Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2006.03166.x

The activity of the immunoregulatory enzyme indoleamine 2,3-dioxygenase is decreased in smokers

M. Pertovaara,*† M. Heliövaara,‡ A. Raitala,* S. S. Oja,§¶ P. Knekt‡ and M. Hurme*¶

*Department of Microbiology and Immunology, University of Tampere, Medical School, Tampere, Finland, †Department of Internal Medicine, Tampere University Hospital, Tampere, Finland, ‡National Public Health Institute, Helsinki, Finland, §Department of Physiology, University of Tampere, Medical School, Tampere, Finland, and ¶The Centre for Laboratory Medicine, Tampere University Hospital, Tampere, Finland

Accepted for publication 28 June 2006
Correspondence: Marja Pertovaara M.D, PhD, Department of Internal Medicine, Tampere University Hospital, PO Box 2000, FIN-33521 Tampere, Finland.
E-mail: marja.pertovaara@uta.fi

Summary

Indoleamine 2,3-dioxygenase (IDO), an enzyme involved in the degradation of the essential amino acid tryptophan (trp) to its main metabolite kynurenine (kyn), suppresses T cell activity. Smoking has marked immunomodulatory effects, above all immunosuppressive, causing a reduction in the levels of immunoglobulins and an increased risk of infections. The immunostimulatory effects of smoking are manifested, for example, in increased autoantibody production. We sought to establish whether IDO activity is involved in the immunomodulatory effects of smoking. To this end we measured the ratio of kyn to trp, reflecting IDO activity, by reverse-phase high-performance liquid chromatography (HPLC) in 784 (464 female, 230 male) subjects of a population-based sample of the adult Finnish population. Serum cotinine concentration as an indicator of active smoking was measured in the patients by radioimmunoassay and detailed data gathered on smoking habits. IDO activity was lower in smokers in this population-based sample compared with non-smokers when active smoking was classified according to serum cotinine concentration or history of smoking habits. Moreover, serum IDO activity correlated inversely with serum cotinine concentration. In conclusion, the activity of the IDO enzyme is decreased in smoking subjects, and the reduction in IDO-dependent immunosuppression could thus be responsible for the known immunostimulatory effects of smoking.

Keywords: indoleamine 2,3-dioxygenase, inflammation, smoking, T cell

Introduction

Smoking has substantial effects on the immune system, affecting both innate and adaptive immunity [1]. Long-term cigarette smoking mainly suppresses immune responses, reducing serum immunoglobulin levels [2] and causing, for example, an increased risk of microbial infections [3]. On the other hand, smoking also has immunostimulatory effects, as demonstrated by increased autoantibody production in smokers and by increased susceptibility to rheumatoid factor-positive rheumatoid arthritis [4–8]. The health risks of tobacco are well documented and smoking is associated with an increased risk of cardiovascular diseases, chronic obstructive pulmonary disease (COPD) and lung cancer. However, epidemiological data indicate that smoking might also reduce the incidence or severity of some diseases of inflammatory nature such as ulcerative colitis, Sjögren's syndrome, sarcoidosis and Parkinson's disease (reviewed in [1]). Tobacco smoke contains thousands of chemicals, and therefore the molecular mechanisms involved in the various immunomodulatory effects of smoking have been difficult to explore [1].

Indoleamine 2,3-dioxygenase (IDO) is a tryptophan (trp)-degrading enzyme, the activation of which leads to a local decrease in trp concentration, thereby suppressing the activation of the surrounding T lymphocytes [9–11]. IDO activity is reflected by the ratio of kynurenine (kyn), the main toxic metabolite of trp, to trp (kyn/trp). IDO is expressed in antigen-presenting cells (APC), such as macrophages and dendritic cells (DC) and also in polymorphonuclear leucocytes. The IDO-dependent regulatory mechanism is thus active at very early stages of the immune response. In animal models cigarette smoke has been shown to decrease the number of DCs in lung tissue [12,13], and in humans smoking ameliorates the function of leucocytes in spite of increasing their quantity [1]. It could thus be postulated that the deleterious effect of smoking on DCs or other

APC would inhibit IDO activity, consequently weakening the immunosuppressive mechanisms of IDO.

