, after immunotherapy: 61.4 ± 20.9 μ mol/l and before immunotherapy: 2.25 ± 0.44 , after

immunotherapy: 1.69 ± 0.70 μ mol/l, respectively; both $p < 0.01$); this was especially true in those responders who also tended to have lower baseline kynurenine concentrations as compared with nonresponders ($p = 0.05$). Finally, a correlation between changes in tryptophan metabolism and neopterin concentrations was observed after immunotherapy.

Conclusions: The decrease in tryptophan and kynurenine concentrations following booster immunotherapy in hay fever patients strengthens the hypothesis that tryptophan metabolism might be involved in the course of allergic responses. However, it is still unclear whether the abnormal tryptophan metabolism in pollinosis patients is related to indoleamine 2,3-dioxygenase and/or to a specific cytokine background.

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Introduction

An immune shift towards Th2-type immunity is considered to be critical in the pathogenesis of allergic asthma and rhinitis. Th2-type cytokines such as interleukin (IL)-4, IL-13 and others are upregulated and suppress Th1-type immune response and Th1-type cytokines such as interferon- γ (IFN- γ) [1, 2]. The latter is a strong inducer of indoleamine 2,3-dioxygenase (IDO), an enzyme

which degrades the essential amino acid tryptophan as part of the antiproliferative strategy of immunocompetent cells to halt the growth of infected and malignant cells within Th1-type immune responses [3, 4]. IDO was also demonstrated to be an important immunoregulatory enzyme that is capable of inducing immunotolerance via the induction of regulatory T cells [5, 6], and IDO has been also suggested to play a role in the pathogenesis of allergic diseases [7–10].

Enhanced IDO activity as is reflected by a decline of tryptophan and an increased ratio of kynurenine to tryptophan (Kyn/Trp) has been documented in a variety of clinical conditions, including infections, autoimmune syndromes and malignant processes [11–13]. Such diseases are characterized by chronic stimulation of the T cell/macrophage system, and during Th1-type immune response, IDO is induced preferentially in monocytes/macrophages and dendritic cells [6, 14, 15]. In these cells, an increased Kyn/Trp is associated with elevated concentrations of neopterin, which, like IDO, is preferentially released in Th1-type immune response [14–17]. By contrast, a lower Kyn/Trp was described in atopic versus nonatopic subjects [18], and an earlier study by von Bubnoff et al. [19] suggests that in allergen-sensitized individuals a higher IDO activity might inhibit allergic reactions. Finally, expression of the IDO gene *INDO* in nasal polyps was found to characterize susceptibility to aspirin-intolerant asthma [20].

We have recently described higher serum tryptophan concentrations in patients with pollinosis compared with blood donors [21]. This finding was confirmed in an independent study reporting higher serum tryptophan concentrations in patients suffering from allergic rhinitis than in controls, and tryptophan concentrations were found to be higher outside the pollen season than in the season [22]. Higher baseline tryptophan concentrations were preferentially observed in nonresponders to specific immunotherapy (SCIT) as compared with responders [21]. However, only tryptophan concentrations but not Kyn/Trp and kynurenine concentrations were of statistical significance, and the changes in tryptophan metabolism were independent of neopterin concentrations [21].

In order to clarify the role of tryptophan as a predictor of response to immunotherapy in patients with pollinosis, we compared serum tryptophan, kynurenine and neopterin concentrations in patients with confirmed grass and birch pollen allergy shortly before and after booster immunotherapy. Booster immunization was introduced for the first time in 1958 [23]. It was applied as a scratch test to reactivate the effects of earlier SCIT. As concerns

SCIT, it is postulated that aside from the Th1/Th2 shift, the blocking effects of IgG antibodies also achieve this goal [24]. For that reason, Pollinex, an allergoid adsorbed to tyrosine, is used with monophosphoryl lipid A as an adjuvant that induces a strong IgG antibody production.

