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Original Research

The role and correlation of IL-35 and type II intrinsic lymphocytes in children with allergic rhinitis

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Abstract: To investigate the role and correlation of IL-35 and ILC2 in children with allergic rhinitis. 50 cases of children with allergic rhinitis admitted to our hospital from February 2018 to March 2020 were selected as the research subjects and set as the study group. During the same period, 50 cases of normal children admitted to our hospital for physical examination were selected as the control group. The differences in the expression of IL-35 and ILC2 between the two groups and the correlation with the severity of allergic rhinitis were compared. In BMI, the study group was significantly lower than the control group, and the difference was statistically significant (P<0.05). IL-35 in the study group was significantly lower than that in the control group, while ILC2, IL4+ILC2, IL-5+ILC2, IL-13+ILC2, IgE and ECP in the study group were significantly higher than those in the control group, the difference was statistically significant (P<0.05). Pearson correlation analysis showed a moderate negative correlation between TNSS score and IL-35 (r =-0.642, P<0.05), was positively correlated with ILC2, IL4+ILC2, IL-5+ILC2, IL-13+ILC2, ECP (r =0.745, 0.713, 0.725, 0.769, 0.746, P<0.05), and was strongly correlated with IgE (r =0.952, P<0.05). Also, It was positively correlated with TGF-β1 (r =0.513, P<0.05). IL-35 was strongly negatively correlated with ILC2, IL4+ILC2, IL-5+ILC2, IL-13+ILC2 (r =-0.845, -0.812, -0.805, 0.823, -0.854, P<0.05). Was negatively correlated with ECP and TGF-β1 (r =-0.795, -0.543, P<0.05). ILC2 was strongly correlated with IgE (r =0.812, P<0.05), and moderately positively correlated with ECP and TGF-β1 (r =0.642, 0.541, P<0.05). ROC curve was used to evaluate the diagnostic value. The results showed that among the five indicators, IgE had the highest sensitivity of 92.23%, while IL-35 had the highest specificity of 92.56%. However, the combined area, sensitivity and specificity of the five indicators were the highest, 0.962, 95.18% and 94.25%, respectively (P<0.05). Both IL-35 and type II intrinsic lymphocytes are highly correlated with the severity of allergic rhinitis in children, the former is negatively correlated with the latter is positively correlated. The detection of these indexes in clinical practice can be helpful for clinical diagnosis.

Key words: IL-35; Type II intrinsic lymphocytes; Children; Allergic rhinitis.

Introduction

Allergic rhinitis in children is a noninfectious inflammatory disease of the nasal mucosa that is mediated by immunoglobulin E after exposure to allergens in susceptible children. In recent years, with the aggravation of air pollution, the incidence of this disease has been increasing year by year, seriously affecting children's health, learning and quality of life (1). The prevalence of AR among children in Inner Mongolia grasslands is extremely high, accounting for 26.6%. The prevalence of boys was 28.8% higher than that of girls and 24.3% (2). The incidence of allergic rhinitis in children is high in China, so in order to improve the quality of life of children and improve their physical and mental health, it is necessary to target this allergic disease. IL-35 is a relatively new regulatory factor, which can inhibit the activity of related inflammatory factors and the mediated inflammatory response to a certain extent (3). Type 2 intrinsic lymphocytes (ILC2) mainly exists in the mucosal barrier and is one of the important effector cells in intrinsic immunity (4). The two factors mentioned above are related to the occurrence and development of allergic rhinitis in children. For example, Ma et al. (5) showed that intranasal administration of IL-35 can alleviate allergic rhinitis. Sun et al. (6) showed a significant increase in the proportion of ILC2 in children with allergic rhinitis and a significant correlation with disease symptom scores. Although there are many studies on the relationship between IL-35, ILC2 and childhood variant rhinitis, there are few studies in China. In order to further enrich the theoretical research on allergic rhinitis in children in China, 50 children with allergic rhinitis admitted to our hospital were selected as the research objects in this study to explore the role and correlation of IL-35 and type II natural lymphocytes in children with allergic rhinitis, so as to provide a reference for the development of clinical prevention and treatment. The results are as follows.

