

Asymptomatic atopy is associated with increased indoleamine 2,3-dioxygenase activity and interleukin-10 production during seasonal allergen exposure

D. von Bubnoff*, R. Fimmers†, M. Bogdanow†, H. Matz*, S. Koch* and T. Bieber*

*Department of Dermatology and †Department of Medical Biometry, Informatics, and Epidemiology, University of Bonn, Bonn, Germany

Summary

Background Indoleamine 2,3-dioxygenase (IDO) is a tryptophan (TRP)-catabolizing enzyme with regulatory effects on T cells. T cell inhibition is achieved through both TRP depletion and TRP metabolite accumulation in specific local tissue microenvironments. The expression of IDO activity by different types of antigen-presenting cells (APCs) has been shown to play a role in many instances of immunoregulation and tolerance induction. Induction of IDO after the engagement of the high-affinity receptor for IgE, FcεRI, on atopic monocytes has been suggested to regulate T cell responses in atopic disorders. Interleukin-10 (IL-10), a cytokine known for its down-regulatory functions in the immune system, has recently been associated with the stable expression of IDO in mature tolerogenic dendritic cells.

Objective This study was devised to understand the role of systemic IDO and IL-10 in the prevention of clinical apparent allergy.

Methods The concentration of TRP and the break-down product kynurenine were measured by high-performance liquid chromatography in- and off-season in sera from patients with seasonal allergic rhinitis ($n = 12$) and from clinically asymptomatic atopic patients sensitized to specific aeroallergens ($n = 12$). Non-atopic (NA) individuals ($n = 12$) served as control. The concentration of plasma IL-10 was determined in parallel from these donors by ELISA in- and off-season.

Results In clinically unresponsive but aeroallergen-sensitized atopic individuals significantly higher systemic activity of IDO and increased plasma IL-10 levels were found during allergen exposure but not off-season compared to symptomatic atopic individuals with allergic rhinitis and NA individuals.

Conclusion Enhanced systemic IDO activity as well as increased systemic levels of IL-10 may contribute to the containment of allergic T cell responses and could be involved in the maintenance of a state of clinical unresponsiveness.

Keywords allergic rhinitis, clinical manifestation, tolerance, tryptophan degradation

Submitted 28 November 2003; revised 10 March 2004; accepted 1 April 2004

Introduction

Atopic allergic individuals are prone to develop one or more atopic diseases such as allergic rhinitis, allergic asthma, or atopic dermatitis (AD) [1]. Under normal circumstances, innocuous proteins delivered through the outer surfaces into regional lymph nodes do not evoke strong immune reactions but induce antigen-specific hypo-responsiveness. The cellular mechanisms that develop after allergen encounter are only poorly understood, and the types of antigen-presenting cells (APCs) involved in T cell hypo-responsiveness toward allergens are not well defined. Recently, it has been shown that during systemic allergen immunotherapy there is a significant increase in cells positive for IL-10 protein, suggesting important regulatory/inhibitory mechanisms for IL-10 in hypo-responsiveness toward allergens [2].

In patients with atopic disorders, periods of disease aggravation alternate with phases of remission, even in the presence of the offending allergen(s). A complex interrelation of genetic, environmental, psychologic, and immunological factors may account for the establishment of atopic disease [3]. Besides its constitutive expression on mast cells and basophils, FcεRI surface expression is found on APCs. On these cells, FcεRI-expression is positively associated with the atopic status of the individual [4]. Asymptomatic atopic (AA) individuals, like symptomatic atopics (SA), often disclose elevated total serum IgE levels (>100 kU/L) and show sensitization toward (aero)allergens. There appears to be a wide array of response mechanisms to allergens, which probably involve early regulatory mechanisms at the time when FcεRI becomes cross-linked by IgE-allergen complexes on APCs and other mediator cells of anaphylaxis.

The activation of FcεRI on APCs leads to well-characterized functions like facilitated antigen focusing and increased production of pro-inflammatory cytokines which account for allergic inflammation [5]. Recently, new immunomodulatory

Correspondence: Dagmar von Bubnoff, Friedrich-Wilhelms-University of Bonn, Sigmund-Freud-Str.25 53105 Bonn, Germany.
E-mail: d.bubnoff@uni-bonn.de

signals have been described that emanate from monocytes after ligation of FcεRI, lead to the rapid production of high levels of IL-10, and cause the conversion of the monocytes into macrophage-like cells with poor T cell stimulatory capacity [6]. Intriguingly, indoleamine 2,3-dioxygenase (IDO) was found to be highly expressed in FcεRI-activated monocytes. IDO is an enzyme that is involved in the catabolism of the essential amino acid tryptophan (TRP) [7]. It is the rate-limiting enzyme in the TRP degradation cascade and is known to be inducible in certain APCs such as macrophages, monocytes, and DCs. It exerts its regulatory function by lowering the TRP level and/or accumulating TRP metabolites such as kynurenine (KYN) [8, 9]. In murine pregnancy, increased TRP catabolism at the site of the placenta has been implicated as a natural immunosuppressive mechanism that prevents rejection of murine allogeneic fetuses by maternal T cells [10]. For FcεRI, it has been shown that ligation of this receptor on monocytes lowers TRP concentrations in the cell culture medium associated with impaired T cell stimulatory capacity [7]. Different mechanisms may contribute to IDO-mediated immune regulation, however, recent *in vitro* studies clearly point to apoptosis of T cells as a major effector mechanism of IDO activity [11].