To investigate whether IDO-dependent regulatory mechanisms are involved in the immunomodulatory effects of smoking we measured kyn/trp, i.e. IDO activity in serum samples from a representative Finnish population-based cohort for which detailed data on smoking habits were also available. Serum cotinine is regarded as the most sensitive and specific biomarker for exposure to tobacco smoke [14] and cut-off points used for separating active smokers from non-smokers are between 100 and 200 μg/l [15]. We measured serum cotinine concentrations in the serum samples and used a concentration of 100 μg/l to discriminate active smokers from non-smokers.

Subjects and methods

Subjects

The Mini-Finland Health Survey, which was carried out in 1978–80, was designed to examine the epidemiology of major health problems in a representative sample of the Finnish adult population (> 30 years of age) [16]. The initial survey cohort comprised 8000 patients, of whom 7217 (90%) eventually participated. The subjects of the current study were 784 participants (464 female, 320 male) in the Mini-Finland Health Survey who were re-examined in 2000–01 as part of the multi-disciplinary epidemiological Finnish Health 2000 Study [17]. The mean age of the patients at the time of randomization was 42 ± 9 years (range 30–72 years) and in 2000 63 ± 9 years (range 50–94 years).

Serum cotinine determinations

The serum cotinine concentration in 2000–01 was determined by a method applying a modification of the Nicotine Metabolite radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA) [18]. A cut-off point of 100 μg/l was used to separate smokers from non-smokers.

Smoking history

Smoking history was elicited by structured detailed interviews, as described previously [17]. Classification of the subjects by smoking history was made by grouping them alternatively into: (1) those who had never smoked during their life-time *versus* those who had smoked; and (2) those who did not smoke currently *versus* those who smoked currently either daily or irregularly.

In addition, the subjects were grouped on the basis of the time since they had last smoked a cigarette (today or yesterday, 2 days–1 month ago, > 1 month–6 months ago, > 6 months–1 year ago, > 1 year–5 years ago, > 5–10 years ago, >10 years ago). In the analyses the subgroups were united so that comparisons were made between those who had smoked today or yesterday and all other smokers.

Trp and kyn determinations

Trp (μmol/l) and kyn (μmol/l) concentrations in peripheral blood were measured by reverse-phase high-performance liquid chromatography (HPLC) as described previously [19]. The concentrations were measured from serum samples taken at the same time-point as the samples for serum cotinine concentrations. Trp was separated with a Shimadzu liquid chromatograph LC-10AD VP (Shimadzu Co, Kyoto, Japan) using a 50-mm BDS Hypersil C 18 5 μm column (Thermo Electron Co, Bellefonte, PA, USA). It was monitored by fluorescence with a Shimadzu RF-10 A XL detector at 266 nm excitation and 366 nm emission wavelengths. Kyn was separated with a Hewlett Packard 1100 liquid chromatograph (Palo Alto, CA, USA) using the Merck LiChroCart 55–4150 mm cartridge containing a Purospher STAR RP-18 3 μm column (Merck Co., Darmstadt, Germany). It was determined by ultraviolet absorption at 360 nm wavelength with a Hewlett Packard G13144 detector. Kyn/trp (μmol/mmol) was calculated by relating concentrations of kyn (μmol/l) to trp (mmol/l), this allowing an estimate of IDO activity.

Statistical analysis

Mann–Whitney *U*-test was applied in comparison of continuous variables, as kyn/trp was not normally distributed. Correlation was calculated with Spearman's rank correlation coefficient. Findings were considered statistically significant at $P < 0{\cdot}05$. Statistical analyses were performed with SPSS version 13·0 for Windows.

Ethical considerations

The study was approved by the Epidemiology and Public Health Division of the Ethical Committee of the Helsinki and Uudenmaa Hospital District. All participants gave written informed consent.

Results

The median (interquartile range, IQR) serum trp and kyn concentrations and kyn/trp in the whole cohort were 79·6 μmol/l (71·0–88·2 μmol/l), 2·41 μmol/l (2·01–2·87 μmol/l) and 29·8 μmol/mmol (25·7–35·7 μmol/mmol), respectively. In female subjects the median concentration of serum trp was 77·0 μmol/l (68·4–85·3 μmol/l) and in males 83·1 μmol/l (75·8–91·6 μmol/l). The serum kyn concentration in females was 2·31 μmol/l (1·92–2·82 μmol/l) and in males 2·52 μmol/l (2·14–2·92 μmol/l). Although both trp and kyn concentrations were significantly higher in males than in females ($P < 0{\cdot}0001$), the kyn/trp between female (29·8 μmol/mmol, IQR 25·5–35·9 μmol/mmol) and male subjects (30·0 μmol/mmol, IQR 26·0–35·4 μmol/mmol) did not differ ($P = 0{\cdot}867$).