Patients and Methods

Patients

Tryptophan, kynurenine and neopterin concentrations were determined retrospectively in 19 allergic patients (6 females, 13 males; mean age 26.2 years; 6 were allergic to grass pollen, 10 to grass/birch, 2 to birch, 1 to plantain). Only patients displaying a clinical response to the booster immunotherapy were included in this analysis. All patients had a history of tree and/or grass pollen allergy of several years and had undergone a 3-year perennial classic SCIT with specific pollen extracts (Alutard SQ, ALK-Abello, Horsholm, Denmark). Patients who responded to SCIT, but whose symptoms of pollinosis recurred after 3 or 4 years of follow-up were asked to participate in a booster immunotherapy study. Patients who interrupted immunotherapy during follow-up were excluded from this analysis as well as patients suffering from inflammatory conditions or any other overt disease. All patients were offered local and oral H₁ antihistamines for symptomatic relief. If they reported obstructive allergic rhinitis, nasal steroids (mometasonefuroate 1–2 sprays into each nostril once a day) were prescribed. After the study, patients were grouped according to the symptoms, which were recorded as: totally disappeared or much better (= responder), unchanged or worse, or new bronchial asthma during booster immunotherapy corresponding to the responsible pollen allergen (nonresponder). Ten patients (3 females and 7 males) were thus classified as responders, and 9 patients as nonresponders.

Serum specimens were obtained before and after the booster immunotherapy (4 injections, days 1, 14, 28 and 56 incremental doses from 300 to 2,000 SU) with the Pollinex Quattro (Bencard) allergoid vaccine. The study was in accordance with the Helsinki Declaration, and patients gave informed consent that parts of their blood specimens obtained during routine procedures were anonymously used for additional analyses within this study.

Specimen Collection and Assay Methods

Tryptophan and kynurenine concentrations of all the specimens were determined by HPLC on the same day using a reversed-phase C₁₈ column, 0.015 mol/l acetic acid/sodium acetate buffer and a flow-rate of 1 ml/min as described earlier [25, 26]. Tryptophan was monitored by its native fluorescence at 285 nm excitation and 360 nm emission wavelengths, kynurenine was determined by UV absorption detection at 360 nm wavelength, Kyn/Trp was calculated. Serum neopterin concentrations were measured using a competitive ELISA with a sensitivity of 2 nmol/l (Brahms, Hennigsdorf, Germany).

Patient tryptophan, kynurenine and neopterin concentrations were compared with those measured in 38 serum specimens from healthy blood donors (23 males and 15 females; 23–42 years) as reported earlier [21]. These individuals were voluntary blood donors and their health status was assessed, but neither total nor specific IgE antibodies were determined. However, they were

