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Decreased expression of indolamine 2,3-dioxygenase in childhood allergic asthma and its inverse correlation with fractional concentration of exhaled nitric oxide

Ying Hu, MD; Zhiqiang Chen, PhD; Ling Jin, MD; Mei Wang, MD; Wei Liao, PhD

The First Affiliated Hospital of the Third Military Medical University, Chongqing, China

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ABSTRACT

Background: The tryptophan metabolic pathway mediated by indolamine 2,3-dioxygenase (IDO), a tryptophan-degrading enzyme, plays an important role in controlling the development of allergic inflammation. The fractional concentration of exhaled nitric oxide (FeNO) is closely associated with the allergic state and is extensively used for the clinical evaluation of airway allergic inflammation. Clinical trials have rarely assessed the expression of IDO in childhood allergic asthma and its correlation with FeNO.

Objective: To evaluate the IDO level in children with childhood allergic asthma and the relation between IDO levels and FeNO.

Methods: Thirty children older than 5 years who were diagnosed the first time with allergic asthma were selected from the pediatric outpatient department. Another 30 healthy children were selected as controls. The subjects were evaluated by complete medical history, pulmonary function test results, skin prick test reaction, FeNO concentration test result, eosinophil count, and a disease severity score. Peripheral venous blood and induced sputum were obtained to measure the concentrations of IDO metabolites (ie, tryptophan and kynurenine).

Results: The IDO levels in the peripheral blood and induced sputum were significantly lower in patients with childhood allergic asthma than in children in the control group. The IDO level was negatively correlated with FeNO but was not significantly correlated with age, sex, blood eosinophil count, or disease severity scale.

Conclusion: The expression of IDO was significantly lower in childhood allergic asthma, particularly in children with high FeNO levels. There was no significant relation between IDO levels and asthma severity.

Trial Registration: Chinese Clinical Trial Register (www.chictr.org.cn) Identifier: ChiCTR-COC-15006080.

Introduction

Allergic asthma is an allergic inflammatory disease characterized by airway hyperresponsiveness. An imbalance in the ratio of T-helper cell type 1 to type 2 (T_H1/T_H2) is a key step in the pathogenesis of asthma, and T_H2 cells in particular play an important role. However, in recent years, T_H17 and regulatory T cells also have been found to have important roles in the pathogenesis of asthma.

Tryptophan is an essential amino acid that functions as an important amino acid substrate used by many human cells. There is growing evidence that the tryptophan metabolic process plays a major role in the development of allergic diseases.[1] Ciprandi et al[2] found that concentrations of plasma tryptophan and its metabolites in patients with pollen allergy were significantly higher than those in healthy blood donor controls. Kositz et al[3] noted that a high basal tryptophan concentration in patients with pollen allergy was associated with hypo-responsiveness to specific immunotherapy with pollen extracts. The key mechanism of specific immunotherapy is immune tolerance. Thus, tryptophan catabolism is believed by some researchers to be an important mechanism in the development of immune tolerance.[4]

Indolamine 2,3-dioxygenase (IDO) is the rate-limiting enzyme of the tryptophan catabolic pathway, with high expression in immune-privileged organs. IDO plays an important role in peripheral immune tolerance. The role of IDO in pregnancy, organ transplantation, and tumor immune tolerance has led to research hotspots in recent years. A clinical trial found that the plasma IDO activity in patients with allergy was significantly lower than that in healthy individuals.[5] Another clinical trial found that in patients with asthma and airborne allergen exposure, the plasma

Reprints: Wei Liao, PhD, 30 Gaotanyan Street, Shapingba District, Chongqing, China; E-mail: liaowei01@163.com.

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IDO activity was significantly higher in patients with asymptomatic allergy than in those with symptomatic allergy and healthy individuals.[6] Maneechotesuwan et al[7] observed lower IDO activity in induced sputum but a significantly higher activity of inhaled corticosteroid treatment in patients with mild to intermittent and mild to moderate persistent asthma compared with healthy subjects, which suggests that glucocorticoids, at least in part, play their anti-inflammatory role by upregulating IDO activity. Thus, IDO-mediated tryptophan catabolism could play an important role in the development and progression of allergic diseases.[8]

Notably, the fractional concentration of exhaled nitric oxide (FeNO), a marker commonly used for noninvasive clinical monitoring of airway allergic inflammation, as recommended by the American Thoracic Society, is closely related to IDO. It has long been confirmed that NO catabolism also plays an important role in allergic diseases.[1] Significantly increased inducible nitric oxide synthase (iNOS) was found in the airway epithelium and inflammatory cells of patients with asthma. The exhaled breath of patients with allergic rhinitis or asthma was shown to contain higher levels of nitric oxide (NO) than that of healthy controls during the non-pollen season. The FeNO of patients with allergic rhinitis was significantly increased during the non-pollen season, and a further increase of FeNO occurred in the pollen season.[9] Therefore, IDO and FeNO are indicators of airway allergic inflammation. The specific interaction between NO and IDO has been reported to be important in asthma and allergic rhinitis.[1] NO production can inhibit IDO expression and activation in symptomatic patients with allergy.[10,11]