Measurement of the plasma KYN per TRP (KYN/TRP) quotient allows an estimate of systemic IDO activity [12]. This quotient is increased in disease states associated with immune activation, i.e. malignancy, infectious diseases and in autoimmune disorders like systemic lupus erythematosus [13]. Specific anergy in peripheral T cells towards potentially harmful antigens is, at least in part, established by the production of IL-10 [14]. Furthermore, cross-linking of FcεRI on atopic monocytes is known to result in the secretion of IDO and IL-10 [4, 7]. Induction of allergen-specific T cell tolerance is an active process mediated by APCs [15]. As the state of clinical unresponsiveness in aerogen-sensitized atopic individuals may involve such specific T cell tolerance, we hypothesized that enhanced IDO activity and IL-10 production were associated with this clinical situation.

Materials and methods

Reagents

22E7, a mouse(m)Ab against the α-chain of FcεRI, was a gift from J. Kochan (Department of Autoimmune Diseases, Hoffmann-La Roche, Nutley, NJ, USA). Mouse anti-CD23 Ab (mIgG1) was from DAKO (Glostrup, Denmark). Unlabelled or FITC/PE-labelled mIgG, anti-CD14-PE, and goat-anti-mouse-FITC-Ab were obtained from BD Immunocytometry Systems (San Jose, CA, USA).

Patients

The study was approved by the local ethics committee and written informed consent was given from all donors. A total of 36 volunteers were recruited for participation: SA individuals with allergic rhinitis (AR) ($n = 12$; mean age 35.5, ranging from 24 to 50 years), AA ($n = 12$; mean age

38.5, ranging from 24 to 53), and non-atopic (NA) individuals ($n = 12$; mean age 40, ranging from 26 to 67).

SA individuals were chosen according to the following criteria: (i) donors had a history of seasonal symptomatic AR toward birch pollen and/or grass pollen; (ii) this was confirmed by elevated specific IgE serum levels toward either one or both of these allergens (specific IgE > 0.7 kU/L, CAP-Class 2); (iii) donors were otherwise healthy and did not show other atopic diseases such as allergic asthma, allergic conjunctivitis, or atopic dermatitis. In the SA group, six individuals were symptomatic during the birch pollen season and sensitized to birch pollen. Two individuals were symptomatic during the grass pollen season and sensitized to grass pollen. Four SA patients were sensitized and symptomatic during both, the birch and grass pollen season. None of these patients had been previously treated with immunotherapy or was receiving steroid or antihistamine treatment before and at the time of investigation. AA individuals were chosen according to the following criteria: (i) they had no history for symptomatic allergic rhinitis or other atopic disease; (ii) donors had elevated specific serum IgE toward birch and/or grass pollen (specific IgE > 0.7 kU/L, CAP-Class 2). In the AA group, five individuals were sensitized solely to birch pollen, two donors were sensitized solely to grass pollen and five were sensitized to both, birch and grass pollen. All SA and AA individuals had stigmata of atopy [16]. All atopic individuals did or did not have a positive family background for atopic diseases. NA (i) never presented with either atopic disease; (ii) did not show specific serum IgE levels to allergens from common pollen (specific IgE ≤ 0.35 kU/L, CAP-Class 0) nor to other environmental allergens such as allergens from house dust mite, food, or animal dander; (iii) there was a lack of family history for atopic diseases, and (iv) no physical findings indicative of allergic diseases.

Flow cytometry

Surface labelling for FcεRI and the low-affinity receptor for IgE, CD23, was performed as reported previously [17]. To quantify receptor expression, the vital and CD14-positive monocyte population was gated by a combination of forward and side scatter (FSC/SSC) and CD14/7-AAD gate sets. The density of these surface antigens was determined as the relative fluorescence index (rFI): The mean fluorescence intensity (MFI) for the antigens FcεRI and CD23 of this population was determined. Relative fluorescence indices (rFIs) were assessed as follows:

$$\text{rFI} = [\text{MFI}(\text{receptor}) - \text{MFI}(\text{control})] / \text{MFI}(\text{control}).$$

Blood samples

All samples were obtained after informed consent from volunteers in accordance with the local ethics committee. Blood samples were collected at 08:00 hours from 12 h fasting subjects to avoid the influence of dietary TRP on measured plasma levels. Plasma concentrations of TRP in the morning are not dependent on TRP intake from the previous evening because it is rapidly cleared from the blood [18]. Serum (for the measurement of IgEs) and plasma samples (for the

measurement of TRP and KYN, and for IL-10) were collected from each patient: both before the birch and grass pollen season (between November 2002 and January 2003) and during the birch and/or grass pollen season. Timing for the latter depended on the period of clinical manifestation (for the SA group: sneezing attacks, watery rhinorrhea, and nasal obstruction) and sensitization of symptomatic and asymptomatic subjects and ranged from mid-February 2002 to July 2002. Plasma and serum samples were stored at -80°C until processing.