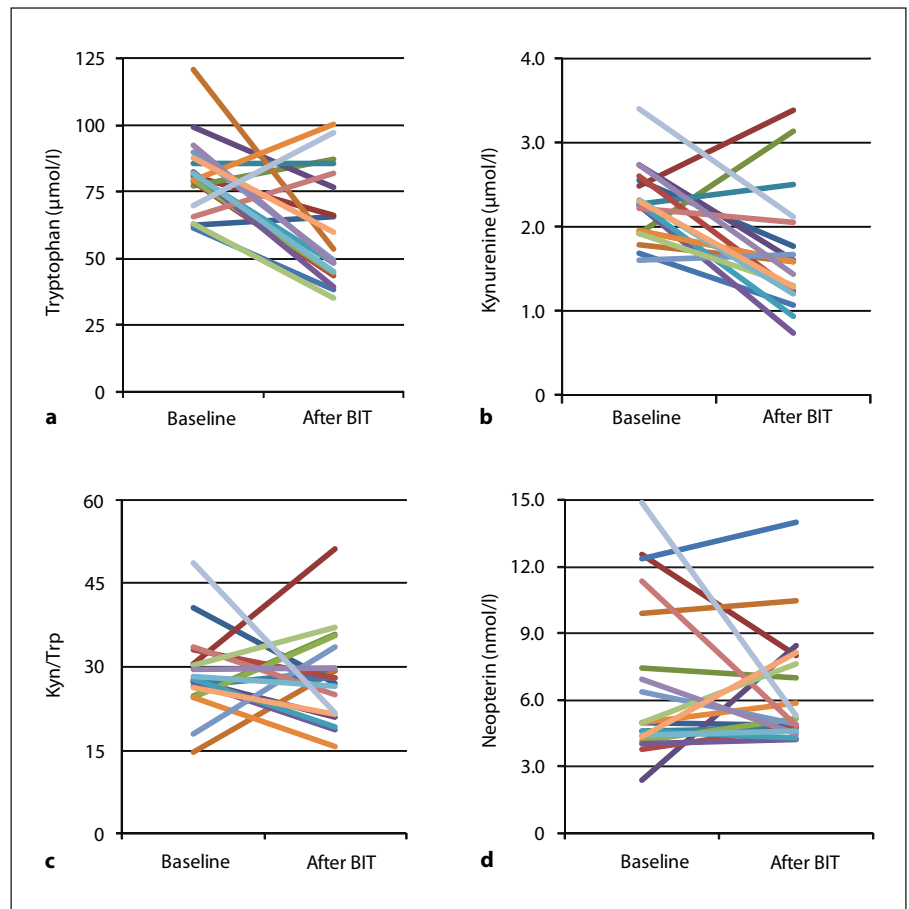


Fig. 1. Changes of tryptophan (**a**; $U = 2.82$, $p < 0.01$), kynurenine (**b**; $U = 2.66$, $p < 0.01$) and neopterin (**d**; n.s.) concentrations and Kyn/Trp; **c**; n.s.) in patients before (baseline) and after booster immunotherapy (BIT).

asked to fill in a questionnaire concerning previous diseases, including allergies, and individuals taking antihistamines or steroids to suppress acute allergic symptoms, like sneezing, rhinorrhea or obstructive rhinitis and conjunctivitis were not accepted.

Statistical Analyses

Results are presented as means $\pm$ SD. As not all the data were normally distributed, the nonparametric Wilcoxon test was used for comparison of grouped data, and associations between variables were calculated with the Spearman rank correlation coefficient (r_s). The Statistical Package for the Social Sciences (SPSS 17.0, Chicago, Ill., USA) was used. p values < 0.05 were considered to indicate significant differences.

Results

Patients' baseline tryptophan concentrations (mean $\pm$ SD) were $81.1 \pm 14.2 \mu\text{mol/l}$ were higher than those of healthy controls [21] ($57.9 \pm 1.21 \mu\text{mol/l}$; $U = 6.45$, $p < 0.001$). Kynurenine concentrations were $2.25 \pm 0.44 \mu\text{mol/l}$ (controls: $1.40 \pm 0.057 \mu\text{mol/l}$; $U = 2.66$, $p <$

0.01), and Kyn/Trp was $28.6 \pm 7.4 \mu\text{mol/mmol}$ (controls: $28.0 \pm 8.3 \mu\text{mol/mmol}$; n.s.). Neopterin concentrations at baseline were $6.8 \pm 3.6 \text{ nmol/l}$ (controls: $5.3 \pm 0.16 \text{ nmol/l}$; n.s.), 5 of 19 patients (26%) had neopterin concentrations outside the normal range (95th percentile is 8.7 nmol/l [17]). Neopterin concentrations correlated with age ($r_s = 0.480$, $p < 0.05$), none of the other parameters showed such a relationship. At baseline, there was no significant correlation between the markers of tryptophan metabolism and neopterin concentrations.

Serum tryptophan concentrations after booster immunotherapy ($61.4 \pm 20.9 \mu\text{mol/l}$) were significantly lower compared with baseline ($U = 2.82$, $p < 0.01$; fig. 1) and were no longer statistically different from the levels observed in controls. After booster immunotherapy, kynurenine concentrations were lower as well ($1.69 \pm 0.70 \mu\text{mol/l}$) than at baseline ($U = 2.66$, $p < 0.01$); accordingly, Kyn/Trp after immunotherapy was 28.0 ± 8.3 and thus had not significantly changed ($p = \text{n.s.}$). Neopterin concentrations remained unchanged as well (after immu-

notherapy: 6.4 ± 2.6 nmol/l; fig. 1), after immunotherapy, only 2 patients (11%) presented with elevated neopterin concentrations.