Materials and Methods

General information

Choose from February 2018 to March 2020 were 50 cases of children allergic rhinitis patients as the research object, and setting up the team, select at the same time to the hospital for a medical in 50 cases normal children as the control group, compared two groups in IL - 35, type II inherent to express differences of lymphocyte and the correlation between the severity of disease and

allergic rhinitis. This study was reviewed and approved by the Ethics Committee of our school.

Inclusion and exclusion criteria

Inclusion criteria: (A) Children in the study group were diagnosed with childhood allergic rhinitis by symptom assessment and positive skin prick test; (B) The patient's age was > 5 years old but < 12 years old; (C) Dust mite allergen was confirmed in all the children by allergen test; (D) All patients and their family members gave informed consent to this study and signed informed consent.

Exclusion criteria :(A) having a long history of living in other places; (B) Recent major events; (C) People with asthma, food allergies and autoimmune diseases; (D) Patients with malignant tumor; (E) Disobeying physician leaders.

Research Methods

The general clinical data of the two groups were collected, including age, gender, BMI, and the Total Nasal Symptomatic Score (TNSS) (25) was evaluated on a 0-4 scale, mainly including nasal congestion, runny nose, nasal itching and sneezing, the higher the score was, the more severe the disease was.

5 ml of fasting elbow venous blood was extracted from children of both groups, and part of the blood was centrifuged at 3000r/min for 15min. The supernatant was extracted, and interleukin-35 (IL-35), Immunoglobulin E (Immunoglobulin E) were determined by enzyme-linked immunoassay. IgE), Eosinophilic cationic protein (ECP), and Transforming growth factor β1 (TGF-β1). The detailed operation instructions can be seen in the kit manual.

The remaining whole blood was taken, and the ILC2 ratio was determined by flow cytometry, including the separation and culture of peripheral blood monocytes and the measurement of the ILC2 ratio by instrument. The separation of peripheral blood monocytes included the stratification of lymphocytes for one month, and then the stratification of lymphocytes was performed. After the monocyte layer liquid was absorbed, the dilution was performed by Hank's solution. Then, the cells were separated at 1000r/min for 5min, the supernatant was discarded and the cells were collected. For cell culture, the isolated cells were placed in 24-well RPMI 1640 culture dish for culture and were stimulated by adding glutamine, penicillin, streptomycin, ionomycin and phobate respectively. After that, the collected mononuclear cells were labeled with antibody mixture. CD2, CD3, C14, CD16, CD56, CD235a, etc., then APC/Cy7-bound CD45 antibody, PE-Cy7-bound CRTH2 antibody and PE-Cy7-bound CD127 antibody were used for cell staining and then placed in flow cytometry to detect ILC2 cells. Then, the intracellular cytokine staining method was applied to determine the proportion of IL4+ILC2, IL-5+ILC2, IL-13+ILC2 cells by flow cytometry (7).

Statistical methods

SPSS20.0 statistical software was used for data processing. All data were tested for normal distribution and homogeneity of variance. The homogeneity of variance was tested by LSD-T, and the heterogeneity of variance was tested by Dunnett's T3 method. Enumeration data were expressed as cases or percentages, and the chi-square test was used. Measurement data were expressed as standard deviation ± mean, and a t-test was used. Pearson correlation analysis was used to explore the correlation between the severity of allergic rhinitis and IL-35 and ILC2, and $P<0.05$ was considered statistically significant.

Results

General information assessment of the two groups of children

There was no statistical significance between the two groups in terms of age and gender ($P>0.05$), but in terms of BMI, the study group was significantly lower than the control group, and the difference was statistically significant ($P<0.05$), as shown in Table 1.

Comparison of IL-35, ILC2 and other indicators between the two groups

IL-35 in the study group was significantly lower than that in the control group, and ILC2, IL4+ILC2, IL-5+ILC2, IL-13+ILC2, IgE, ECP and TGF-β1 were

Table 1. General information assessment for the two groups of children.