Determination of TRP degradation *in vivo*

To quantify the enzymatic activity of IDO *in vivo*, TRP and KYN concentrations in plasma samples were determined twice per season. The KYN/TRP quotient was calculated as an estimate of the TRP degradation rate *in vivo*. Although TRP concentrations in females are 10% lower than that in males, the TRP degradation rates do not differ [14]. In addition, by comparing values in- and off-season from the same individual within these groups, changes in the concentrations of plasma TRP and KYN and in the KYN/TRP quotient become apparent independently of other factors such as age and gender.

High-performance liquid chromatography (HPLC)

For the determination of L-TRP and L-KYN in human plasma, a modified HPLC method was used [19]. Accordingly, L-TRP was determined at +850 mV and 50 nA and L-KYN at +850 mV and 10 nA.

Measurement of total and specific IgE

Total and specific IgE levels in the serum were determined by the ImmunoCAP System of Pharmacia (Pharmacia & Upjohn, Uppsala, Sweden).

Determination of IL-10 in the plasma

For determination of IL-10 by ELISA, plasma samples from NA, SA, and AA individuals were collected in- and off-season and were stored at -80°C until cytokine measurement. The ELISAs were conducted in duplicates according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). The sensitivity of detection for was 7.8 pg/mL.

Data analysis

Differences between groups were compared using the Mann-Whitney-Wilcoxon Test. Comparisons between summer and winter are based on the signed-rank test applied to the differences between the seasons. Box-Whisker plots indicate 25th and 75th quartiles, median, minimum, and maximum of the distributions. Otherwise, data are mean ($\pm$ SD). *P*-values of <0.05 are considered as statistically significant.

The Pearson's correlation coefficient (r_p) was calculated to determine the degree of correlation between TRP degradation (as expressed as the KYN/TRP quotient) and IL-10 in the plasma during the seasons. Significant correlation was accepted when $P < 0.05$ on Pearson's correlation analysis.

Results

Expression of FcεRI on monocytes from atopic individuals is upregulated during allergen exposure

The surface expression of FcεRI and CD23 on monocytes from SA individuals with allergic rhinitis, AA individuals, and NA individuals was analysed by flow cytometry, both in- and off-season (Fig. 1). The cell surface densities for these antigens were determined using the rFIs as described in Materials and methods. Monocytes from atopic donors expressed significantly higher amounts of FcεRI on their monocyte surface than NA, in-season (SA/NA: $P = 0.0011$; AA/NA: $P = 0.0004$), and off-season (SA/NA: $P = 0.0077$; AA/NA: $P = 0.0010$). Monocytes from AA expressed higher amounts of FcεRI than monocytes from SA, in-season and off-season. Interestingly, there was a significant upregulation of FcεRI during the pollen season on monocytes from SA and AA. The expression of the low affinity receptor, CD23, was comparable in all three groups and did not show significant variation with differing seasons. In summary, monocytes from SA, AA, and NA displayed FcεRI-expression in a distinct manner on their cell surface during the seasons.

Significant increase of total and allergen-specific IgE in SA during exposure to pollen

Total IgE and specific IgE were measured in the serum of all donors, in- and off-season. The level of total IgE was always significantly higher in the groups of atopic individuals (SA/AA) than of NA individuals (Fig. 2). The levels of total IgE between the SA and AA group were, however, not significantly different during either season ($P = 0.977$ in-season; $P = 0.707$ off-season). There was a significant up-regulation

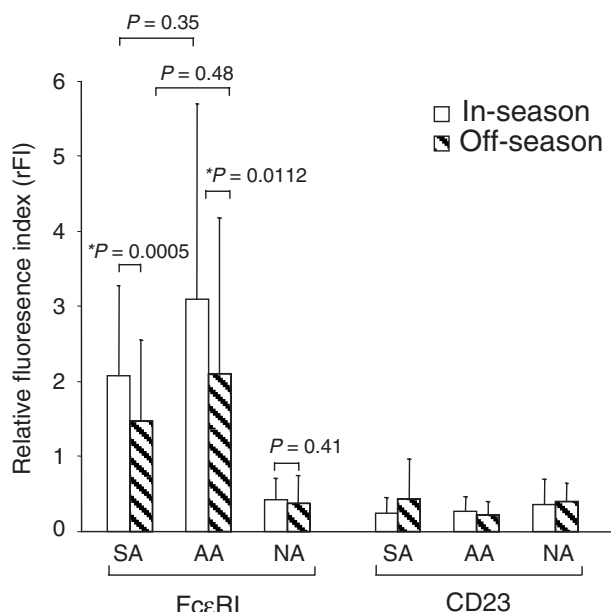


Fig. 1. Flow cytometric analysis of monocytes from symptomatic atopic (SA), asymptomatic atopic (AA), and non-atopic (NA) donors. The mean fluorescence intensity (MFI) after staining with specific Abs for FcεRI and CD23 was compared with that of a corresponding isotype control Ab. Relative fluorescence indices (rFIs) were determined accordingly as described in Materials and methods. **P*, statistical significance.