After booster immunotherapy, there was a rather strong association between tryptophan and kynurenine levels ($r_s = 0.754$, $p < 0.001$). Neopterin concentrations correlated with kynurenine ($r_s = 0.478$, $p < 0.05$) but not with tryptophan concentrations ($r_s = 0.319$) or with Kyn/Trp ($r_s = 0.353$); neither correlation was statistically significant).

When patients were grouped according to their clinical responses, the baseline kynurenine concentrations in nonresponders (2.42 ± 0.46 μ mol/l) strongly tended to be higher than those of responders (2.07 ± 0.34 μ mol/l; $U = 1.96$, $p = 0.05$). None of the other variables showed any significant difference between the two groups. In the responder group, the kynurenine concentrations dropped significantly after the booster immunotherapy (2.07 ± 0.34 vs. 1.68 ± 0.63 μ mol/l; $U = 2.19$, $p < 0.03$), and in the nonresponders, tryptophan concentrations also decreased after the booster immunotherapy (77.6 ± 9.9 μ mol/l) as compared with baseline values (63.0 ± 24.0 μ mol/l; $U = 2.31$, $p < 0.03$). The alterations in Kyn/Trp and neopterin concentrations were not significant in any of the groups compared.

Discussion

Our study reports high tryptophan and kynurenine concentrations in patients with seasonal rhinoconjunctivitis caused by tree or grass pollen, but in the absence of allergen exposure, the levels declined towards the normal range when patients underwent successful booster immunotherapy. These results confirm and extend our earlier findings [22] and those of others [19], and further strengthen the hypothesis that tryptophan metabolism is involved in the pathogenesis of allergic responses and symptoms. Consequently, tryptophan concentrations may serve as a simple monitoring parameter for successful immuno. Moreover, significant relationships also existed between the responses to immunotherapy and tryptophan metabolism in this independent collection of specimens: in fact, the decline in kynurenine concentrations before and after immunotherapy was significant only in the responders. However, this result has to be regarded with some caution because the number of patients was very small for such an analysis of subgroups.

The observed decline in tryptophan concentrations under booster immunotherapy may be due to an influ-

ence on specific cytokine cascades and, as a consequence, the activity of tryptophan-degrading enzyme IDO may change. Allergic asthma and rhinitis are related to a preponderance of Th2-type immunity that is exaggerated upon exposure to an allergen, Th2-type cytokines like IL-4 and IL-13 are predominantly produced and induce a hyperproduction of IgE [1, 26]. In parallel, during an allergic response, the Th1-type immune response is thought to be suppressed by the same cytokines [1, 2]. As a consequence, the production of the Th1-type cytokine IFN- γ , the main inducer of IDO, declines, and a decreased expression of IFN- γ receptor and an attenuated IFN- γ response by dendritic cells was reported in patients with atopic dermatitis [29]. Thus, under such circumstances the activation of IDO and of neopterin production is likely to become suppressed, which would explain the rise in tryptophan concentrations in atopic subjects. However, to address this issue properly, it would be necessary to measure both the concentrations of Th2 cytokines like IL-4 and/or IL-13 in the sera of treated patients and IDO induction in patients' peripheral blood monocytes or in specific nasal secretions before and after SCIT. Unfortunately, such data were not accessible in our study. Moreover, in our patients the mean serum neopterin concentrations, another indicator of Th1-type immunity, measured before booster immunotherapy was even slightly higher than those found in larger populations of blood donors and healthy controls [17, 30]; approximately one quarter of our patients presented with neopterin concentrations clearly above the upper limit of the normal range in healthy controls (>8.7 nmol/l). Kynurenine concentrations were high too and, consequently, Kyn/Trp was not subnormal before booster immunotherapy; in addition, neopterin and Kyn/Trp did not change during immunotherapy, and the difference of kynurenine levels before and after treatment, albeit significant, was rather small in absolute terms, which is comparable to that observed in our first report, where the difference was not significant, however. Accumulation of kynurenine might occur when vitamin B₆, the necessary cofactor of converting enzyme, is deficient [31]. However, such information was not available from our patients.

