The correlation between IL-20 and the Th2 immune response in human asthma

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Summary

Background: **Interleukin-20 (IL -20) belongs to the IL-10 family, which has been shown to be crucial in immune responses, regulation of inflammatory responses, hemopoiesis, and epidermal cell and keratinocyte differentiation. However, the role of IL-20 in asthma remains unclear. Here, our aim is to evaluate the correlation between IL-20 and the Th2 immune response in human.**

Methods: **In this study, we recruited 100 asthma patients and 100 control subjects for collection of serum samples and biopsy material. Firstly, We observed the expression of IL-20 in the airway epithelium of asthma patients by immuno-histochemical analysis, and then used an enzyme-linked immune-sorbent assay (ELISA) to analyze the serum levels of IL-20, IL-4, IL-5 and IL-13 in patients with asthma.**

Results: **We found high levels of expression of IL-20 in the airway epithelium of asthma patients. We also found that the concentrations of IL-20 and the Th2 cytokines, IL-4, IL-5 and IL-13, were significantly higher in patients with asthma and a positive correlation were found between IL-20 and Th2 cytokines (IL-4, IL-5, and IL-13). Furthermore, levels of IL-20 gradually increased according to the severity of the asthma.**

Conclusions: **IL-20 levels are increased in the epithelium and serum of asthmatic patients. The correlation between IL-20 levels and Th2 cytokines suggests that IL-20 may play a patho-physiologic role in the Th2 immune response in human asthma and may be a potential biomarker of asthma severity.** ***(Asian Pac J Allergy Immunol 2014;32:316-20)***

Keywords: *IL-20, Th2 immune response, IL-4, IL-5, IL-13, asthma severity*

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Introduction

Asthma is a chronic disease characterized by bronchial hyper-responsiveness (BHR) and lung inflammation. The development of a Th2 immune response and its associated production of cytokines are known to play an important role in the pathogenesis of allergic asthma. Th2 cells release interleukin-4 (IL-4), IL-5, and IL-13, driving IgE production, stimulating basophils and eosinophils, increasing mucus production, and enhancing mast cell differentiation.[1, 2] Recently, a number of studies have suggested that lung epithelial cells produce specific cytokines, such as thymic stromal lymphopoietin (TSLP),[3] IL-33,[4] IL-6,[5] and that these cytokines can promote a Th2 response.

IL-20 is a member of the IL-10 family of cytokines,[6] and is mainly secreted by myeloid cells and epithelial cells.[7] IL-20 can enhance tissue remodeling and wound-healing activities and, to maintain tissue integrity, restore the homeostasis of epithelial layers during infection and inflammatory responses.[7] A number of studies have revealed that IL-20 is involved in psoriasis, rheumatoid arthritis (RA), inflammatory bowel diseases (IBD), lupus nephritis,[8] and lung cancer.[9] IL-19 and IL-20 are active via the receptor complex consisting of IL-20R1 and IL-20R2, and IL-20 also binds to the receptor complex composed of IL-22R1 and IL-20R2.[8] An independent group showed that human IL-19 has a potential role in the induction of Th2 responses.[10] Another group found IL-19 induces Th2

Cytokines and was up-regulated in asthma patients.[11] However the relationship between IL-20 and asthma in human is not clear. Since IL-20 and IL-19 share the common receptor (IL-20R2), we hypothesise that IL-20 is also associated with the pathogenesis of asthma.

Methods

Subjects and patients

A total of 100 asthmatic patients were recruited from the Respiratory Clinic of Qilu Hospital, Shandong University (Ji'nan, Shandong, China), in accordance with the guidelines for prevention and cure for bronchial asthma established by the asthma group of the 2006 Global Initiative for Asthma (GINA). The patients recruited for this study had no other atopy-related or allergy diseases. Patients with COPD were excluded on the basis of incomplete reversibility of airflow obstruction. In addition, 100 healthy subjects, matched for age, were recruited from Qilu Hospital, where they had undergone pulmonary function tests, as a result of which neither doctor-diagnosed asthma nor a history of asthma or other pulmonary diseases were identified. Blood samples were obtained from all participants.