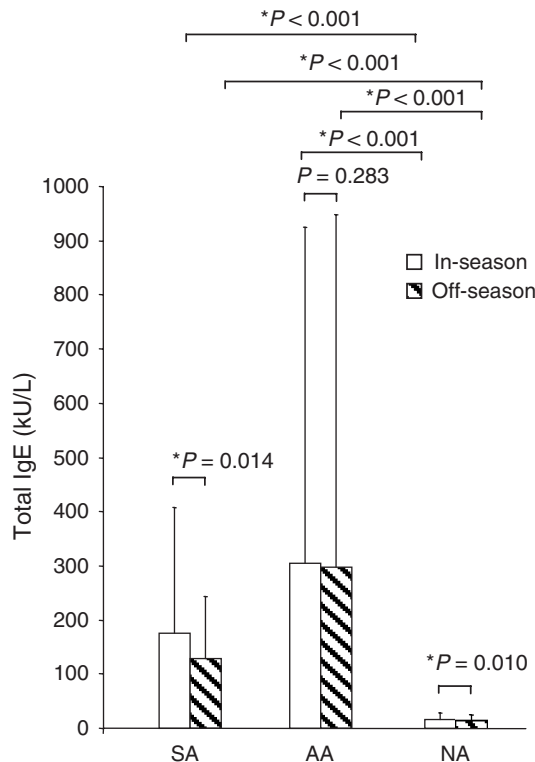


Fig. 2. Seasonal change in total serum IgE levels of symptomatic atopic (SA), asymptomatic atopic (AA) and non-atopic (NA) individuals. Values are mean ($\pm$ SD). *P, statistical significance.

of total IgE in the SA group during the pollen season, whereas this difference was not seen in the AA group. NA individuals showed low total IgE levels in either season. As absolute levels of total IgE in this group are low, relatively small changes between in- and off-season are effective and therefore indicate statistical significance. Allergen-specific IgE values between the groups of SA and AA were not statistically significant in either season, for birch pollen ($P = 0.130$ in-season; $P = 0.540$ off-season) and for grass pollen ($P = 0.317$ in-season; $P = 0.384$ off-season). In the group of SA, there was a small but statistically significant seasonal increase in specific IgE toward birch pollen ($P = 0.012$) but not toward grass pollen ($P = 0.109$). In the group of AA, there were no significant seasonal changes in specific IgE to either pollen ($P = 0.069$ for birch pollen; $P = 0.225$ for grass pollen).

Increased IDO activity in AA during allergen exposure

Plasma concentrations of TRP, KYN, and the corresponding KYN/TRP quotient were determined from donors in all three groups, in- and off-season (Fig. 3). During allergen exposure, markedly lower TRP concentrations were found in the plasma of the AA group compared to those found in the SA group although this difference just failed to reach statistical significance. Lower TRP concentrations were also measured in the group of NA than in the SA group. At the same time, during exposure to aeroallergens, the TRP degradation product KYN increased in the plasma of AA over the values from SA and NA. Determination of the KYN/TRP quotient clearly disclosed a significant increase in

systemic IDO activity during the in-season period in the AA group over the SA and NA group. In the absence of aeroallergens, TRP and KYN plasma levels as well as systemic IDO activity expressed as the KYN/TRP quotient did not significantly differ among the three groups and were lower than during the in-season period. Table 1 shows an overview of the P -values of the differences in KYN, TRP, and the KYN/TRP quotient between in- and off-season within one group. In the group of AA and NA, plasma TRP levels were significantly decreased in-season over off-season. However, the concentration of KYN, the product of IDO-dependent TRP degradation, was elevated significantly in-season over off-season only in the AA group. Similarly, the KYN/TRP quotient was increased only in this group. Together, these results suggest that increased IDO activity during allergen exposure are associated with the regulation of unwanted allergic reactions in allergen-sensitized individuals.

Increased plasma IL-10 levels in AA during allergen exposure

We assumed that IL-10 was involved in the establishment of a clinically asymptomatic aeroallergen-sensitized atopic phenotype during periods of high exposure to natural allergens. Therefore, levels of plasma IL-10 were determined twice in each individual of all groups, both in- and off-season (Fig. 4). Interestingly, in-season, plasma levels of IL-10 were increased in all groups compared to off-season. However, in the group of AA this difference was most obvious and only there it reached statistical significance. During the in-season period, plasma IL-10 levels were significantly increased in the group of AA over the group of NA, although these differences in the levels of IL-10 to the SA group just failed to reach statistical significance. During the off-season period, lower and comparable levels of IL-10 were found among individuals of all groups.

These results demonstrate that an increase in the level of systemic IL-10 during allergen exposure might be involved in the down-regulation of specific T cells in allergen-sensitized, yet asymptomatic individuals. This mechanism may act in concert with an enhanced IDO activity and contribute to clinical unresponsiveness.

