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ORIGINAL RESEARCH

Coinhibitory Molecule VISTA Play an Important Negative Regulatory Role in the Immunopathology of Bronchial Asthma

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Objective: To investigate the significance of VISTA in bronchial asthma and its impact on the disease.

Methods: Human peripheral blood of asthma children was gathered. The expression concentrations of VISTA, IL-4, IL-6, CD25, CD40L, and PD-L2 in peripheral blood plasma were detected by ELISA. We established the mouse model of asthma and intervened with agonistic anti-VISTA mAb (4C11) and VISTA fusion protein. ELISA, flow cytometry, and Western blotting were performed to detect the expression levels of Th1, Th2, and Th17 cell subsets and related characteristic cytokines, as well as the protein levels of MAPKs, NF-κB, and TRAF6 in lung tissues. In addition, the infiltration of eosinophils and inflammatory cells, airway mucus secretion, and VISTA protein expression in lung histopathological sections of different groups of mice were analyzed.

Results: The concentration of VISTA in human asthma group decreased significantly ($p < 0.05$); A positive correlation was observed between VISTA and CD40L. The intervention of 4C11 mAb and fusion protein respectively during the induction period increase the differentiation of Th1 cells and the secretion of IFN-γ, and inhibit the differentiation of Th2 and Th17 cells, as well as the secretion of IL-4, IL-5, IL-13 and IL-17, partially reduce the pathological changes of asthma in mouse lungs and correct the progress of asthma. The MAPK, NF-κB, and TRAF6 protein levels were the middle range in the 4C11 mAb and fusion protein groups ($p < 0.05$).

Conclusion: The findings suggest VISTA may play a negative regulatory role in the occurrence and development of bronchial asthma.

Keywords: VISTA, bronchial asthma, Th2 cells, cytokines, anti-VISTA mAb, VISTA fusion protein

Introduction

Bronchial asthma, commonly known as asthma, has a very high incidence rate in the present society and is a serious threat to the physical and mental health of human beings.

The immune, neurological, psychological, endocrine, and genetic factors exert different degrees of influence throughout the course of the disease, the immunological factor being the most prominent. Asthma is currently classified into two types by researchers: Th2-high (eosinophilic) asthma and Th2-low (non-eosinophilic) asthma.[1] Most childhood asthma belongs to the Th2-high type, and its immune mechanism is primarily related to the functional imbalance of the Th1/Th2 cells. Th17/Treg immune imbalance is also an important pathogenesis of asthma. Regulatory T cells (Treg) can reverse airway inflammation and airway hyper reactivity(AHR), thereby inhibiting the progression of asthma. In addition, studies have reported that Treg differentiation in asthma patients is significantly inhibited.[2,3] IL-4,[4] IL-6,[5] CD25,[6] Cluster of Differentiation 40 Ligand (CD40L),[7] and Programmed Death Ligand 2 (PD-L2)[8] participate in the regulation of asthma by inhibiting or promoting Treg differentiation in different ways.

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Th cell differentiation needs T cell activation as the premise, while T cell activation and immune response initiation need to be achieved through dual signal stimulation.[9] The B7 family consists of structurally related, cell-surface protein ligands, which bind to receptors on lymphocytes that regulate immune responses. Interaction of B7-family members with costimulatory receptors augments immune responses, and interaction with coinhibitory receptors attenuates immune responses. There are currently seven known members of the family: B7.1 (CD80), B7.2 (CD86), Inducible Costimulator Ligand (ICOS-L), Programmed Death Ligand 1 (PD-L1), PD-L2, B7-H3, and B7-H4.[10] VISTA (short for V-domain Ig suppressor of T cell activation, also known as PD-1H, B7-H5, c10orf54, SISP1, Gi24, Dies-1, and DD1 α) is a new member of the B7 family, with 85.6% homology between mice and humans. The distribution of VISTA exhibits evident concentration; it is mainly distributed in hematopoietic tissues, lymphocytes, and tissues with high infiltration of leukocytes, which is closely related to immune response.[11] The biological function of VISTA is currently a hot research topic. Several reports on the role of VISTA in negative regulation are available. VISTA fusion proteins inhibit the differentiation and activity of $CD4^+$ and $CD8^+$T cells, block the secretion of IL-2, IL-10, Tumor Necrosis Factor-α (TNF-α), and Interferon-gamma (IFN- γ), and upregulate the synthesis of the early activation markers of the T cells (CD69, CD25); however, the VISTA fusion proteins do not participate in the regulation of T cell apoptosis.[12,13] Bharaj et al[14] observed through gene enrichment analysis that VISTA could induce TNF- α secretion, increase the opening of NF- κB and other pro-inflammatory signaling pathways,produce more pro-inflammatory cytokines and ultimately leading to inflammation. Xu et al[15] stimulated peritoneal macrophages isolated from wild and VISTA-KO mice in vitro using Toll like receptor (TLR) agonists CpG (TLR9) and R848 (TLR7), and found that CpG and R848 significantly upregulated the gene expression and protein production of IL-12, IL-6, and IFN-γ in VISTA-KO mouse macrophages. At the same time, VISTA could inhibit the activation of MAPKs/AP-1 and IKK/NF-κB signaling cascades after TLR stimulation, thereby blocking VISTA from triggering endogenous TLR/MyD88 mediated pro-inflammatory responses.The correlation between VISTA and the pathological process of asthma is not clear, and related reports are lacking to date. The mechanism of VISTA in an asthma model has not yet been reported.

Herein, we studied the differential expression of VISTA in the peripheral blood of children with asthma and the control group, analyzed its correlation with relevant cytokines, and explored the significance of the differential expression of VISTA in asthma and its impact on disease. The immune mechanism of VISTA in asthma was also explored, especially the mechanism of Th1/Th2/Th17 cell differentiation imbalance, and a preliminary study was conducted on the signal pathway of VISTA, providing a new target and direction for the biological treatment of asthma, by making an ovalbumin(OVA)-induced mouse asthma model and adding the intervention of mouse IgG, agonistic anti-VISTA mAb (4C11), and VISTA fusion protein separately during the induction period.

Materials and Methods

Research Objects

A total of 51 children with asthma hospitalized in the respiratory department of Children's Hospital of Soochow University, from May 2020–September 2020 were selected as the asthma group, including 35 boys and 16 girls. Their age ranged from 2 y and 1 m to 12 y and 4 m, and the average age was 6.11 ±2.77 y. The enrolled children met the diagnostic criteria for acute attacks of bronchial asthma. The asthma diagnosis of children under 6 years was evaluated with reference to the asthma prediction index. No glucocorticoids and drugs affecting the platelet activity had been used in the past 4 w, and no immune deficiency disease or respiratory tract congenital malformation was noticed in them. A total of 37 children undergoing elective surgery (such as circumcision, umbilical hernia, and hemangioma) in our hospital during the same period were selected as the control group, including 22 boys and 15 girls. Their ages ranged from 1 y and 8 m to 11 y and 9 m, with an average age of 5.84 ±2.66 y. The children in the control group had no history of allergic diseases and allergies, and congenital diseases. They were healthy in the past month and were not on any drugs. Peripheral blood was collected from all selected subjects for examination within 24 h of admission. No statistical difference was found between the two groups in terms of age, sex, and other basic data ($p > 0.05$). The research was conducted in accordance with the Declaration of Helsinki. The research involving human participants was reviewed and approved by the Institutional Human Ethical Committee of Children's Hospital of Soochow University with judgment's

reference number 2020CS039. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

All enrolled subjects were provided with the routine treatment of asthma, such as aerosol inhaled glucocorticoids and bronchodilators, according to the diagnosis and treatment criteria for children with asthma. In severe cases, glucocorticoids were given orally or intravenously. Simultaneously, relevant interventions such as oxygen inhalation, appropriate fluid infusion, antibiotics, and maintenance of water-electrolyte and acid–base balance were provided based on individual conditions. The treatment period was 7–15 d.