A seasonal influence on our results can be widely ruled out because all specimens were collected before and after the specific booster immunotherapy that was initiated in the symptom-free interval in late autumn. So the therapeutic protocol itself appears to be involved in the decline in tryptophan concentrations, and this decline does not necessarily depend on the modulation of cytokine cascades, at least not those that are important for

regulating tryptophan degradation and neopterin production.

Tryptophan breakdown and neopterin production were induced in parallel both in vivo and in vitro and were found to correlate strongly in several groups of patients with, e.g. infections, autoimmune syndromes or malignancies [13]. There was no correlation between changes in neopterin and tryptophan metabolism in our patients before treatment: neopterin and kynurenine concentrations correlated significantly only after booster immunotherapy. As no seasonal influence on neopterin concentrations was observed in thousands of blood donations collected over more than 1 year [30], it seems that the absence of any correlation between neopterin and Kyn/Trp in atopic subjects is due to an abnormal tryptophan metabolism outside the pollen season, which, however, seems to be independent of a specific cytokine background and returns to normal during allergen exposure in the pollen season. Corresponding changes of neopterin concentrations were only minor as compared with tryptophan levels. These results are in good agreement with our earlier findings in two independent groups of patients with pollinosis [21, 22]; a slight increase in neopterin was observed but still there is no good explanation for the divergent development of neopterin and Kyn/Trp in the population of atopic subjects. Any increase in tryptophan concentrations in our patients could only have persisted as a result of immunoregulatory events that occurred in the pollen season and a slowed down IDO activity outside the season, persisting throughout the rest of the year when exposure has ceased.

A specific interaction between nitric oxide (NO) and IDO could play a particular role in the development of higher tryptophan concentrations in patients with allergic rhinitis. Enhanced NO synthesis has been reported in patients with asthma and allergic rhinitis [32]. Exhaled-breath NO is increased in asthmatics versus healthy controls [33] and serum nitrite concentrations were found to be higher in allergic patients outside the pollen season than in the season [34]. Likewise, it is well documented that NO slows down the expression and activity of the heme enzyme IDO [35]. Thus, our observation is in line with the view that NO might suppress IDO activity and lead to increased tryptophan concentrations. Notably, no such effect of NO is known for GTP-cyclohydrolase I, the key enzyme for neopterin production [36]. Thus, this background corresponds well with the independent development of Kyn/Trp and neopterin concentrations in patients with allergic rhinitis when, e.g. mast cells would begin to produce IFN- γ [37] and stimulate neopterin pro-

duction but not IDO activity, which is arrested by NO. Von Bubnoff et al. [19] showed that higher IDO activity should be able to suppress the allergic response. Accordingly, inhibitors of inducible NO synthase (iNOS) are candidates for antiallergic therapy that may decrease the production of NO and thus abrogate the arrest of IDO [38]. However, selective iNOS did not achieve any measurable effect on allergen challenge in asthma [39]. Based on those observations and our own observations, it will be very important to decide when to treat patients with iNOS inhibitors, a treatment that should be effective in reducing tryptophan concentrations in patients outside the pollen season but not so much in the season, when tryptophan declines endogenously.

In conclusion, our study confirms high serum tryptophan concentrations in atopic subjects out of the pollen season. Like allergen exposure in the pollen season, booster immunotherapy slows down tryptophan concentrations. The background for this observation is still unclear, but it seems to be related to the interaction between Th1- and Th2-type cytokines and their immunobiological consequences, such as enhanced NO production, which may selectively downregulate IDO, thereby strengthening the response to allergens. However, our study population was small and thus the findings can only be regarded as preliminary. Clearly, a large population of patients is necessary to assess the value of tryptophan measurements during SCIT.