Significant correlation between systemic IDO activity and IL-10 production in AA during allergen exposure

The results of correlational analysis between the systemic degradation of TRP (expressed as the KYN/TRP quotient) and systemic IL-10 during allergen exposure are summarized in Fig. 5. The increase in TRP degradation was significantly correlated with the production of systemic IL-10 during allergen exposure only in the group of AA. The regression coefficient for these values in-season was much higher in the group of AA (4.008) than in the group of SA (0.440) and NA (-0.148). Off-season, there was no significant correlation between systemic TRP degradation and IL-10 production in either group (AA: $r_p = 0.0514$; $P = 0.8808$; regression coefficient 0.355; SA: $r_p = 0.274$; $P = 0.3888$; regression coefficient -1.444 ; NA: $r_p = 0.067$; $P = 0.8353$; regression coefficient 0.302; data not shown).

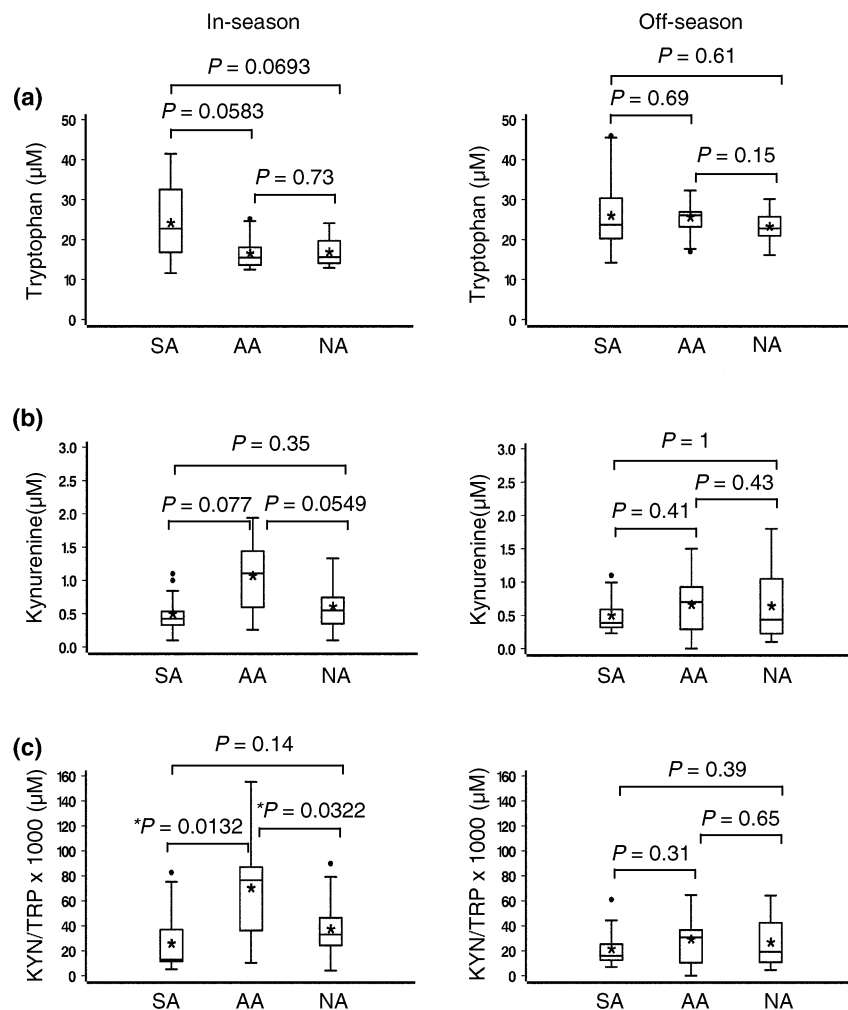


Fig. 3. Plasma tryptophan levels (a), kynurenine levels (b), and kynurenine per tryptophan quotients $\times 1000$ (c) among symptomatic atopic (SA), asymptomatic atopic (AA), and non-atopic (NA) individuals, in- and off-season. Box-Whisker plots indicate 25th and 75th quartiles, median, minimum, and maximum of the distributions. **P*, statistical significance.

Table 1. Overview of the *P*-values of the differences in tryptophan (TRP), kynurenine (KYN), and the KYN/TRP quotient between in- and off-season within the group of symptomatic atotics (SA), asymptomatic atotics (AA), and non-atotics (NA).

	Tryptophan	Kynurenine	KYN/TRP quotient
SA (<i>n</i> = 12)	0.15	0.89	0.73
AA (<i>n</i> = 12)	0.0010*	0.0098*	0.001*
NA (<i>n</i> = 12)	0.0010*	0.87	0.18

**P*, statistical significance.

Discussion

In this study, we provide the first evidence that increased systemic IDO activity may contribute to inhibition of specific allergic reactions in allergen-sensitized individuals without clinically apparent disease. We show that during aeroallergen-exposure a significant increase of the KYN/TRP quotient was seen among individuals of the AA group compared to individuals of the SA and NA group, respectively. In contrast, off-season, there was no significant variation in

systemic IDO activity among symptomatic, asymptomatic, and NA individuals. The levels of systemic IL-10 paralleled this pattern. In particular, in the group of asymptomatic aeroallergen-sensitized atopic individuals during allergen-exposure, systemic IL-10 values were increased over the values of the SA and NA group. During the winter season, levels of IL-10 decreased to comparable amounts in all three groups. The correlation between systemic IDO activity and IL-10 production in season was only significant in the group of AA, and off-season no correlation was found between these values in either group.