Blood Sample Collection and Processing

Within 24 h after admission of an individual, 3~5 mL of venous blood was collected, anticoagulated with ethylene diamine tetraacetic acid (EDTA), centrifuged at 2500 rpm at 4 °C for 5 min to obtain the plasma, and stored in a refrigerator at – 80 °C to provide sample support for the subsequent tests. The concentrations of expressed VISTA, IL-4, IL-6, CD25, CD40L, PD-L2, total IgE, and specific IgE in peripheral blood were detected by enzyme-linked immuno sorbent assay(ELISA).

Mouse Model of Asthma

Fifty specific pathogen free(SPF) grade C57BL/6 experimental mice aged 6–8 w were stochastically separated into five groups: PBS control group, asthma group, IgG group, 4C11 mAb group, and fusion protein group, with 10 mice in each group. They were raised under normal environmental conditions and fed freely. The research was conducted in accordance with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the United National Institutes of Health. The protocols were approved by the Ethics Committee of Soochow University (ECSU) (approval number: SUDA20210512A01). All experiments were repeated three times.

Mice in the asthma, IgG, 4C11 mAb, and fusion protein groups were sensitized and stimulated with OVA (mice were sensitized by intraperitoneal injections of 0.25mL OVA suspension (containing OVA 100 μg and aluminum hydroxide gel 400 μg) on days 0 and 7, then 5mL 10% OVA (10g OVA dissolved in 1000mL physiological saline) was inhaled by atomization for 30 mins on days 15, 16, 17, and 18), and the PBS control group was sensitized and stimulated with the same dose of PBS. The IgG, 4C11 mAb, and fusion protein groups were given intraperitoneal injections of IgG (50ug), anti-VISTA mAb (4C11) (50ug), and VISTA fusion protein (50ug) on days 1, 5, 8, 12, and 15 (1 h before the challenge) (Figure 1).

Detection of Inflammatory Cytokines, Cells, and Pathway Proteins in Mice

After the last excitation for 24 h, each experimental group was anesthetized, and 1–2 mL of eyeball blood from all of the 10 mice in each group was collected with EDTA. Plasma was collected after centrifugation at 2500 rpm at 4 °C for 5 min, and samples were stored at –80 °C for testing. Thereafter, the mice were euthanized by neck detachment. The right lung of mice was ligated with surgical suture from the root of the right lung, and physiological saline was instilled into the left lungs thoroughly. Then, the collected bronchoalveolar lavage fluid (BALF) from all of the 10 mice in each group was centrifuged to pellet the cells, and the supernatant was kept at −80 °C until it was used for cytokine analysis. The experiment used an ELISA method to detect the cytokines (IFN-γ, IL-4, IL-5, IL-13, and IL-17) in mouse plasma and BALF. We randomly selected 3 mice from each group for flow cytometry and Western blotting. Flow cytometry was used

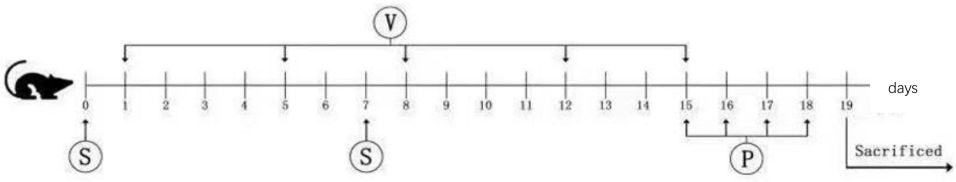

Figure 1 Mouse model production. **(S)** Intraperitoneal injections of PBS or OVA according to different groups. **(P)** inhalation of PBS or OVA according to different groups. **(V)** Intraperitoneal injections of IgG, anti-Vista mAb (4C11), or Vista fusion protein.

to detect the Th1, Th2, and Th17 cell subpopulations in the mouse peripheral blood. Kaluza Analysis 2.1 software was used to analyze cell related fluorescence expression. Western blotting was used to determine the expression levels of MAPKs (short for mitogen-activated protein kinases), NF-κB (short for nuclear factor kappa-B), and TRAF6 (short for tumor necrosis factor receptor-associated factors 6) in lung tissues. The collected image data was processed using the Quantity One tool, and the relative values were calculated before being imported into GraphPad for further statistics.

Pathological Section Staining of Mouse Lung Tissue

For histological staining, the collected fresh right lung tissue from 3 mice in each group was fixed in 4% paraformaldehyde and embedded in paraffin. Then, 3-μm tissue sections were prepared to be stained with hematoxylin and eosin staining (HE), periodic acid-Schiff stain (PAS), and immunohistochemical staining. Image-Pro Plus 6.0 image analysis software was used to analyze the immunohistochemical sections.

Statistical Analysis

SPSS 25.0 software was used for statistical analysis of data. The measurement data of human plasma cytokine levels was described by ($\overline{X}\pm$ SD). The difference between groups conforming to normal distribution was tested by the *t*-test, and the correlation was tested by Pearson's method. The measurement data of non-normal distribution were tested by the Wilcoxon rank sum test, and the correlation was tested by Spearman's straight-line method. The measurement data of mice from different groups was also described by ($\overline{X}\pm$ SD). One Way ANOVA analysis was used to compare multiple groups of data. LSD-*t* test was used for comparison between two sets. The level of significant difference was set as $p < 0.05$.

Results

Characteristics of Children with Bronchial Asthma

The age range of 51 children, including 35 boys and 16 girls in the asthma group, was from 2 y 1 m to 12 y 4 m, with an average age of 5.70 ±2.76 y. All children in the asthma group had cough and wheezing symptoms on admission, with 30 children (58.8%) suffering from nasal congestion and runny nose, 17 children (33.3%) suffering from fever, 35 children (68.6%) suffering from shortness of breath and 22 children (43.1%) suffering from dyspnea and triple concave syndrome. Among the 51 children, 38 (74.5%) had a history of eczema at an early age, 28 (54.9%) had a family history of asthma or allergy, and 34 (66.7%) had a history of allergic rhinitis. In total, 45 children underwent blood allergen tests, and 41 (91.1%) children tested positive. Among them, the highest detection rates corresponded to dust mites (82.2%), house dust (48.9%), and eggs (28.9%). A total of 41 children underwent a total IgE test, of which 34 children showed an increase in total IgE (82.9%) (Table 1).

Table 1 Population Distribution and Clinical Characteristics of Children with Asthma

Basic information	
Age (years)	5.70±2.76
Height(cm)	116.10±17.63
Weight (kg)	23.18±10.80
Sex for Male [n,(%)]	35 (68.6%)
Hospitalization Days	6.2±1.8
Clinical symptoms	
Nasal Congestion and Runny Nose[n,(%)]	30 (58.8%)
Fever[n,(%)]	17 (33.3%)
Shortness of Breath[n,(%)]	36 (68.6%)
Dyspnea and Triple Concave Syndrome[n,(%)]	22 (43.1%)

(*Continued*)

Table 1 (Continued).