Table 1. Serum kynurenine (kyn) to tryptophan (trp) ratio (median, interquartile range) in subjects grouped by serum cotinine concentration.

Kyn/trp (μmol/mmol)	Subjects with serum cotinine ≥ 100 μg/l	*n*	Subjects with serum cotinine < 100 μg/l	*n*	*P*
All	26·8 (23·9–31·5)	116	30·3 (26·0–36·4)	668	< 0·0001
Female	26·8 (23·9–31·1)	58	30·2 (25·8–36·5)	406	0·001
Male	26·8 (23·8–32·0)	58	30·7 (26·5–36·3)	262	< 0·0001

Statistics: Mann–Whitney *U*-test.

Of the subjects, 95% with serum cotinine concentration ≥100 μg/l belonged to the current smoker group. When the subjects were grouped into those with serum cotinine ≥100 μg/l and <100 μg/l, kyn/trp was significantly lower in active smokers, i.e. in those with high cotinine concentration (Table 1). In parallel with this finding, kyn/trp was significantly lower in ever-smokers (29·1 μmol/mmol, IQR 25·3–34·4 μmol/mmol, $n = 497$) compared with never-smokers (30·6 μmol/mmol, IQR 25·9–38·3 μmol/mmol, $n = 259$, $P = 0·006$) as grouped by the self-reported history of smoking. Moreover, kyn/trp was significantly lower in current daily or irregular smokers than in those who were not smoking currently at all (Table 2).

Kyn/trp was significantly lower in subjects who had smoked today or yesterday compared with other ever-smokers, i.e. subjects who had smoked from 2 days to over 10 years ago (Table 3). There was also a significant inverse correlation with kyn/trp and serum cotinine concentrations in subjects who had smoked today or yesterday, both in females and in males (Table 4).

Discussion

Tobacco smoke contains thousands of chemical compounds of which, for example, nicotine has been found to affect the adaptive immune responses [12]. Cotinine is the main metabolite of nicotine and its concentration has been shown to correlate better with biological measures of tobacco than self-reported history of smoking [14]. We used the serum cotinine concentration to discriminate active smokers from non-smokers. IDO activity was significantly lower in smoking than in non-smoking subjects in a representative sample of the adult population of Finland. In parallel with findings of lowered IDO activity in smokers based on cotinine concentrations, IDO activity was also lower in ever-smokers compared to never-smokers when the classification was made by self-reported detailed history of smoking habits. The finding of lowered IDO activity in smokers compared with non-smokers is novel; this is the first study to report on the subject.

As the half-life of elimination of cotinine is only 17 h, its concentration reflects ongoing rather than long-standing exposure to environmental tobacco smoke [14]. In line with this biological fact it could further be observed that IDO activity was significantly lower in subjects who were current smokers than in those who were not and, in particular, in subjects who had smoked today or yesterday when compared with other ever-smokers, i.e. smokers who had last smoked >2 days and up to >10 years ago. Moreover, IDO activity correlated inversely with serum cotinine concentration in subjects who had smoked today or yesterday. The level of IDO in subjects who had smoked, but not today or yesterday, i.e. over 2 days and up to 10 years ago (Table 2), was actually comparable to the level of IDO in never-smokers (median

Table 2. Serum kynurenine (kyn) to tryptophan (trp) ratio (median, interquartile range) in subjects who smoke daily or irregularly currently and current non-smokers.

Kyn/trp (μmol/mmol)	Current daily or irregular smokers	*n*	Current non-smokers	*n*	*P*
All	26·5 (23·5–31·4)	120	30·6 (26·2–37·6)	497	< 0·0001
Female	26·8 (23·9–31·0)	61	30·2 (25·9–37·3)	303	< 0·0001
Male	26·0 (23·1–31·9)	59	31·9 (26·8–37·7)	194	< 0·0001

Statistics: Mann–Whitney *U*-test.

Table 3. Serum kynurenine (kyn) to tryptophan (trp) ratio (median, interquartile range) in subjects who smoked today or yesterday and other ever-smokers.

Kyn/trp (μmol/mmol)	Subjects who smoked today or yesterday	*n*	Other ever-smokers	*n*	*P*
All	26·7 (23·8–31·5)	104	30·0 (26·0–36·6)	253	< 0·0001
Female	27·0 (23·9–31·0)	52	28·6 (24·9–33·9)	96	0·061
Male	26·3 (23·3–32·0)	52	31·9 (27·2–37·9)	157	< 0·0001

Statistics: Mann–Whitney *U*-test.