Increased systemic IDO activity has been found in conditions where immunoregulatory functions are of major importance in the maintenance of T cell homeostasis. For example, in an *in vivo* model for experimental autoimmune encephalitis (EAE), decreased IDO mRNA levels in the spinal cord were observed during periods of relapse while an increase was seen during periods of remission [20]. These and other data suggest that IDO regulates autoantigen-specific T cell responses during the course of EAE and may down-regulate the activity of autoreactive lymphocytes in other autoimmune diseases. IDO-expressing APCs and

systemic IDO activity have also been observed during chronic infections like the AIDS, in pregnancy, after transplantation and in various cancers [21].

In our study, we found increased levels of IL-10 in asymptomatic individuals during allergen exposure. IL-10 is a cytokine of potent immunoregulatory capacity, although its potential may be described as ambivalent. IL-10, produced by activated Th2 cells, T regulatory cells (Tregs), and monocytes/macrophages can counteract the development of a Th1 response, probably by reducing the production of IL-12 by APCs [22]. This effect could serve to drive the immune response further toward Th2, which in turn would promote allergy. On the other hand, IL-10 has in a number of recent studies been shown to act as a mediator of the containment and down-regulation of a T cell response; by this mechanism IL-10 might curb an allergic response. Recent evidence indicates that IL-10 exerts this limiting effect by the production of Tregs, a relatively new functional class of T

cells that can contain T cell responses and prevent unwanted or overly strong reactions [23]. Repeated stimulation of T cells in the presence of IL-10 *in vitro* leads to the generation of IL-10 producing Tregs or Tr1 [24]. A further intriguing discovery was the finding that Tregs induce IDO in APCs and may thus use IDO as an effector mechanism [25]. In this study, we saw an association of both IL-10 and IDO activity with the status of asymptomatic atopy during allergen exposure. *In vitro*, IL-10 was shown to down-regulate TRP degradation in PBMCs by down-regulating Th1-type cytokine production [26]. However, it is conceivable that *in vivo*, IL-10 supports the development of Tregs that counter-regulate the allergic response by production of IL-10 and induction of IDO in APCs. Figure 6 provides a model of how the maintenance of clinical unresponsiveness could be achieved in sensitized AA individuals.

Immune suppression by IDO has two aspects, the deprivation of TRP and the increase in TRP catabolites [8]. From studies *in vitro*, it can be concluded that TRP deficiency, as a sole mechanism for IDO-induced T cell suppression is questionable [27]. In particular, the lower plasma levels of TRP in-season compared with off-season in the group of AA and NA are probably not sufficient for T cell suppression. The serum concentrations of the metabolite KYN in the group of AA were within the normal range of healthy individuals (0–3 μM) [12]. However, serum KYN levels do not necessarily reflect the concentration at the site of production. Moreover, in addition to KYN, other TRP metabolites like picolinic acid, 3-hydroxyKYN or quinolinic acid may be produced in defined compartments like lymph nodes or spleen [8]. These might act synergistically to down-regulate T cell activation in clinically healthy but sensitized individuals. The combination of lowering the levels of TRP with concomitant increase of TRP catabolites may additively enhance the inhibitory potential of IDO for T cells in AA donors. In contrast, in patients symptomatic for allergic rhinitis, endogenous APC and Th2-dependent cytokines or other mechanisms may favour an allergic response rather than actually inducing anergy [28].

In NA no increase in systemic IL-10 or IDO activity (as measured by the KYN/TRP quotient) during the pollen season was seen. Local IDO induction at sites of the immune system with the environment (e.g. in the airway epithelium) is an attractive hypothesis to be part of a tolerance induction

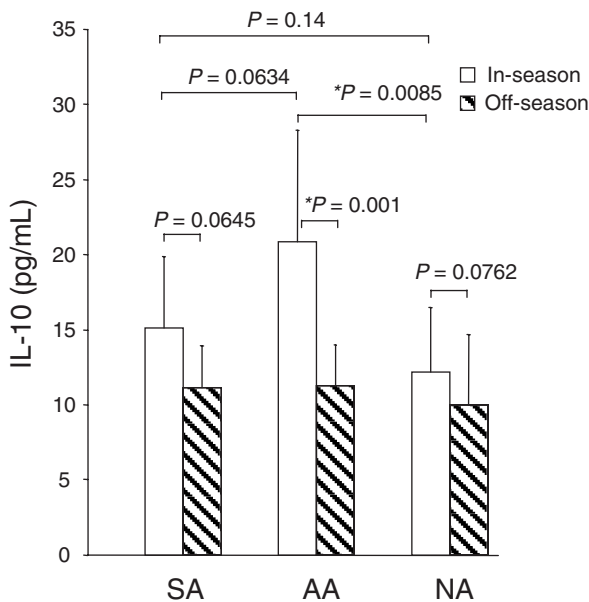


Fig. 4. Quantification of plasma IL-10 from symptomatic atopic (SA), asymptomatic atopic (AA) and non-atopic (NA) individuals, in- and off-season. Values are expressed as the mean ($\pm$ SD) of all individuals per group. **P*, statistical significance.

