



Short communication

Indoleamine 2,3-dioxygenase (IDO) activity is lower in atopic than in non-atopic individuals and is enhanced by environmental factors protecting from atopy

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Abstract

Tryptophan catabolism activated by the indoleamine 2,3-dioxygenase (EC 1.13.11.42) (IDO) enzyme in antigen presenting cells has a central role in induction of mechanisms suppressing T cell activation or clonal expansion. There is evidence suggesting that IDO activity is mainly upregulated by typical Th1-differentiating signals such as interferon- γ and bacterial lipopolysaccharide (LPS). Therefore, we hypothesized that IDO activity would be lower in a Th2-associated disease such as atopy and that it would be higher in the presence of environmental factors known to favor Th1 differentiation. Here we show that this was the case. Concentrations of tryptophan (trp) and kynurenine (kyn), the main metabolite, were determined by reverse phase liquid chromatography from serum samples of a cohort of 392 non-asthmatic individual of whom 149 were atopics (one or more positive skin test when tested with a panel of 22 allergens). Kyn/trp ratio, as an indicator of IDO activity, was significantly lower in atopic than in non-atopic individuals. The cohort was stratified according to two known atopy-protecting factors, presence of antibodies against *Helicobacter pylori* or anamnestic information about childhood on a farm environment. As expected, IDO activity was significantly higher in their presence than absence.

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1. Introduction

Tryptophan catabolism-dependent mechanisms are novel immunoregulatory functions, which have raised great interest during recent years (reviewed by Mellor and Munn, 2003, 2004). In brief, tryptophan is catabolized along two distinct biochemical pathways. The first to serotonin is initiated by tryptophan 5-monooxygenase (EC 1.14.16.4) and the second to kynurenine derivatives by two enzymes, tryptophan 2,3-dioxygenase (EC 1.13.11.11) and indoleamine-pyrrole 2,3-dioxygenase (EC 1.13.11.42) (IDO) (Mellor and Munn,

2003, 2004). IDO is expressed in the cells of the immune system, e.g. on the antigen presenting dendritic cells (DC), and is therefore responsible for the induction of the various immunoregulatory functions. There is increasing amount of data, mainly from in vitro experiments, demonstrating the role of IDO activity in direct downregulation of T cell activity (i.e. suppression by starvation) or in the induction of various T regulatory cells (Mellor and Munn, 2003, 2004).

Hayashi et al. (2004) have recently demonstrated that induction of IDO activity with bacterial components via toll-like receptors inhibited the Th2-cell driven murine experimental asthma. This finding is in line with the popular “hygiene hypothesis”, i.e. that frequent contacts with microbial components in childhood favor differentiation of T cells

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towards the Th1 direction and thus reducing the risk of Th2-associated diseases, such as atopy, in later life (reviewed by Liu and Murphy, 2003). We now hypothesized that the effect of atopic phenotype and that of the risk-modifying environmental factors could be reflected as differences in IDO activity, which is determined from serum by quantitation of the kynurenine/tryptophan (kyn/trp) ratio. This method was originally used in the report demonstrating IDO-activation during pregnancy (Schröcksnadel et al., 2003).

2. Materials and methods

Kyn and trp concentrations and *Helicobacter pylori* IgG antibodies were investigated from the serum samples derived from a cohort of 392 non-asthmatic adults of whom 149 were atopics (51 males, 98 females). Detailed information about this cohort has been published previously (Karjalainen et al., 2002). A person was defined as atopic if one or more of the 22 allergens tested in skin test gave a positive response. In our cohort, 148 individuals (57 males, 91 females) gave the anamnestic information about the childhood on a farm.

Kyn and trp concentrations in the serum samples were measured by reverse-phase high-performance liquid chromatography (Laich et al., 2002). Trp was monitored by fluorescence detection at 266 nm excitation wavelength and 366 nm emission wavelength; kyn was measured by ultraviolet absorption at 360 nm wavelength. Kyn/trp ratio was calculated by relating concentrations of kyn ($\mu\text{mol/l}$) to trp (mmol/l) allowing an estimate of IDO activity [6]. *H. pylori* IgG antibodies were measured by enzyme immunoassay (Pyloriset-EIA-G, Orion Diagnostica, Espoo, Finland).

The statistical method selected is indicated in Section 3 as used. The analyses were carried out with Statistica software (ver. Win 6.1, StatSoft Inc., Tulsa, OK). The findings were considered significant in case of a p -value less than 0.05.