Basic information	
Past history and family history	
Eczema[n,(%)]	38 (74.5%)
Family history of Asthma or Allergy[n,(%)]	28 (54.9%)
Allergic Rhinitis[n,(%)]	34 (66.7%)
Dust Mite Allergy[n,(%)]	37/45 (82.2%)
House Dust Allergy[n,(%)]	22/45 (48.9%)
Egg Allergy[n,(%)]	13/45 (28.9%)
Laboratory Examination	
Total IgE (IU/L)	512.80±359.86
$CD19^{+}$ $CD23^{+}$ (%)	9.24±4.52
Peripheral Blood Eosinophil ($\times 10^{9}$ /L)	0.491±0.416
CRP (mg/L)	9.31±9.98
FeNO (ppb)	12.96±10.03
IgG (g/L)	8.69±2.46
IgM (g/L)	1.13±0.41

Comparison of Human Plasma Cytokine Levels Between the Asthma and Control Groups

Compared with the control group, the asthma group showed a decrease in VISTA (P=0.03). The levels of CD25 (p<0.001), CD40 (p= 0.007) and PD-L2 (p<0.001) decreased significantly in asthma group. The levels of IL-4 (p=0.007) and IL-6 (p=0.002) in the asthma group were significantly higher than those in the control group (Figure 2).

Correlation Analysis of VISTA with IL-4, IL-6, CD25, CD40L, and PD-L2 and Clinical Characteristics in Children with Asthma

VISTA was positively correlated with CD40L in children with asthma ($r_p = 0.508$, $p < 0.01$), but no correlation was found with the parameters such as IL-4, IL-6, CD25, PD-L2, eosinophil, total IgE, $CD19^{+}CD23^{+}$B cells, hospitalization days, and age (Table 2 and Figure 3).

Animal Behavior Observation

The behavior of the PBS control group mice showed no significant changes after daily nebulization. The asthma group mice showed symptoms of restlessness, accelerated breathing, frequent scratching of the face and neck skin, and forelimb retraction and lifting after nebulization for approximately 5 min on the first day (15^{th} day) of stimulation. On the 17^{th} day, the above symptoms appeared a few seconds after nebulization and the response was even stronger, including nodding breathing, slow response, and urinary and fecal incontinence. The behavior of the IgG group was similar to that of the asthma group. The behavioral manifestations in the 4C11 mAb and fusion protein groups were similar to those in the asthma group on the 15^{th} and 16^{th} days of stimulation. The symptoms were significantly reduced from the 17^{th} day compared to those in the asthma group.

Comparison of IFN-γ, IL-4, IL-5, IL-13, and IL-17 in the BALF and Plasma of Mice

Compared with the PBS control group, the levels of IFN-γ in BALF in the asthma and IgG groups were significantly reduced, while the levels of IL-4, IL-5, IL-13, and IL-17 were significantly increased ($p < 0.05$). No difference in various cytokines was observed between the asthma and IgG groups ($p > 0.05$). Compared with the IgG group, the expression levels of IL-4, IL-5, IL-13, and IL-17 in the 4C11 mAb and fusion protein groups significantly decreased, while the results for the IFN-γ were opposite. Statistically significant differences were observed in all except for IL-5. The results

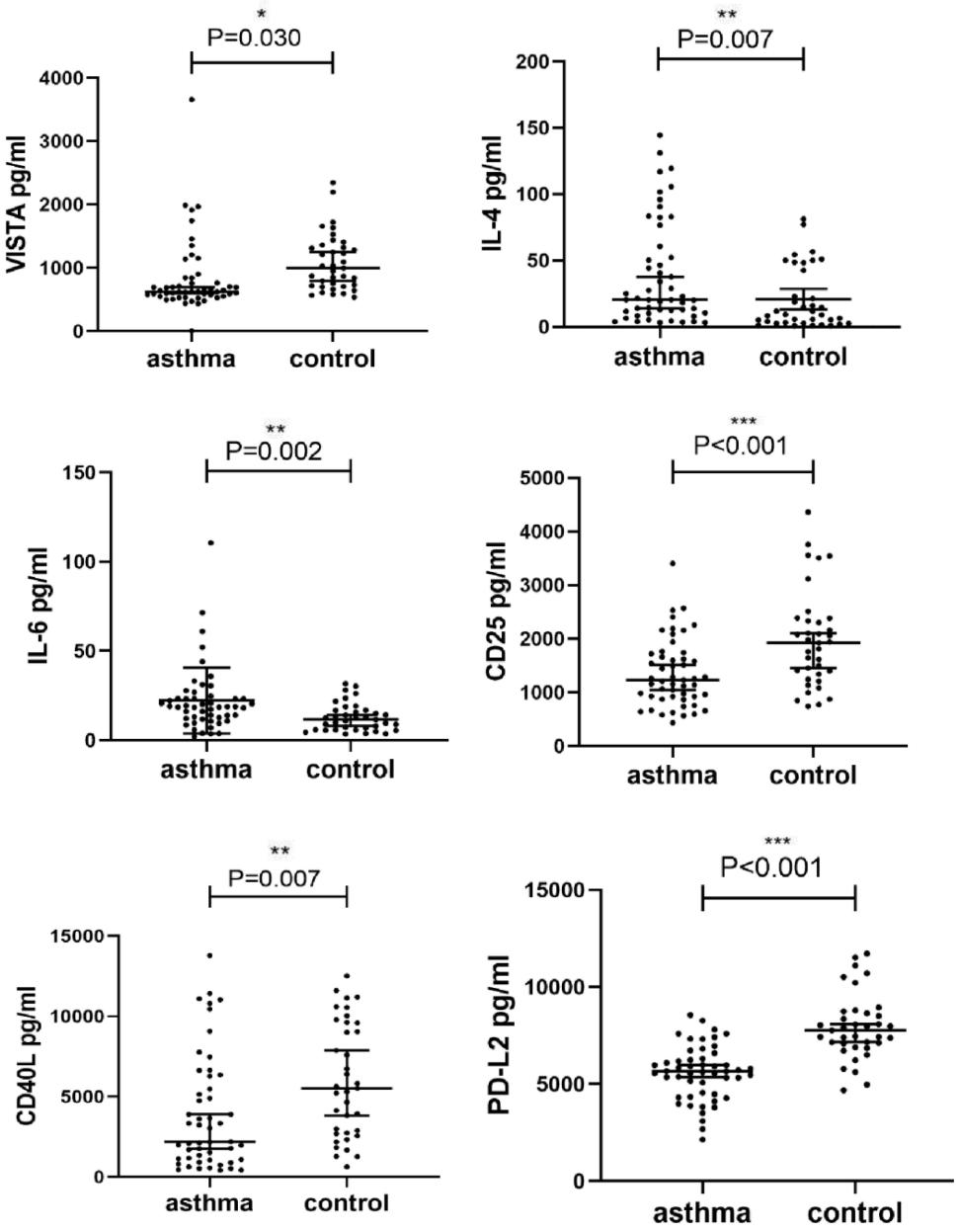

Figure 2 Comparison of Vista, IL-4, IL-6, CD25, CD40L and PD-L2 in asthma and control group. Experimental subjects were divided into the asthma group and control group. The plasma cytokine levels of the two groups were detected by ELISA, including VISTA, IL-4, IL-6, CD25, CD40L, and PD-L2. In the asthma group, the levels of VISTA, CD25, CD40L, and PD-L2 significantly decreased compared to those in the control group, while the levels of IL-4 and IL-6 increased. (*P<0.05, **P<0.01, ***P<0.001).

also revealed that compared to the PBS control group, the IFN-γ levels in the 4C11 mAb and fusion protein groups still decreased. The levels of IL-4, IL-5, IL-13, and IL-17 were increased ($p < 0.05$). However, no statistical difference was observed between the 4C11 mAb and fusion protein groups with respect to various cytokines ($p > 0.05$) (Figure 4A).