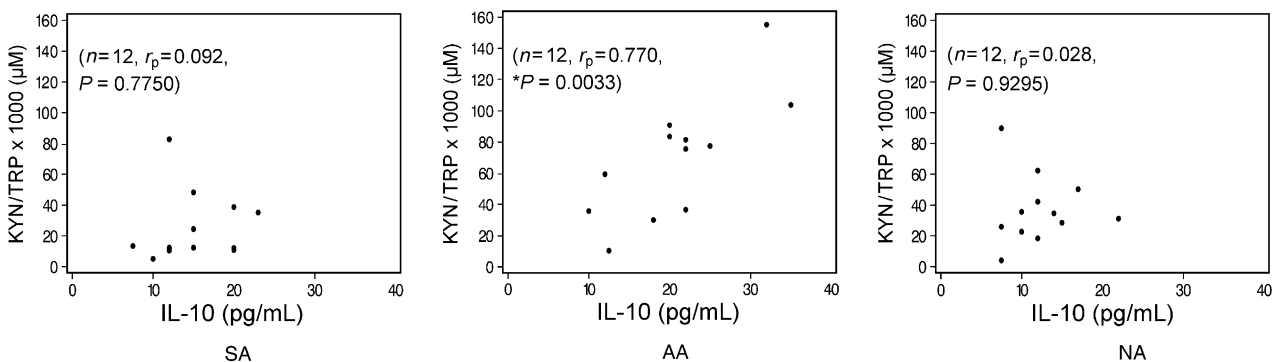


Fig. 5. Diagram of correlation between the increase of systemic IDO activity (as expressed as the KYN/TRP quotient) and systemic IL-10 production during allergen exposure. SA, symptomatic atotics; AA, asymptomatic atotics; NA, non-atopic individuals; r_p , Pearson's correlation coefficient; **P*, statistical significance.

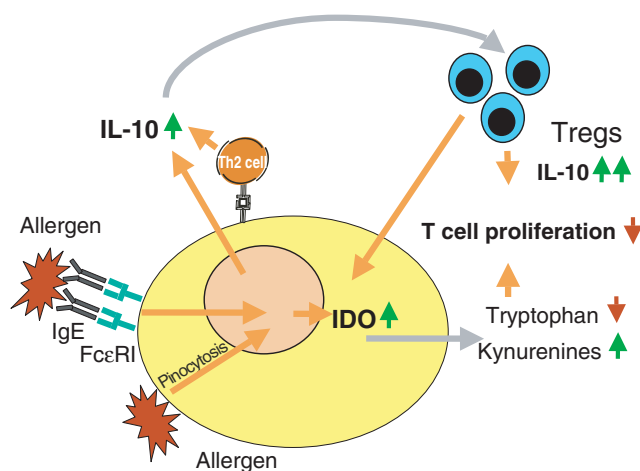


Fig. 6. Potential regulatory mechanism that leads to clinical unresponsiveness in allergen-sensitized individuals. The uptake of IgE-loaded allergen may induce the production of IL-10 in antigen-presenting cells (APCs). In addition, the presentation of antigenic peptides to Th2 cells leads to the induction and secretion of IL-10 from these cells. The presence of high amounts of IL-10 may lead to the differentiation of T regulatory cells (Tregs), which in turn induce the production of IDO in APCs. The lowering of (TRP) and increase of TRP catabolites and/or the production of IL-10 are possibilities of how Tregs may down-regulate allergen-specific Th2 cells.

mechanism also in NA, since systemic immune activation or production of specific IgE does not occur [29, 30].

In summary, our results clearly point to major differences in regulatory mechanisms of SA vs. AA individuals. The specific events that alter allergen processing and presentation in defined compartments and those that establish tolerance in asymptomatic individuals are not yet clear. Our study suggests that systemic and/or local IDO and IL-10 production may be important factors in mediating T cell anergy in asymptomatic but allergen-sensitized individuals. However, although our study points to a protective role of IDO in the prevention of progression from atopy to allergy, this link will have to be analysed further by using cellular models or by using patients undergoing systemic immunotherapy (SIT).

Acknowledgements

We are very grateful to Professor Rao and Christine Frahnert, Department of Neuropsychiatry, University of Bonn, for the measurements of TRP and KYN by HPLC. We especially thank Georg Häcker, MD, Institute for Medical Microbiology, Immunology, and Hygiene, Technical University of Munich, Munich, Germany and Brian Hall, MD, Mayo Clinic, Rochester, MN, USA for their critical reading of the manuscript. This work was supported by a grant from the University of Bonn, Germany.