3. Results and discussion

The data shown in Table 1 demonstrate that atopics had a significantly lower kyn/trp ratio than non-atopics. This was observed in both males and females. *H. pylori* is known to be one of the infectious agents (or a surrogate marker of poor hygienic conditions) known to decrease the risk of atopy (Liu and Murphy, 2003). In line with the previous publications, atopy was less frequent in *H. pylori* seropositives (33.2 versus 44.3%, $p = 0.022$, χ^2 -test). Kyn/trp ratio was significantly elevated by the presence of *H. pylori* antibodies (Table 1). Childhood on a farm is also a factor known to be associated with a reduced atopy risk, probably being related to an increased exposure to LPS and/or increased rate of orofecal infections (Liu and Murphy, 2003). However, reduction of the atopy risk by this factor in this material was not significant ($p = 0.445$, χ^2 -test), but its effect on the kyn/trp ratio was clear (Table 1). It should be noticed that in our cohort *H. pylori* and farm effects were independent: childhood on a farm did not increase the *H. pylori* seropositivity rate (data not shown).

The presented data imply that IDO activity is closely associated with the immune mechanism involved in the pathogenesis of atopy. Moreover, the change in IDO activity is so strong that it is measurable from the serum (as a difference in the ratio of the main metabolite to the substrate, i.e.

Table 1

Kynurenine/tryptophan ($\mu\text{mol}/\text{mmol}$) ratio in a cohort of 392 non-asthmatic adults stratified according to the presence of atopy (one or more positive skin tests) or to the presence of *H. pylori* IgG antibodies or according to the anamnestic information about childhood on a farm

Group (n)	Sex	Kyn/trp (median, interquartile range)	p (Mann–Whitney test)
Atopics (149)		24.67 (19.53–29.62)	
Non-atopics (243)		26.95 (21.39–32.00)	0.005
Atopics (98)	F	25.38 (19.73–30.85)	
Non-atopics (151)	F	27.80 (22.09–33.55)	0.037
Atopics (51)	M	23.54 (18.40–27.04)	
Non-atopics (92)	M	26.40 (20.74–30.03)	0.049
<i>H. pylori</i> seropositives (201)		27.00 (22.19–32.72)	
<i>H. pylori</i> seronegatives (191)		25.13 (19.50–29.55)	0.002
<i>H. pylori</i> seropositives (117)	F	29.00 (22.49–33.55)	
<i>H. pylori</i> seronegatives (132)	F	25.21 (20.05–29.90)	0.005
<i>H. pylori</i> seropositives (84)	M	26.19 (21.25–30.03)	
<i>H. pylori</i> seronegatives (59)	M	25.13 (17.81–28.27)	0.083
Childhood on a farm, yes (148)		26.99 (22.18–32.78)	
Childhood on a farm, no (244)		25.69 (20.34–30.60)	0.031
Childhood on a farm, yes (91)	F	28.98 (22.49–36.22)	
Childhood on a farm, no (158)	F	25.60 (20.72–31.23)	0.024
Childhood on a farm, yes (57)	M	26.15 (21.39–28.19)	
Childhood on a farm, no (86)	M	25.82 (18.81–29.67)	0.431

kyn/trp), in analogy to the change observed during pregnancy (Schröcksnadel et al., 2003). Mechanistically, the observed variation in the kyn/trp ratio is in line with the Th1/Th2 paradigm. In antigen presenting cells, IDO is known to be effectively induced by LPS and interferon- γ (IFN- γ), i.e. strong activators of the Th cell differentiation towards the Th1 direction (Mellor and Munn, 2003, 2004). Thus, the reduced incidence of atopy by high IDO activity could be due to the cross-regulation of the Th2 activity by these cells (or by the T regulator cells activated by an IDO-dependent mechanism). This explanation would be perfectly in line with the role of IDO in the experimental Th2-associated disease model mentioned above (Hayashi et al., 2004). However, von Bubnoff et al. (2003) have recently demonstrated that monocytes from atopic individuals express higher IDO levels than monocytes from non-atopic individuals after Fc ϵ RI cross-linking or after IFN- γ treatment. At present it is difficult to reconcile this observation with the Th differentiation effects stated above and also with the role of LPS and IFN- γ in the induction of differentiation of another type of antigen presenting cells, DCs, towards a more IDO-positive state.

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