Compared with the PBS control group, the plasma IFN-γ levels in the asthma and IgG groups decreased, while IL-4, IL-5, IL-13, and IL-17 levels increased ($p < 0.05$). No significant difference was observed in the various cytokine levels between the two groups ($p > 0.05$). Compared with the IgG group, the 4C11 mAb and fusion protein groups showed an increase in IFN-γ levels, while the levels of IL-4, IL-5, IL-13, and IL-17 decreased ($p < 0.05$). The results showed significant statistical differences except for IL-5. We also found that the IFN-γ levels in the 4C11 mAb and fusion protein groups were still lower than those in the PBS control group, while IL-4, IL-5, IL-13, and IL-17 were slightly higher ($p <$

Table 2 Correlation Analysis of VISTA with IL-4, IL-6, CD25, CD40L, PD-L2 and Clinical Characteristics in Children with Asthma

	VISTA (n=51)	
	r_p	P
IL-4 (pg/mL)	0.048	0.738
IL-6 (pg/mL)	0.053	0.710
CD25 (pg/mL)	−0.156	0.276
CD40L (pg/mL)	0.508	0.000
PD-L2 (pg/mL)	−0.236	0.096
Peripheral Blood Eosinophil ($\times 10^9$ /L)	0.028	0.848
Total IgE (IU/L)	−0.106	0.539
$CD19^+$ $CD23^+$ (%)	−0.017	0.921
Hospitalization Days	−0.010	0.945
Age (years)	0.183	0.198

Note: *r_p: Correlation coefficient of linear correlation analysis.

0.05). There was no statistical difference between the 4C11 mAb and fusion protein groups with respect to the levels of various cytokines ($p > 0.05$) (Figure 4B).

Comparison of Th1, Th2, and Th17 Cell Levels in the Plasma of Mice

The plasma level of Th1 cells ($CD4^+IFN\text{-}\gamma^+$) in the PBS control group was the highest, while those of Th2 ($CD4^+IL\text{-}4^+$) and Th17 ($CD4^+IL\text{-}17^+$) cells were the lowest. The levels of Th1 cells were the lowest in the asthma and IgG groups, while the levels of Th2 and Th17 cells were the highest. No statistically significant difference in cell levels was observed between the asthma and the IgG groups ($p > 0.05$). The levels of Th1, Th2, and Th17 were in the middle of those in the 4C11 mAb and fusion protein groups and were statistically significant compared to those in the asthma and IgG groups ($p < 0.05$). There was no statistical difference between the 4C11 mAb and fusion protein groups with respect to each cell subgroup level ($p > 0.05$) (Figure 5).

Comparison of MAPK, NF-κB, and TRAF6 Protein Levels in Lung Tissue of Mice

The levels of MAPK, NF-κB, and TRAF6 proteins were the lowest in the lung tissue of the PBS control group and highest in the asthma and IgG groups. The levels of MAPK, NF-κB, and TRAF6 proteins were in the middle in the 4C11 mAb and fusion protein groups, with statistically significant differences ($p < 0.05$). No statistically significant difference was observed between the asthma and IgG groups, as well as between the 4C11 mAb and fusion protein groups ($p > 0.05$) (Figure 6).

Comparative Analysis of Lung Histopathology of Mice

Comparative Analysis of HE Staining

Comparison of the HE staining of the lung tissue in each group revealed that the lung tissue structure in the PBS control group was clear and no change in the alveolar septum was observed. In addition, no eosinophils and inflammatory cell aggregation were found. The lung tissue in the asthma and IgG groups showed airway stenosis, columnar epithelial hyperplasia, significant eosinophils, and inflammatory cell infiltration in the airway and around the alveoli. Similar manifestations were observed in the 4C11 mAb and fusion protein groups compared to the asthma and IgG groups, but the occurrence was significantly reduced (Figure 7).

A specially-assigned person was responsible for observing the infiltration of inflammatory cells and a score was assigned based on the Bronchial Pulmonary Inflammation Pathology Scale Score (Table 3), within a range of 0–10 points. The average score of the PBS control group was 1.3 points, while those of the asthma and IgG groups were 8.3