Endobronchial biopsies from the 20 asthma patients (selected from the 100 asthma patients) were obtained at Qilu Hospital of Shandong University. The 20 healthy control biopsy specimens (from the above 100 healthy subjects) were also obtained at Qilu Hospital of Shandong University and were from pneumoresections. Subjects' characteristics are shown in Table 1.

The study was approved by the Ethics Review Committee for Human Studies at Qilu Hospital, Shandong University. Each subject provided written, informed consent.

ELISA

Five milliliters of venous blood were collected from each patient. The blood samples were centrifuged and the serum was collected and stored in aliquots at -80°C before analysis. The serum from healthy age-matched controls was similarly prepared.

Human serum IL-20, IL-4, IL-5, and IL-13 were detected by means of ELISA (R&D Systems), according to the manufacturer's instructions.

Immunohistochemistry

Immunohistochemical staining was performed as described in our former article.[12] The primary antibody used was anti-IL-20 (PL-C21, sc-134365) (1:200 dilution). The secondary antibody and the chromogenic agent used in this study was goat anti-mouse IgG-HRP (sc-2005, 1 : 200, Santa Cruz, CA, USA) and DAB, 50X (sc-24982, Santa Cruz, CA, USA). The intensity of labeling was evaluated in a blind manner by 2 independent investigators and graded by using a 5-scale system (0, no signal; 1, weak; 2, moderate; 3, strong; 4, very strong).[13]

Statistical analysis

Statistical analysis was performed using the SPSS 12.0 software package (*SPSS Inc., Chicago, IL*, USA). Data were analyzed using Student's t-test. Correlations between different parameters were made using Pearson's correlation test. Significance was defined as $p < 0.05$.

Results

IL-20 was upregulated in the airway epithelium of patients with asthma.

Immunohistochemical analysis of bronchial biopsy specimens showed that IL-20 was expressed in the epithelium (Figure 1A). The scores for

Table 1. Subject characteristics of asthmatic patients based on clinical severity

	Normal	Mild	Mild/Moderate	Severe	*P* value
Case (n)	100	29	33	38	
Sex (M/F), (%)	57/53(57/43)	15/14(52/48)	19/14(58/42)	18/20(53/47)	.215
Age (±SD)	40.8±7.3	41.7±9.5	37±8.5	35±10.7	.631
Smoking status(n) Smoking/nonsmoking	37/63	13/16	14/19	16/22	.121
FEV1 % predicted (±SD)	99.7±8.8	91±6.2	87±7.4	57±6.9	< .01
FEV1 reversal % (±SEM)	4.3 ±2.7	9.1±2.3	11±2.2	32±3.4	< .01

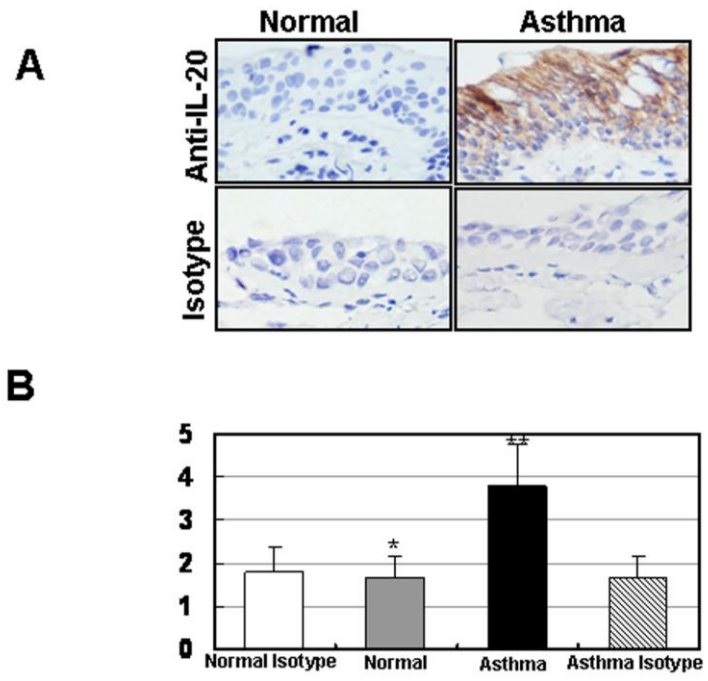

Figure 1. Protein expression of IL-20 in human asthmatic airway epithelial tissue. The expression of interleukin-20 (IL-20) in the airway epithelium of 20 asthma patients by immunohistochemical analysis A Magnification, 400×. B Bimodal H score distribution of IL-20 immunoperoxidase reactions, p*>0.05 vs isotype groups in both controls and asthma patients, p**<0.01 vs isotype group and control group.