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References

- 1 Lucey DR, Clerici M, Shearer GM: Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. *Clin Microbiol Rev* 1996;9:532–562.
- 2 Romagnani S: Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol* 2004;113:395–400.
- 3 Yoshida R., Imanishi J, Oku T, Kishida T, Hayaishi O: Induction of pulmonary indoleamine 2,3-dioxygenase by interferon. *Proc Natl Acad Sci USA* 1981;78:129–132.
- 4 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;81:908–912.

- 5 Moser M, Murphy KM: Dendritic cell regulation of TH1-TH2 development. *Nat Immunol* 2000;3:199–205.
- 6 Mellor AL, Munn DH: IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004;4:762–774.
- 7 Hayashi T, Beck L, Rossetto C, Gong X, Takikawa O, Takabayashi K, Broide DH, Carson DA, Raz E: Inhibition of experimental asthma by indoleamine 2,3-dioxygenase. *J Clin Invest* 2004;114:270–279.
- 8 Xu H, Oriss TB, Fei M, Henry AC, Melgert BN, Chen L, Mellor AL, Munn DH, Irvin CG, Ray P, Ray A: Indoleamine 2,3-dioxygenase in lung dendritic cells promotes Th2 responses and allergic inflammation. *Proc Natl Acad Sci USA* 2008;105:6690–6695.
- 9 Taher YA, Piavaux BJ, Gras R, van Esch BC, Hofman GA, Bloksma N, Henricks PA, van Oosterhout AJ: Indoleamine 2,3-dioxygenase-dependent tryptophan metabolites contribute to tolerance induction during allergen immunotherapy in a mouse model. *J Allergy Clin Immunol* 2008;121:983–991.
- 10 Le AV, Broide DH: Indoleamine-2,3-dioxygenase modulation of allergic immune responses. *Curr Allergy Asthma Rep* 2006;6:27–31.
- 11 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 symptoms. *J Acquir Immune Defic Syndr* 1990;3:873–876.
- 12 Huengsborg M, Winer JB, Gompels M, Round R, Ross J, Shahmanesh M: Serum kynurenine-to-tryptophan ratio increases with progressive disease in HIV-infected patients. *Clin Chem* 1998;44:858–862.
- 13 Schroeksadel K, Wirleitner B, Winkler C, Fuchs D: Monitoring tryptophan metabolism in chronic immune activation. *Clin Chim Acta* 2006;364:82–90.
- 14 Werner ER, Bitterlich G, Fuchs D, Hausen A, Reibnegger G, Szabo G, Dierich MP, Wachter H: Human macrophages degrade tryptophan upon induction by interferon-gamma. *Life Sci* 1987;41:273–280.
- 15 Wirleitner B, Reider D, Ebner S, Böck G, Widner B, Jaeger M, Schennach H, Romani N, Fuchs D: Monocyte-derived dendritic cells release neopterin. *J Leukoc Biol* 2002;72:1148–1153.
- 16 Huber C, Batchelor JR, Fuchs D, Hausen A, Lang A, Niederwieser D, Reibnegger G, Swetly P, Troppmair J, Wachter H: Immune response-associated production of neopterin – Release from macrophages primarily under control of interferon-gamma. *J Exp Med* 1984;160:310–316.
- 17 Murr C, Widner B, Wirleitner B, Fuchs D: Neopterin as a marker for immune system activation. *Curr Drug Metab* 2002;3:175–187.
- 18 Raitala A, Karjalainen J, Oja SS, Kosunen TU, Hurme M: Indoleamine 2,3-dioxygenase (IDO) activity is lower in atopic than in non-atopic individuals and is enhanced by environmental factors protecting from atopy. *Mol Immunol* 2006;43:1054–1056.
- 19 von Bubnoff D, Fimmers R, Bogdanow M, Matz H, Koch S, Bieber T: Asymptomatic atopy is associated with increased indoleamine 2,3-dioxygenase activity and interleukin-10 production during seasonal allergen exposure. *Clin Exp Allergy* 2004;34:1056–1063.
- 20 Sekigawa T, Tajima A, Hasegawa T, Hasegawa Y, Inoue H, Sano Y, Matsune S, Kurono Y, Inoue I: Gene-expression profiles in human nasal polyp tissues and identification of genetic susceptibility in aspirin-intolerant asthma. *Clin Exp Allergy* 2009;39:972–981.