References

- Peroni DG, Piacentini GL, Alfonsi L et al. Rhinitis in pre-school children: prevalence, association with allergic diseases and risk factors. *Clin Exp Allergy* 2003; 33:1349–54.
- Nasser SM, Ying S, Meng Q, Kay AB, Ewan PW. Interleukin-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy. *Eur J Immunol.* 2001; 31:3704–13.
- Ring J, Krämer U, Schäfer T, Behrendt H. Why are allergies increasing? *Curr Opin Immunol* 2001; 13:701–8.
- Novak N, Tepel C, Koch S, Brix K, Bieber T, Kraft S. Evidence for a differential expression of the FcεRIγ chain in dendritic cells of atopic and nonatopic donors. *J Clin Invest* 2003; 111:1047–56.
- Maurer D, Ebner C, Reiniger B et al. The high affinity IgE receptor (Fc epsilon RI) mediates IgE-dependent allergen presentation. *J Immunol* 1995; 154:6285–90.
- Novak N, Bieber T, Katoh N. Engagement of Fc epsilon RI on human monocytes induces the production of IL-10 and prevents their differentiation in dendritic cells. *J Immunol.* 2001; 167:797–804.
- Von Bubnoff D, Matz H, Frahnert C et al. FcεRI induces the tryptophan degradation pathway involved in regulating T cell responses. *J Immunol* 2002; 169:1810–6.
- Terness P, Bauer TM, Röse L et al. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J Exp Med* 2002; 196:447–57.
- Grohmann U, Fallarino F, Puccetti P. Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol* 2003; 24:242–8.
- Munn DH, Zhou M, Attwood JT et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 1998; 281:1191–3.
- Fallarino F, Grohmann U, Vacca C et al. T cell apoptosis by tryptophan catabolism. *Cell Death Differ* 2002; 9:1069–77.
- Widner B, Fuchs D. Immune activation and degradation of tryptophan. *Mod Asp Immunobiol* 2000; 3:105–8.
- Widner B, Sepp N, Kowald E et al. Enhanced tryptophan degradation in systemic lupus erythematosus. *Immunobiology* 2000; 201:621–30.
- Seewaldt S, Alferink J, Forster I. Interleukin-10 is crucial for maintenance but not for developmental induction of peripheral T cell tolerance. *Eur J Immunol* 2002; 32:3607–16.
- Charbonnier AS, Hammad H, Gosset P et al. Der p 1-pulsed myeloid and plasmacytoid dendritic cells from house dust mite-sensitized allergic patients dysregulate the T cell response. *J Leukoc Biol* 2003; 73:91–9.
- Leung DYM, Bieber T. Atopic dermatitis. *Lancet* 2003; 361:151–60.
- Wollenberg A, Kraft S, Hanau D, Bieber T. Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema. *J Invest Dermatol* 1996; 10:446–53.
- Shotwell MA, Kilberg MS, Oxender DL. The regulation of neutral amino acid transport in mammalian cells. *Biochim. Biophys. Acta* 1983; 737:267–84.
- Meyer-Lindenberg A, Rao ML. Interrelationship between blood serotonin levels and affinity of platelet imipramine binding sites in healthy subjects. *Pharmacopsychiatry* 1993; 26:15–19.
- Sakurai K, Zou JP, Tschetter JR, Ward JM, Shearer GM. Effect of indoleamine 2,3-dioxygenase on induction of experimental autoimmune encephalomyelitis. *J Neuroimmunol* 2002; 129:186–96.
- Brown RR, Ozaki Y, Datta SP, Borden EG, Sondel PM, Malone DG. Implications of interferon-induced tryptophan catabolism in cancer, autoimmune diseases and AIDS. *Adv Exp Med Biol* 1991; 294:425–35.
- Ria F, Penna G, Adorini L. Th1 cells induce and Th2 inhibit antigen-dependent IL-12 secretion by dendritic cells. *Eur J Immunol.* 1998; 28:2003–16.
- Cottrez F, Hurst SD, Coffman RL, Groux H. T regulatory cells 1 inhibit a Th2-specific response in vivo. *J Immunol* 2000; 165:4848–53.
- Groux H, O'Garra A, Bigler M et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997; 389:737–42.

- 25 Fallarino F, Grohmann U, Hwang KW et al. Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol* 2003; 4:1206–12.
- 26 Weiss G, Murr C, Zoller H et al. Modulation of neopterin formation and tryptophan degradation by Th1- and Th2-derived cytokines in human monocytic cells. *Clin Exp Immunol.* 1999; 116:435–40.
- 27 Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrata GB. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *J Exp Med* 2002; 196:459–68.
- 28 Hagendorens MM, Ebo DG, Schuerwegh AJ et al. Differences in circulating dendritic cell subtypes in cord blood and peripheral blood of healthy and allergic children. *Clin Exp Allergy* 2003; 33:633–9.
- 29 Siroux V, Oryszczyn MP, Paty E et al. Relationships of allergic sensitization, total immunoglobulin E and blood eosinophils to asthma severity in children of the EGEA Study. *Clin Exp Allergy* 2003; 33:746–51.
- 30 Swanson KA, Zheng Y, Heidler KM, Mizobuchi T, Wilkes DS. CD11c+ Cells Modulate Pulmonary Immune Responses by Production of Indoleamine 2,3-dioxygenase. *Am J Respir Cell Mol Biol.* 2003.