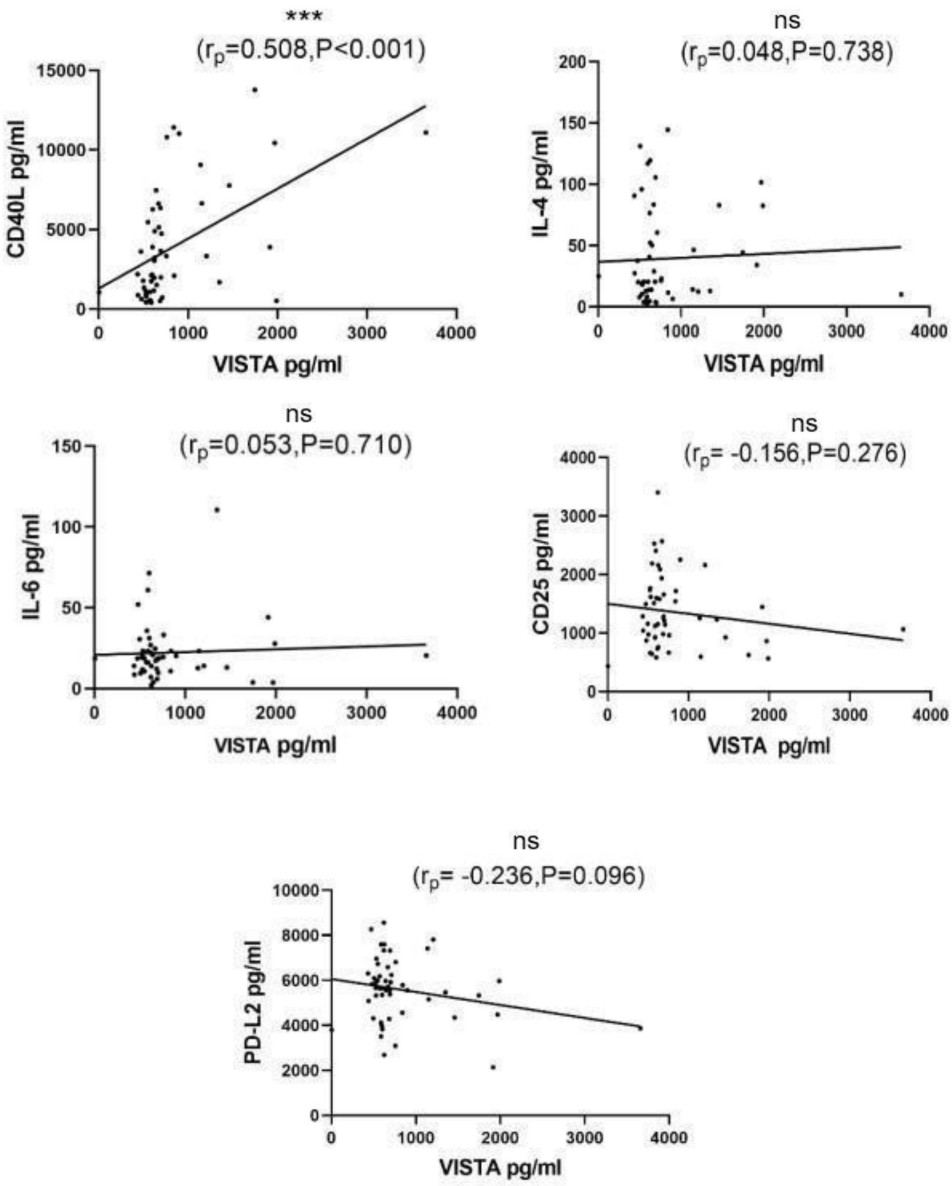

Figure 3 Correlation analysis of VISTA with IL-4, IL-6, CD25, CD40L, and PD-L2 in the asthma group. Our experiment analyzed the correlation between the plasma VISTA and IL-4, IL-6, CD25, CD40L,PD-L2 in the asthma group. The plasma VISTA in the asthma group was only positively correlated with IL-4. No correlation was observed between the plasma VISTA and IL-6, CD25, CD40L, PD-L2. (ns=no significance, ***P<0.001).

and 9.0 points, respectively. The 4C11 mAb group and fusion protein groups had similar scores of 3.7 and 4.0 points, respectively, which were significantly lower than those of the asthma and IgG groups ($p < 0.05$) (Figure 7).

Comparative Analysis of Lung Tissue Mucus Secretion in PAS Staining

No mucus secretion was observed in the PBS control group after PAS staining, while significant red acidic mucus secretion was observed in the airways of the asthma and IgG groups. In addition, a small amount of mucus secretion was observed in the 4C11 mAb and fusion protein groups (Figure 8).

In addition to detecting PAS staining, the specially-assigned person was responsible for observing the secretion of airway mucus and scoring in a range of 0–5 points. The numerical scores for the abundance of PAS-positive mucus-containing cells in each airway were determined as follows: 0, <5% PAS-positive cells; 1, 5%–25%; 2, 25%–50%; 3, 50%–75%; 4, >75%. The average score of the PBS control group was 0, while the asthma and IgG groups had similar

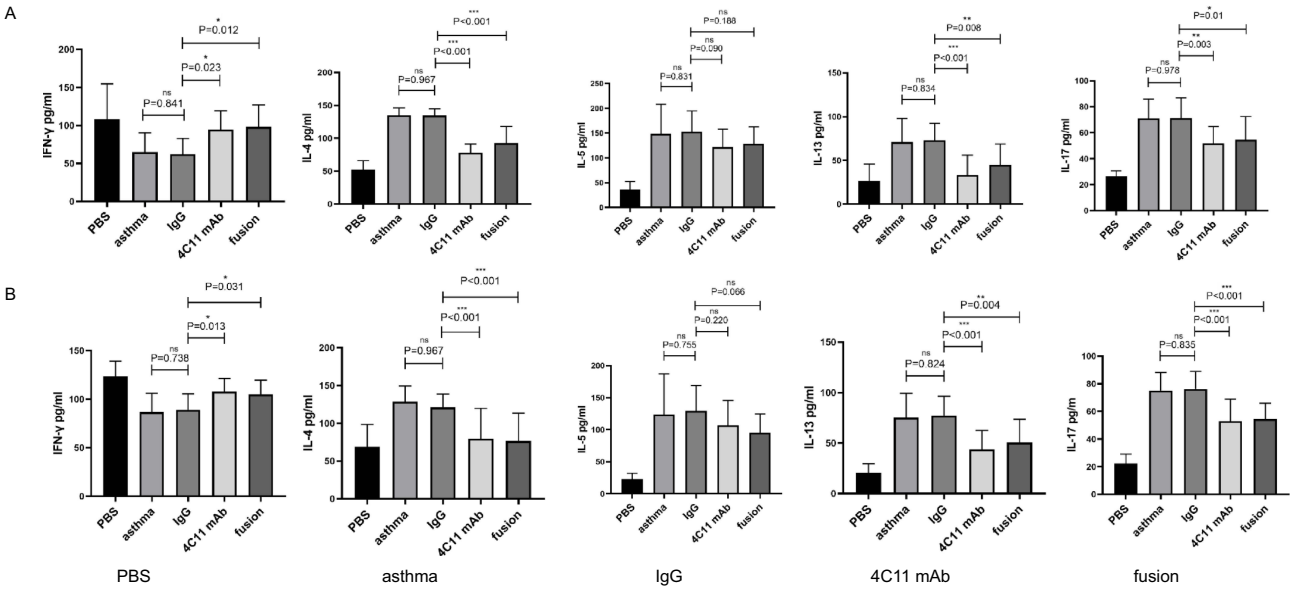

Figure 4 IFN-γ, IL-4, IL-5, IL-13, and IL-17 levels in each group. Our experiment established a mouse asthma model. Based on the different intervention measures, they were divided into five groups, including the PBS control group, asthma group, IgG group, 4C11 mAb group, and fusion protein group. The BALF **(A)** and plasma **(B)** were collected from each group of mice, and the levels of IFN-γ, IL-4, IL-5, IL-13, and IL-17 were compared among the groups. The levels of IFN-γ in the asthma and IgG groups decreased compared to the PBS group, while the levels of IL-4, IL-5, IL-13, and IL-17 increased compared to the PBS group. No statistical difference between the asthma and IgG groups was observed. The IFN-γ levels in the 4C11 mAb and fusion protein groups were higher than those in the IgG group, while the levels of IL-4, IL-13, and IL-17 were all lower than those in the IgG group except for IL-5. (ns=no significance,*P<0.05, **P<0.01, ***P<0.001).