immunostaining showed a significant difference between IL-20 staining in asthmatic patients and healthy control subjects (*p*<0.01*) (Figure 1B)

IL-20 level is elevated in asthmatic patients and correlated with an elevated IL-4, IL-5 and IL-13

To explore whether IL-20 is associated with asthma, we compared the average serum levels of IL-20 in 100 asthmatic patients with the average levels in 100 healthy adults. We found that asthmatic patients had higher levels of serum IL-20 (221.21±22.69 vs 191.17±14.13 pg/ml, *p<0.001*) (Figure 2A).

To detect whether a higher IL-20 level in asthmatic patients correlated with Th2 cytokines, we analyzed the serum level of IL-4, IL-5 and IL-13. As expected, upregulation of IL-4 (10.55±3.73 vs 4.84±1.72 pg/ml, *p<0.01*), IL-5 (113.07±27.99 vs 48.77±16.99pg/ml, *p*<0.001*) and IL-13 (76.24±12.76 vs 37.02±13.92, *p*<0.001*) were detected in serum (Figure 2B) and the data showed that the IL-20 level correlated with the levels of IL-4, IL-5 and IL-13 in the asthmatic patients (R^2=0.809 for IL-4, R^2=0.783 for IL-5, R^2=0.752 for IL-13) (Figure 2C).

Increased IL-20 in blood is associated with increased severity of asthma.

To identify whether a higher IL-20 level in asthmatic patients was associated with the severity of asthma, all of the 100 asthma patients were further divided into mild, mild/moderate and severe asthma as previously described.[14] We found that IL-20 gradually increases according to the severity of asthma. IL-20 levels differed among groups and the difference was statistically significant (Figure 3).

Discussion

Recently, evidence has suggested that the pathogenesis of the asthmatic airway responses is mainly explained by an imbalance between Th1 and Th2 (IL-4-, IL-5–, IL-13–producing) cells.[15] IL-4, IL-5 and IL-13 are the major cytokines produced by Th2 cells. IL-4 is essential to IgE production, IL-5 can drive the differentiation and survival of eosinophils, and IL-13 can promote mucus secretion, tissue remodeling, and airway hyper-responsiveness (AHR).[16] The present study has demonstrated that high expression of IL-20 is positively correlated with levels of IL-4, IL-5 and