These findings indicate that IDO-mediated tryptophan catabolism could play an important role in the development of allergic diseases. In allergic diseases, NO can inhibit IDO expression, leading to high tryptophan levels. The relation among IDO, NO, and allergic diseases has become a research hotspot. Asthma mainly develops in children, whose basic characteristics are growth and development. Allergy and immune tolerance in vivo can change with advancing age and with environmental changes. No researchers have been involved in clinical trials on IDO, FeNO, and childhood allergic asthma. Therefore, we investigated IDO levels in childhood allergic asthma and compared the results with those in normal children. Furthermore, we explored the possible relation of the IDO level with the FeNO level and disease severity in children with childhood allergic asthma.

Methods

The trial was registered at and approved by the Chinese Clinical Trial Register (registration number ChiCTR-COC-15006080). This trial also was reviewed and approved at a meeting of the ethics committee of Southwest Hospital, Chongqing, China. Family members of all participating children signed an informed consent form.

Subjects

The subjects were recruited at the pediatric outpatient department of Southwest Hospital. Thirty children (sample size calculated from the expected significant difference) older than 5 years who had allergic asthma (retrospective grading based on disease severity) were included. Another 30 age- and sex-matched children who had a physical examination during the same period were selected as normal controls. The representativeness and universality of age and sex were taken into account during the selection of patients and controls to decrease the offset and make the distribution as normal as possible. The clinical data of the 2 groups were comparable.

Determination of sample size

The sample size was calculated using the superiority test formula for a 2-sample mean comparison based on a 2-group, parallel, controlled, randomized blocks design:

$$N = \frac{\left(Z_\alpha + Z_\beta\right)^2 \times \left(\sigma_1^2 + \sigma_2^2\right)}{\left(\mu_1 - \mu_2 - \delta\right)^2}$$

The observation indicator was IDO activity. According to relevant studies in China and other countries, the characteristic value of IDO activity, which is the ratio of kynurenine to tryptophan (percentage), was 12.8 in the experimental group and 7 ± 1 in the control group ($\alpha = .025$ [unilateral], $1 - \beta = .90$, $\mu_1 = 12.8$, $\mu_2 = 7$, ${\sigma^2}_1 = 2^2$, ${\sigma^2}_2 = 1^2$, and $\delta = 3$). The sample size of each group was calculated to be 29. Considering interference factors such as the dropout of subjects during the clinical trial, we determined that each group should have 30 subjects.

Selection criteria for observation group

1. Selection criteria for patients in the observation group
   a. Patients older than 5 years
   b. Patients diagnosed with asthma for the first time or previously diagnosed with asthma but without standard treatment for asthma
   c. Patients did not receive inhaled glucocorticoid or leukotriene receptor antagonist treatment within 2 weeks
   d. Patients were classified by disease severity according to the Global Initiative for Asthma 2016 guidelines for the diagnosis and treatment of childhood bronchial asthma after 6 months of follow-up as outpatient treatment
2. Diagnostic criteria for asthma
   a. Recurrent wheezing, cough, shortness of breath, and chest tightness, mostly related to contact allergens, cold air, physical and chemical stimuli, respiratory infections, and sports and often with onset or exacerbation at night and (or) early morning
   b. Scattered or diffuse, mainly expiratory, wheezing in the 2 lungs at onset, with an extended expiratory phase
   c. Effective or spontaneous remission of these symptoms and signs after antiasthma treatment
   d. Exclusion of wheezing, coughing, shortness of breath, and chest tightness caused by other diseases
   e. Atypical clinical manifestations (eg, no obvious wheeze), with at least 1 of the following (those who met criteria a–d or d and e were diagnosed with asthma):
      i. Positive results from the bronchial challenge test or exercise challenge test
      ii. Confirmed reversible airflow limitation from a positive result on the bronchial dilation test: greater than 12% increase in forced expiratory volume in the first second (FEV_1) at 15 minutes after inhalation of the short-acting β_2-receptor agonist salbutamol sulfate
      iii. 20% daily variability of peak expiratory flow (continuous monitoring for 1–2 weeks)
3. The classification of childhood asthma severity was performed as follows.[12] The severity of the asthma condition was subjected to retrospective assessment and classification based on the level of treatment required to achieve asthma control. The assessment was usually performed a few months after the standard treatment with control drugs. Mild persistent asthma was defined as asthma that was well controlled by treatment with a level 1 or 2 escalation therapy regimen (1 point); moderate persistent asthma was defined as asthma that was well controlled by treatment with the level 3 escalation therapy regimen (2 points); and severe