Group		Research group (n=50)	Control group (n=50)	Statistics	P
Age		27.45±2.13	26.62±2.15	0.923	0.542
Gender	Male	23	22	0.841	0.715
	Female	27	28		
BMI		15.15±1.16	17.21±1.15	5.412	0.013
TNSS Score		7.52±2.13	-	-	

Table 2. Comparison of IL-35, ILC2 and other indicators between the two groups.

Group	Study group (n=50)	Control group (n=50)	T	P
IL-35	138.52±50.13	426.45±80.15	10.537	0.024
ILC2	0.097±0.065	0.025±0.016	6.842	0.001
IL4+ILC2	0.074±0.046	0.027±0.015	4.531	0.001
IL-5+ILC2	0.081±0.053	0.017±0.006	3.224	0.001
IL-13+ILC2	0.078±0.056	0.015±0.006	9.832	0.001
IgE	278.42±5.12	31.45±4.11	5.312	0.019
ECP	61.32±12.54	18.12±5.32	6.145	0.011
TGF-β1	6.15±0.22	2.23±0.12	7.542	0.008

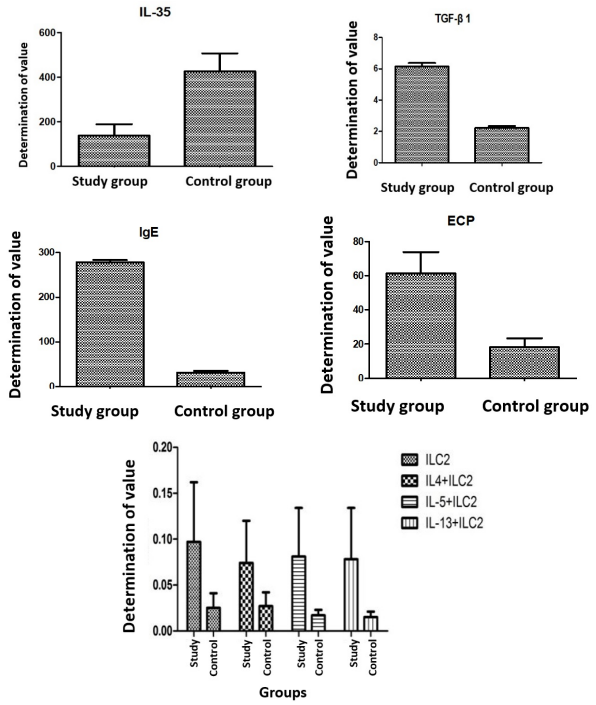

Figure 1. Comparison of IL-35, ILC2 and other indicators.

significantly higher than those in the control group, with statistical significance (P<0.05), as shown in Table 2. Figure 1.

Correlation between severity of allergic rhinitis and IL-35, ILC2 and other indicators

Pearson correlation analysis was used to explore the correlation between the severity of allergic rhinitis, IL-35 and ILC2 and other indicators. The results showed that TNSS score was moderately negatively correlated with IL-35 (r =-0.642, P<0.05). Was positively correlated with ILC2, IL4+ILC2, IL-5+ILC2, IL-13+ILC2, ECP (r =0.745, 0.713, 0.725, 0.769, 0.746, P<0.05), and was strongly correlated with IgE (r =0.952, P<0.05). Was positively correlated with TGF-β1 (r =0.513, P<0.05). IL-35 was strongly negatively correlated with ILC2, IL4+ILC2, IL-5+ILC2, IL-13+ILC2 and IgE (r =-0.845, -0.812, -0.805, 0.823, -0.854, P<0.05). Was negatively correlated with ECP and TGF-β1 (r =-0.795, -0.543, P<0.05). ILC2 was strongly correlated with IgE (r =0.812, P<0.05) and moderately positively correlated with ECP and TGF-β1 (r =0.642, 0.541, P<0.05), as shown in Table 3.

ROC curve was used to evaluate the diagnostic efficacy of the above indicators for allergic rhinitis in children

ROC curve was used to evaluate the diagnostic value. The results showed that among the five indicators, IgE had the highest sensitivity of 92.23%, while IL-35 had the highest specificity of 92.56%. However, the area, sensitivity and specificity under the combined curve of the five indicators were the highest, which were 0.962, 95.18% and 94.25% (P<0.05). (Table 4).