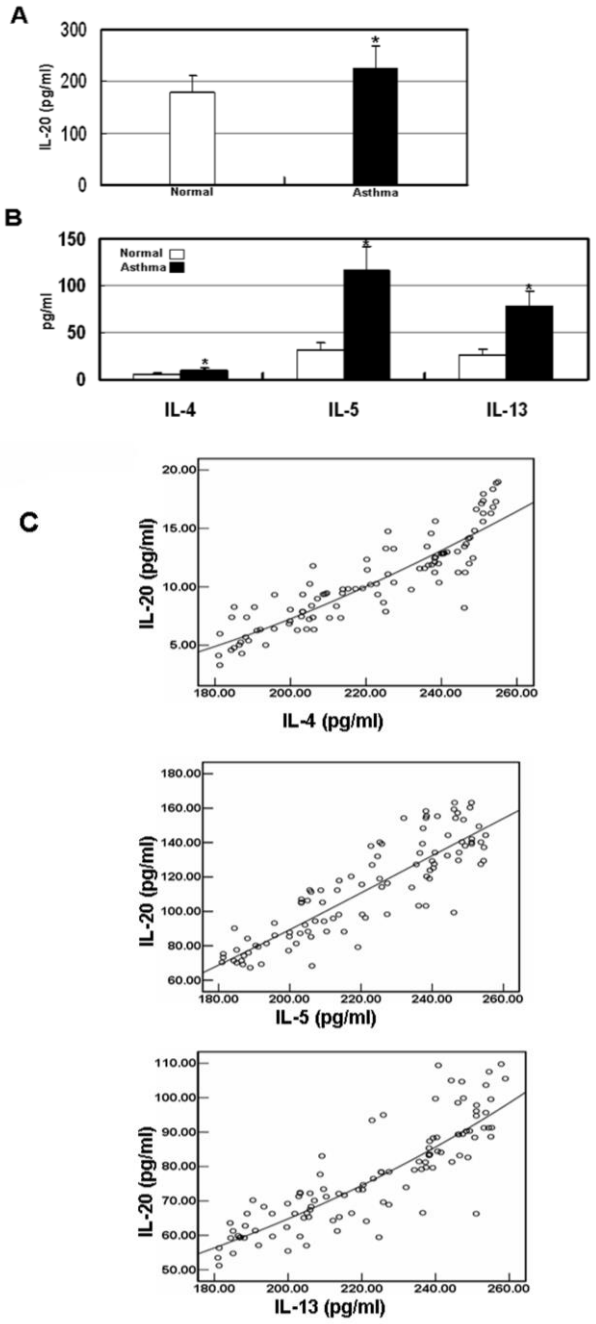

Figure 2. Comparison of serum IL-20 and Th2 cytokines (IL-4, IL-5, and IL-13) in the serum of asthmatic patients and healthy controls. Blood samples were collected from asthma patients (n = 100) and healthy subjects (n = 100). **A**. Level of IL-20 (p*<0.001 vs control group) in the serum was analyzed by ELISA kit. **B.** IL-4 (p*<0.01 vs control group), IL-5 (p*<0.001 vs control group), and IL-13 (p*<0.001 vs control groups) levels were compared with those of healthy individuals using an IL-4, IL-5 and IL-13 ELISA kits. **C.** IL-4, IL-5 and IL-13 serum level were positively correlated with the IL-20 level in asthmatic patients. (IL-4, Interleukin-4; IL-5, Interleukin-5; IL-13, Interleukin-13)

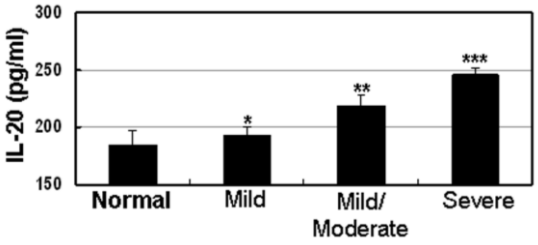

Figure 3. IL-20 expression in serum obtained from asthma patients with different degrees of severity. IL-20 gradually increased in association with the severity of asthma. IL-20 levels differed among groups and these differences were statistically significant (p*<0.05 vs normal, mild to moderate, severe; p**<0.05 vs normal, mild, severe, p***<0.05 vs normal, mild, mild to moderate).

IL-13. This suggests that IL-20 might play a role in the pathogenesis of asthma.

IL-20 is mainly expressed by monocytes and skin keratinocytes. In addition, expression of IL-20 has been found in healthy human bronchial epithelial cells [17] and occurs in cells of the immune system such as monocytes, T cells and maturing dendritic cells.[18,19] In this study, increased expression of IL-20 was found in the airway epthelium of lung tissue. Similarly, we also observed that serum IL-20 was up-regulated in asthma patients. The data presented here suggest that IL-20 may play an important role in the pathogenesis of asthma. There is no doubt that we detected higher levels of IL-4, IL-5 and IL-13, and this result is similar to the observations by Chris J et al.[13] We also found that IL-20 was positively correlated with the Th2 cytokines (IL-4, IL-5, and IL-13). The above results indicate that IL-20 is closely related to the Th2 immune response in asthma. IL-19 and IL-20 share partial common receptors and IL-19 induces Th2 cytokines and was up-regulated in asthma patients.[10, 11] Whether or not IL-20 induces the Th2 immune response or has another role in asthma must be determine by further studies.

Recently, several independent groups presented evidence that some important factors, including epithelial eotaxin-2, eotaxin-3[20] and ADRB25'-UTR methylation[21] may contribute to asthma severity. Here we found increased IL-20 expression was associated with the severity of asthma; this suggests

that IL-20 may be a vital factor involved in the severity of asthma.

In summary, we demonstrated that IL-20, another member of the IL-10 family, was associated with asthma by its positively correlation with Th2 cytokines and asthma severity. The study provides further understanding of the mechanisms of asthma.

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