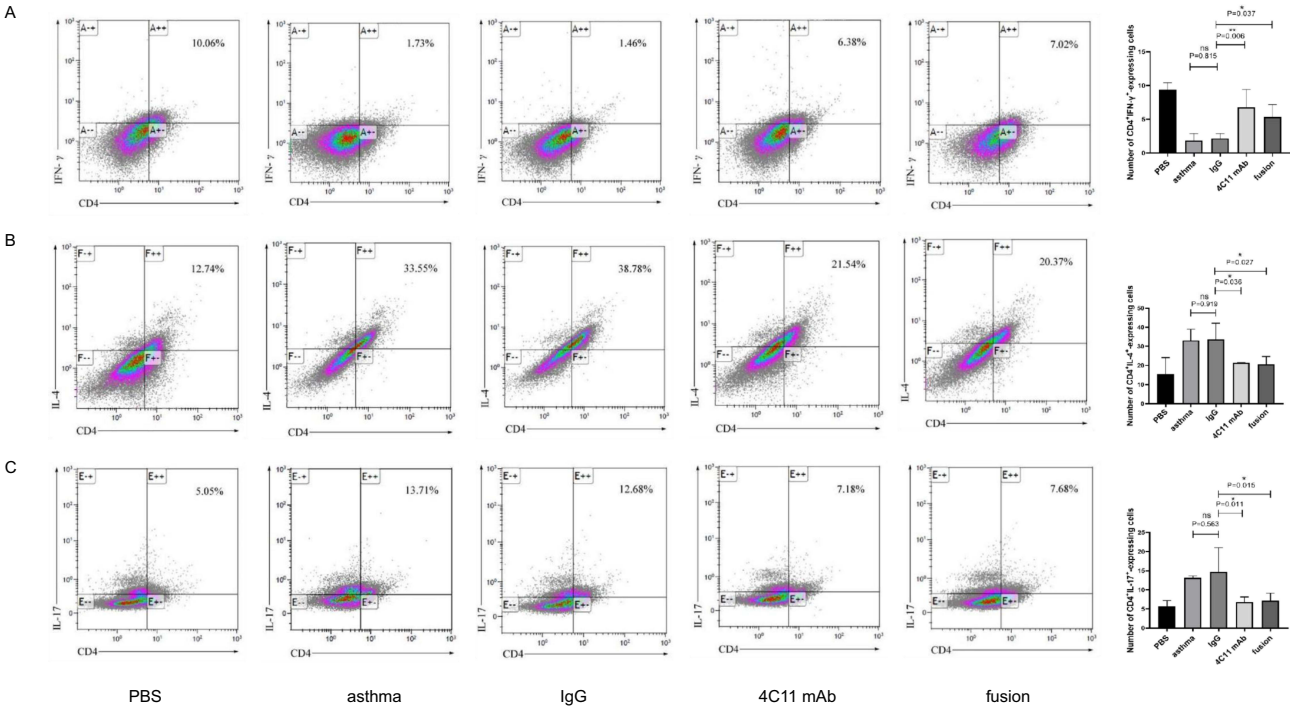

Figure 5 The Th1, Th2, and Th17 cell levels in the plasma of mice in each group. We established a mouse asthma model. Based on different intervention measures, they were divided into five groups: PBS control group, asthma group, IgG group, 4C11 mAb group, and fusion protein group. The Th1, Th2, and Th17 cell levels in the plasma of each group were detected. (ns=no significance,*P<0.05, **P<0.01). **A**. Compared to the PBS group, the Th1 cell ($CD4^+IFN\text{-}\gamma^+$) levels decreased in the asthma and IgG groups, and no statistically significant difference was observed between the two groups. The Th1 cell ($CD4^+IFN\text{-}\gamma^+$) levels in the 4C11 mAb and fusion protein groups were higher than those in the IgG group. (**B** and **C)**. Compared to the PBS group, the levels of Th2 ($CD4^+IL\text{-}4^+$) and Th17 ($CD4^+IL\text{-}17^+$) cells in the asthma and IgG groups increased, and no statistically significant difference was observed between the two groups. The Th2 ($CD4^+IL\text{-}4^+$) and Th17 ($CD4^+IL\text{-}17^+$) cell levels in the 4C11 mAb and fusion protein groups decreased than those in the IgG group.

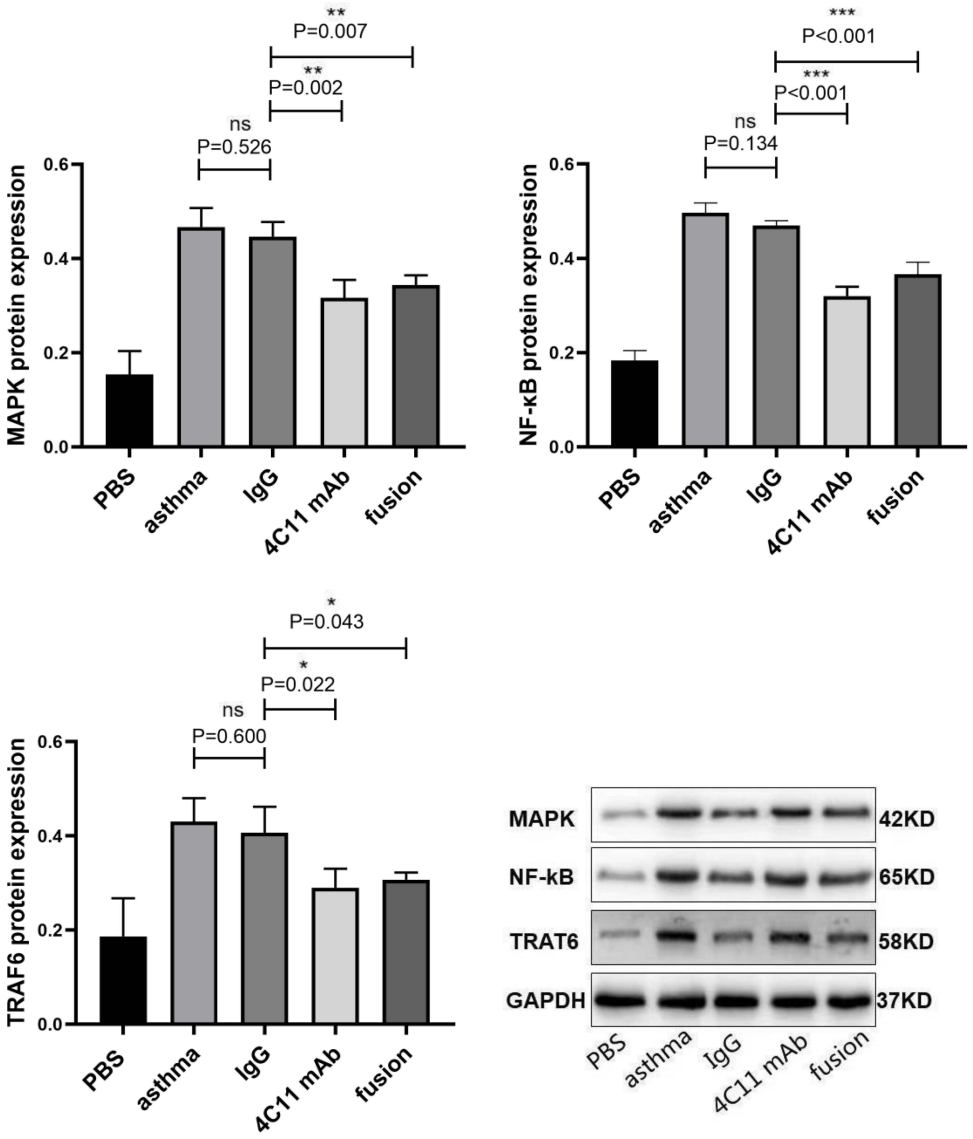

Figure 6 MAPK, NF-κB, and TRAF6 protein levels in lung tissue of mice. The MAPK, NF-κB, and TRAF6 protein levels in the lung tissue of mice in each group were determined. Compared to the PBS group, the levels of MAPK, NF-κB, and TRAF6 protein increased in the asthma and IgG groups, and there was no statistically significant difference between the two groups. However, the MAPK, NF-κB, and TRAF6 protein levels decreased in the 4C11 mAb and fusion protein groups than those in the IgG group. (ns=no significance,*P<0.05, **P<0.01, ***P<0.001).

scores of 2.7 and 3.3, respectively. The 4C11 mAb and fusion protein groups had similar scores of 1.7 and 1.3, respectively, which were significantly lower than those of the asthma and IgG groups ($p < 0.05$) (Figure 8).