- 21 Kositz C, Schroeksadel K, Grander G, Schennach H, Kofler H, Fuchs D: Serum tryptophan concentration in patients predicts outcome of specific immunotherapy with pollen extracts. *Int Arch Allergy Immunol* 2008;147:35–40.
- 22 Ciprandi G, De Amici M, Tosca M, Fuchs D: Tryptophan metabolism in allergic rhinitis: the effect of pollen allergen exposure. *Hum Immunol* 2010;71:911–915.
- 23 Chardonnet Y: Study of the experimental immunity of guinea pigs against mumps virus. II. Research on the mechanism of appearance of complement fixing antibodies after secondary immunization (booster phenomenon) (in French). *Rev Immunol Ther Antimicrob* 1958;22:191–221.
- 24 Ebner C, Kraft D, Ebner H: Booster immunotherapy (BIT). *Allergy* 1994;49:38–42.
- 25 Widner B, Werner ER, Schennach H, Wachter H, Fuchs D: Simultaneous measurement of serum tryptophan and kynurenine by HPLC. *Clin Chem* 1997;43:2424–2426.
- 26 Laich A, Neurauder G, Widner B, Fuchs D: More rapid method for simultaneous measurement of tryptophan and kynurenine by HPLC. *Clin Chem* 2002;48:579–581.
- 27 Eder W, Ege MJ, von Mutius E: The asthma epidemic. *N Engl J Med* 2006;355:2226–2235.
- 28 Weiss G, Murr C, Zoller H, Haun M, Widner B, Ludescher C, Fuchs D: Modulation of neopterin formation and tryptophan degradation by Th1- and Th2-derived cytokines in human monocytic cells. *Clin Exp Immunol* 1999;116:435–440.
- 29 Gros E, Petzold S, Maintz L, Bieber T, Novak N: Reduced IFN- γ receptor expression and attenuated IFN- γ response by dendritic cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 2011;128:1015–1021.
- 30 Mayersbach P, Fuchs D, Schennach H: Performance of fully automated quantitative neopterin measurements in a routine voluntary blood donations setting. *Clin Chem Lab Med* 2009;48:373–377.
- 31 Chen Y, Guillemin GJ: Kynurenine pathway metabolites in humans: disease and healthy states. *Int J Tryptophan Res* 2009;2:1–19.
- 32 Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ: Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994;343:133–135.
- 33 Kharitonov SA, Rajakulasingam K, O'Connor B, Durham SR, Barnes PJ: Nasal NO is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 1997;99:58–64.
- 34 Ciprandi G, Tosca M, Fuchs D: Nitric oxide metabolites in allergic rhinitis: the effect of pollen allergen exposure. *Allergol Immunopathol* 2011;39:326–329.
- 35 Thomas SR, Terentis AC, Cai H, Takikawa O, Levina A, Lay PA, Freewan M, Stocker R: Post-translational regulation of human indoleamine 2,3-dioxygenase activity by nitric oxide. *J Biol Chem* 2007;282:23778–23787.
- 36 Ciprandi G, Fuchs D: Tryptophan, neopterin and nitrite in allergy. *Allergy* (in press).
- 37 Sun J, Sukhova GK, Wolters PJ, Yang M, Kitamoto S, Libby P, MacFarlane LA, Malen-St Clair J, Shi GP: Mast cells promote atherosclerosis by releasing proinflammatory cytokines. *Nat Med* 2007;13:719–724.
- 38 Koarai A, Ichinose M, Sugiura H, Yamagata S, Hattori T, Shirato K: Allergic airway hyperresponsiveness and eosinophil infiltration is reduced by a selective iNOS inhibitor, 1400W, in mice. *Pulm Pharmacol Ther* 2000;13:267–275.
- 39 Singh D, Richards D, Knowles RG, Schwartz S, Woodcock A, Langley S, O'Connor BJ: Selective inducible nitric oxide synthase inhibition has no effect on allergen challenge in asthma. *Am J Respir Crit Care Med* 2007;176:988–993.