Discussion

Allergic rhinitis is a common ear, nose and throat disease mediated by IgE with incidence increasing year by year in developed countries, affecting the normal life of 40% of the world's population, and becoming a global health problem (8). The incidence of allergic rhinitis in China is also rising steadily, causing serious economic and social burdens. Symptoms of the disease typically include itchy nose, runny nose and sneezing, and can lead to anxiety, insomnia and general discomfort. It reduces the quality of life of patients. And because of the more complex pathogenesis of allergic rhinitis, the treatment of allergic rhinitis is more, such as allergen avoidance, drug therapy, allergen therapy, etc. However, there are still some patients whose conditions cannot be improved after adequate treatment, mainly because the mechanism of the disease is relatively complex, which

Table 3. Correlation between severity of allergic rhinitis and IL-35, ILC2 and other indicators.

Indicators	TNSS Score		IL-35		ILC2	
	r	P	r	P	r	P
IL-35	-0.642	0.021	-	-	-0.845	0.005
ILC2	0.745	0.008	-0.845	0.005	-	-
IL4+ILC2	0.713	0.006	-0.812	0.011	0.942	0.001
IL-5+ILC2	0.725	0.008	-0.805	0.015	0.958	0.001
IL-13+ILC2	0.769	0.019	-0.823	0.023	0.923	0.001
IgE	0.952	0.001	-0.854	0.012	0.812	0.007
ECP	0.746	0.022	-0.795	0.025	0.642	0.018
TGF-β1	0.513	0.041	-0.543	0.032	0.541	0.022

Table 4. ROC curve evaluates the diagnostic efficacy of the above indicators in children with allergic rhinitis.

Indicators	Area under curve	Sensitivity	Specificity	P
IL-35	0.913	90.15	92.56	0.001
ILC2	0.923	91.16	91.14.	0.001
IgE	0.947	92.23	90.52.	0.001
ECP	0.903	92.15	76.55	0.001
TGF-β1	0.745	76.52	58.23	0.012
Joint indicators	0.962	95.18	94.25	0.011

Note: The combined index was the combined judgment of IL-35, ILC2, IgE, ECP and TGF-β1.

makes it difficult to carry out comprehensive and accurate treatment (9). Therefore, it is of high practical value and research significance to explore the pathogenesis of allergic rhinitis in this study and find new targets for precise treatment.

The results of this study were mainly to explore the correlation between the progression degree of allergic rhinitis in children, IL-35, type II intrinsic lymphocytes and other indicators. The results showed that IL-35 in the study group was significantly lower than that in the control group. ILC2, IL4+ILC2, IL-5+ILC2, IL-13+ILC2, IgE, ECP and TGF-β1 were significantly higher than those in the control group. Erazab et al. (10) showed that allergen-specific immunotherapy can reduce the expression level of IL-17 and increase the expression level of IL-35 in patients with allergic rhinitis sensitive to house dust mites. Fan et al. (24) demonstrated that patients with allergic rhinitis sensitive to house dust mite or mugwort allergen had different phenotypic and functional characteristics in ILC2S frequency. ILC2S mediates major type 2 immunity during the development of allergic rhinitis and may be a potential therapeutic target. Ruan et al. (11) showed that plasma IL-35 level was significantly negatively correlated with serum eosinophil cationic protein (ECP) level and eosinophil count, and serum IL-35 or miR-223 level was negatively or positively correlated with TNSS, respectively. Levels of miR-223 and IL-35 were associated with Th1/Th2 cytokines, eosinophil count, and clinical severity. The above research results are consistent with the results of this study, which all confirm the involvement of IL-35, ILC2, ECP and IgE in the pathological mechanism of childhood allergic rhinitis.