Table 4. Correlations between serum kynurenine (kyn) to tryptophan (trp) ratio and serum cotinine concentrations in subjects who smoked today or yesterday.

Variable	Correlation (r) for cotinine	*P*-value
Kyn/trp, all ($n = 104$)	− 0·316	0·001
Kyn/trp, female ($n = 52$)	− 0·323	0·019
Kyn/trp, male ($n = 52$)	− 0·347	0·012

30·6 μmol/mmol). It seems that the effect of smoking on IDO activity is strong and immediate, but is of short duration and reversible if smoking is not continued.

These findings would imply that nicotine rather than, for example, reactive oxygen species (ROS) produced by tobacco smoke, would cause the lowered IDO activity. As smoking is a potent ROS inducer and IDO can utilize ROS to catalyse trp catabolism [20], it would have been expected that smoking would enhance rather than down-modulate IDO activity if the effect of smoking on IDO activity was due to ROS.

Our hypothesis was that as IDO is expressed in APCs and as tobacco smoke has been shown to decrease the number of DCs in the lung tissue in animal models [12,13], and to have an immunosuppressive effect on DC functions [21], smoking could possibly cause inhibition of IDO activity and thereby reduction of immunosuppressive mechanisms, i.e. immunostimulation. Our hypothesis proved correct: smoking did lower IDO activity and IDO could thereby be the mechanism also explaining at least in part the known immunostimulatory effects of smoking. It could be postulated that the immunostimulatory effects of smoking, such as increased rheumatoid factor production in smokers demonstrated in epidemiological studies [4,5,22], might be mediated by the decreased immunoregulatory effect of IDO. Nicotine has also been shown to affect receptor-mediated endocytosis in monocyte-derived DCs leading to a defective Th1 response including reduced production of interferon (IFN)-γ [21], which in turn is a strong inducer of IDO activity. Thereby smoking could also indirectly affect IDO induction by reducing IFN-γ levels.

Our finding of lowered IDO activity in smokers is also in line with previous indications that smoking alters immunity by impeding Th1 and favouring Th2 responses [23,24]. We have found increased IDO activity previously in Sjögren's syndrome [25], a primarily Th1-mediated rheumatic autoimmune disease [26]. Interestingly, Manthorpe and colleagues have observed that in smoking Sjögren's syndrome patients, the salivary gland histology is less severe and the presence of anti-SSA and anti-SSB antibodies decreased compared with non-smoking patients with Sjögren's syndrome [27]. Circumstantially, as IDO activity in Sjögren's syndrome is increased and as smoking, on the other hand, protects from Sjögren's syndrome, the protective effect of smoking could be mediated by decreased IDO in smokers.

Cigarette smoke has been found to suppress Th1 cytokine production in a neonatal mouse model [23] and soluble compounds extracted from tobacco smoke have been found to suppress the function of DCs and favour the development of Th2 responses [24]. Our finding of lowered IDO activity in smokers and previous data on Th2-directed responses associated with smoking are also in line with a recent observation of lowered IDO activity in a Th2 disease, such as atopy [28].

Wolf and co-workers have found overexpression of IDO in inflammatory bowel disease; kyn/trp was higher in colonic explant cultures, particularly in patients with Crohn's disease, but also in lesions in patients with ulcerative colitis compared with samples from healthy controls [29]. Our current finding of decreased IDO levels in smokers is also in line with this finding, as epidemiological studies have shown that smoking reduces the risk of ulcerative colitis [30], and even the therapeutic use of nicotine in ulcerative colitis has been investigated [31].

In conclusion, smokers have decreased activity of the immunosuppressive IDO enzyme. Thereby IDO-dependent mechanisms could explain, at least in part, the immunostimulatory effects of tobacco smoke shown clinically, for example, by increased rheumatoid factor production in smokers. On the other hand, the lower frequency of diseases such as ulcerative colitis and Sjögren's syndrome in smokers might also be explained by the low IDO activity caused by smoking.

Acknowledgements

This study was supported by the Tampere Tuberculosis Foundation, the Academy of Finland and the Medical Research Fund of Tampere University Hospital, Tampere, Finland. We thank Ms Raija Repo, Ms Sinikka Repo-Koskinen and Ms Eija Spåre for skilful technical assistance.

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