Comparative Analysis of the Immunohistochemical Results of Lung Tissue

3.3-diaminobenzidine (DAB) color rendering and hematoxylin staining were performed. The dendritic cells expressing VISTA were stained brown. Analysis of the staining results showed that the PBS control group exhibited the highest proportion of VISTA-positive cells, while the asthma and IgG groups exhibited only a small proportion. A moderate number of VISTA-positive cells were observed in the 4C11 mAb and fusion protein group.

By using the Image-Pro Plus 6.0 tool to process the collected images, the average grayscale value of the positive view was determined based on the software functional module. The results showed that the expression of VISTA was the highest in the PBS control group, with extremely low levels of expression in the asthma and IgG groups and moderate levels of VISTA expression in the 4C11 mAb and fusion protein groups (Figure 9).

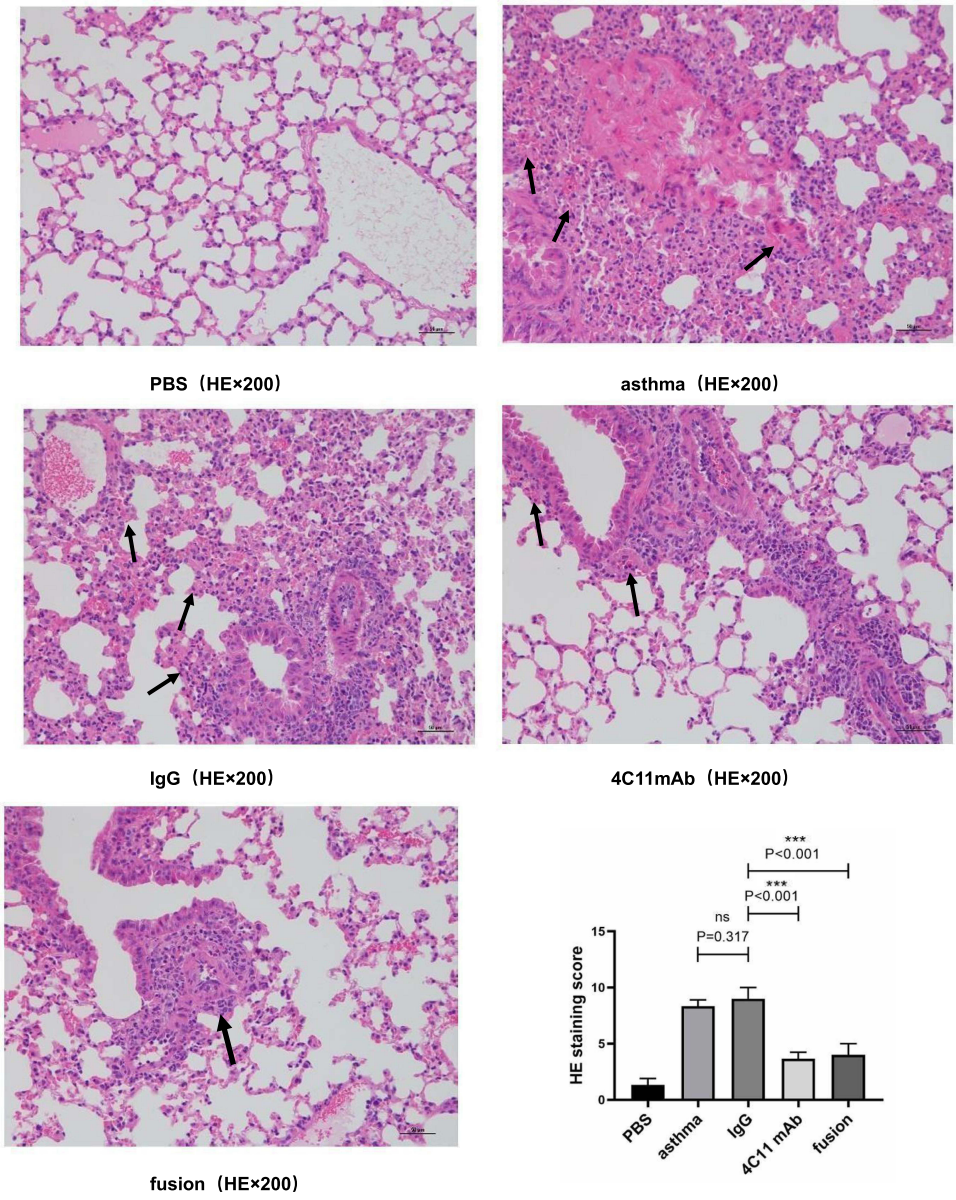

Figure 7 HE staining of lung tissue in each group. In the PBS control group, the lung tissue structure was clear and no change in the alveolar septum. In other groups, the arrows indicated the airway stenosis, columnar epithelial hyperplasia, significant eosinophils and inflammatory cell infiltration in the airway and around the alveoli. (ns=no significance, ***P<0.001).

Discussions

With the economic development and environmental changes in China, the incidence rate of asthma is increasing, which has aroused widespread concern in society. Relevant statistical results show that the prevalence of asthma in the youth group has reached 2.6% in China. Moreover, a significant gender difference is observed, with boys having a higher incidence rate.[16] With the standardization of asthma diagnosis and treatment, though the hospitalization and mortality rates of asthma have significantly decreased, they exert adverse effects on individuals, families, and society, thus requiring targeted interventions.

A close relationship exists between asthma and abnormal immune factors, among which the costimulatory molecules play an important role in activating or inhibiting T cells by regulating the balance of Th1/Th2 cells in asthma patients to worsen or alleviate asthma symptoms.[17] The B7 family is an important component of costimulatory molecules. Fourth cDNA of cytolytic T-lympnocyte-associated antigen (CTLA-4), Programmed Cell Death 1(PD-1), B7-H3, B7-H4, and

Table 3 Bronchial Lung Tissue Inflammation Pathology Scoring Table

Reference Indicators	Grade	Feature Description
Inflammatory Cell Infiltration	0	Normal, none or occasional inflammatory cell infiltration
	1	Mild, scattered infiltration, occasional aggregation into clusters
	2	Moderate, clustered, but not invaded the submucosa
	3	Severe, massive aggregation, invasion of submucosa or even mucosa
Bronchial Epithelial Cell Detachment	0	Normal, none or occasional squamous epithelial metaplasia
	1	Mild, occasional epithelial detachment
	2	Moderate, partial shedding, exposing lamina propria
	3	Severe, falling off in pieces, destruction of lamina propria
Alveolar Septal Thickening	0	Normal, no alveolar septal thickening
	1	Mild, 1–2 times the normal thickness
	2	Moderate, 3–5 times normal thickness
	3	Severe, more than 5 times the normal thickness
Bronchospasm	0	Normal
	1	Mild, scattered, with most lumens of normal size
	2	Moderate, with spasms visible under the microscope and narrowing of the lumen
	3	Severe, severe spasms, severe narrowing of the lumen

Human endogenous retrovirus subfamily H Long terminal repeat Associating protein 2(HHLA2) can play a role in the progression of asthma by regulating the activity of T cells.[18]

To understand the role of the synergistic inhibitory molecule VISTA in immune imbalance in children with asthma, we compared the differential expression of cytokines such as VISTA, IL-4, IL-6, CD25, CD40L, and PD-L2 in the asthma and control groups. The results show that the levels of VISTA, CD25, CD40L, and PD-L2 in the plasma of children with asthma are significantly reduced compared to the control group, while the levels of IL-4 and IL-6 are significantly increased. Th2 cell dysfunction and Treg differentiation inhibition in children with asthma are speculated. The plasma levels of VISTA in children with asthma are positively correlated with CD40L, while no significant correlation was observed with IL-4, IL-6, CD25, and PD-L2. Further research is necessary to investigate the mechanism of positive correlation between VISTA and CD40L in children with asthma.