Previous studies have suggested that the pathogenesis of allergic rhinitis is generally a type I allergic response to IgE, that is, initial sensitization leads to the production of IgE antibodies, which subsequently bind to basophils and mast cells in the tissue. When the allergen is combined with IgE antibody again, the sensitizing mediators are released, thus triggering clinical reactions (12), which is consistent with the results of this study, both of which confirmed that IgE is strongly positively correlated with TNSS scores. IgE is synthesized by B cells, and studies have also confirmed that Th2 cells can participate in humoral immunity by secreting cytokines such as IL-4, IL-5 and IL-13, thereby inducing the production of IgE, so Th2 cells are closely associated with the occurrence of allergic rhinitis (13). ILC2 is a type of innate lymphocytes distributed in the skin, intestinal tract, adipocytes and lung tissues and organs, and is a key cell group in the process of allergic inflammatory reaction (14). Related studies have found that allergens can directly participate in the occurrence of allergic reactions through epithelium-derived cytokines and ILC2S. It does not depend on the activation of dendritic cells, T and B cells (15). At the same time, some studies have confirmed that IC2 has the function of important Th2 cytokines such as IL-4, IL-5 and IL-13. Among the above cytokines, IL-5 can induce the increase of eosinophils, the expansion of B1 cells and the generation of IgM by B cells, and the production of IL-9 activates autocrine, while IL-9 can promote the growth of mast cells. Both IL-4 and IL-13 can regulate the IgE response of B cells, mucus secretion of epithelial cells and remodeling of extracellular matrix, and are also key factors in the induction of eosinophilic chemokine production by epithelial cells (16-18). Therefore, ILC2, IL4+ILC2, IL-5+ILC2, IL-13+ILC2 in the study group were higher than those in the control group. And there was a strong correlation between ILC2 and IgE, and a moderate positive correlation between ILC2 and ECP in the study group.

IL-35, a member of the IL-12 family, is an inflammatory cytokine. Research results indicated that IL-35 is a negative regulatory protein, which may exert physiological activity by inhibiting the growth and effector function of T lymphocytes (19). Studies have also shown that in a variety of models of non-fatal autoimmune response diseases when the expression of functional IL-35 is down-regulated, the inhibitory function of Treg cells is significantly reduced, and the inflammatory response of the body is further aggravated (20). Liu et al. (21) showed that the expression of IL-35 and the proportion of ITR35 were decreased in patients with allergic rhinitis compared with normal people, while ILC2 and type II cytokines were increased. IL-35 inhibits ILC2 differentiation and the production of type II cytokines by regulating IL-12Rβ2 and gp130. IL-35 promotes ITR35-induced costimulatory molecule expression and ILC2-induced costimulatory molecule ligand expression. IL-35-treated AR mice showed a decrease in the frequency and function of intranasal ILC2, confirming that the ILC2 response was inhibited by intercontact between IL-35 and ILC2 in AR, either directly or through ITR35. As a result of the above research mechanism, IL-35 was strongly negatively correlated with ILC2, IL4+ILC2, IL-5+ILC2, IL-13+ILC2 and IgM, and moderately negatively correlated with TNSS. TGF-β1 increased in the study group and was mostly moderately or weakly correlated, which may be mainly related to the airway epithelial cell response caused by dust mite allergen and Th17 /Tregs in the pathophysiological process (22-28). In this regard, a comprehensive study of the genome (29) can be effective. IL-35 is a relatively new regulatory factor that can inhibit the activity of related inflammatory factors thereby causing cancer (30).

ROC curve was used to evaluate the diagnostic value. The results showed that among the five indicators, IgE had the highest sensitivity of 92.23%, while IL-35 had the highest specificity of 92.56%. However, the area, sensitivity and specificity under the combined curve of the five indicators were the highest, which were 0.962, 95.18% and 94.25%, respectively. The results of this study confirmed that IL-35 and ILC2 have high sensitivity and specificity for allergic rhinitis in children, and can be used as a diagnostic index for allergic rhinitis in children. But the effect of combined detection will be better applied.

In conclusion, both IL-35 and type II intrinsic lymphocytes are highly correlated with the severity of allergic rhinitis in children, with the former being negatively correlated and the latter being positively correlated. The detection of these indicators in clinical practice can be helpful for clinical diagnosis.

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