In addition to the immune factors, allergies, genetics, and environmental factors also contribute to the complex physiological and pathological mechanisms of asthma. The heritability of asthma ranges between 36% and 95% and is closely related to racial differences.[19] Our research findings also suggest that most children with asthma had a history of eczema when they were young (74.5%). Moreover, 66.7% of them had allergic rhinitis and 54.9% had a history of asthma or allergy in family history. In addition, 91.1% tested positive for allergens, mainly including dust mites, house dust, and eggs. These findings indicate that allergic, genetic, and environmental factors also play a significant role in the progression of asthma.

The main pathological changes of asthma are the infiltration of eosinophils and other inflammatory cells in the airway, abnormal secretion of mucus, and notable proliferation of goblet cells, which eventually lead to airway obstruction.[20] The classic theory in immunological mechanisms is Th1/Th2 immune imbalance. Asthma in children is mostly caused by excessive differentiation or Th2 cell activity stimulated by allergens, the overactive synthesis and secretion of IgE, and type 2 cytokines such as IL-4, IL-5, and IL-13, leading to the development of asthma.[21] IL-4 is a multifunctional

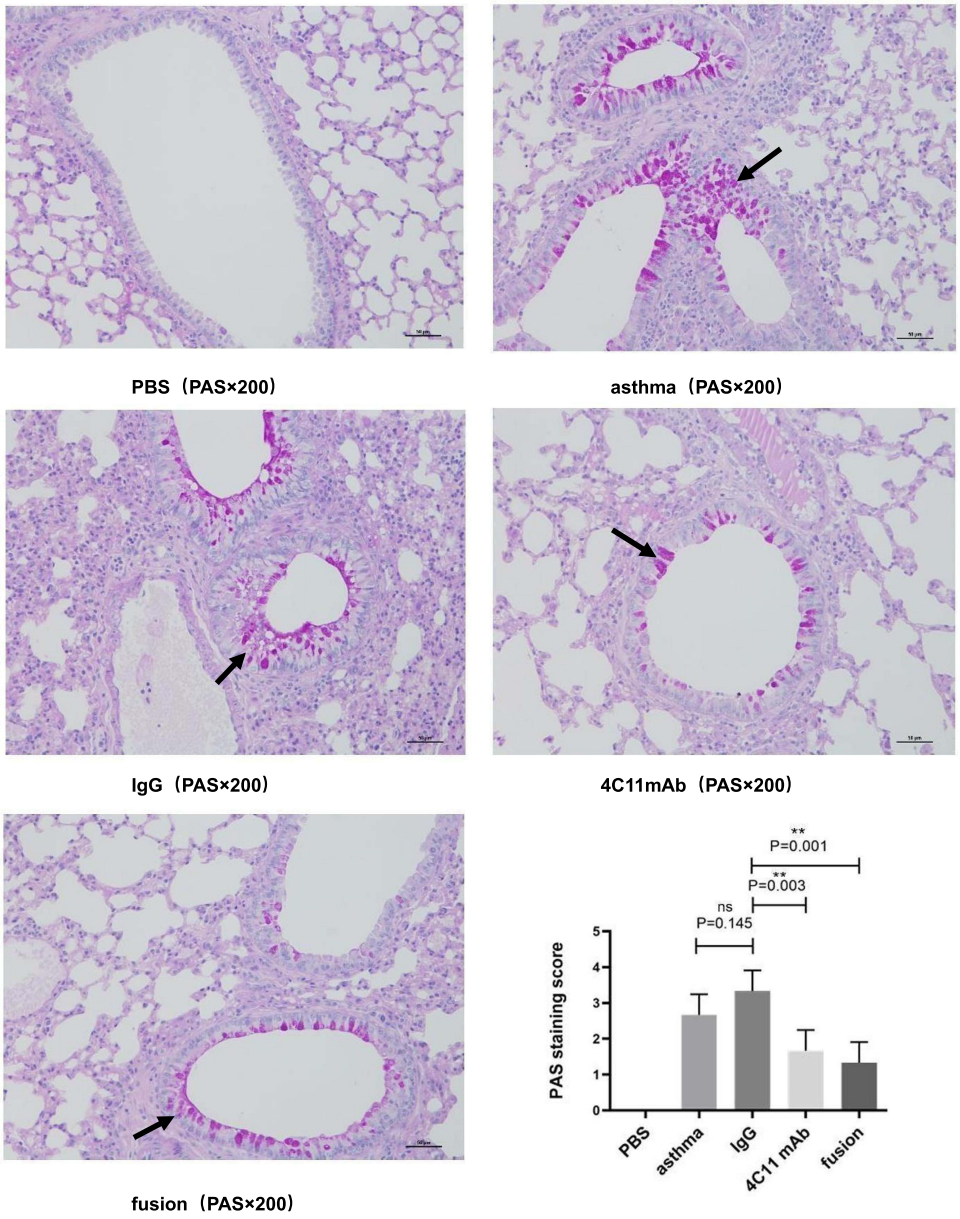

Figure 8 PAS staining of the lung tissue mucus secretion in each group. In the PBS control group, no mucus secretion was observed. The arrows indicated significant red acidic mucus secretion observed in the asthma and IgG groups. The arrows also indicated small amounts of mucus secretion observed in the 4C11 mAb and fusion protein groups. (ns=no significance, **P<0.01).

glycoprotein secreted by $CD4^{+}$T cells, B cells, innate lymphocytes (such as NK T cells), and myeloid cells. It is a marker cytokine of Th2 cells and induces hyperdifferentiation of Th2 cells while hindering Th17 cell differentiation. In addition, it can induce B cells to release IgE, leading to chronic airway inflammation and airway obstruction.[22] Vaccination against IL-4 reduces the secretion of IgE, inhibits the proliferation of mast cells, and prevents the occurrence of asthma in mice.[23] Makieieva et al[24] reported that the plasma IL-4 levels in children with asthma were higher than those in the healthy control group, and the IL-4 levels were higher in children with recurrent wheezing. IL-6 is a cytokine with multiple regulatory functions secreted by cells such as monocytes, macrophages, activated T cells, and B cells. It is secreted in large quantities when the body balance is disrupted, leading to the accelerated synthesis of substances such as C-reactive protein, complement C3, and fibrinogen, and inhibition of albumin synthesis. The inhibited albumin synthesis promotes B cells to synthesize immunoglobulin (Ig), inducing increased airway mucus secretion, and upregulation of Th17/Treg cell balance by binding to transforming growth factor-β (TGF-β).[25–27] Esty et al[28] reported two cases of

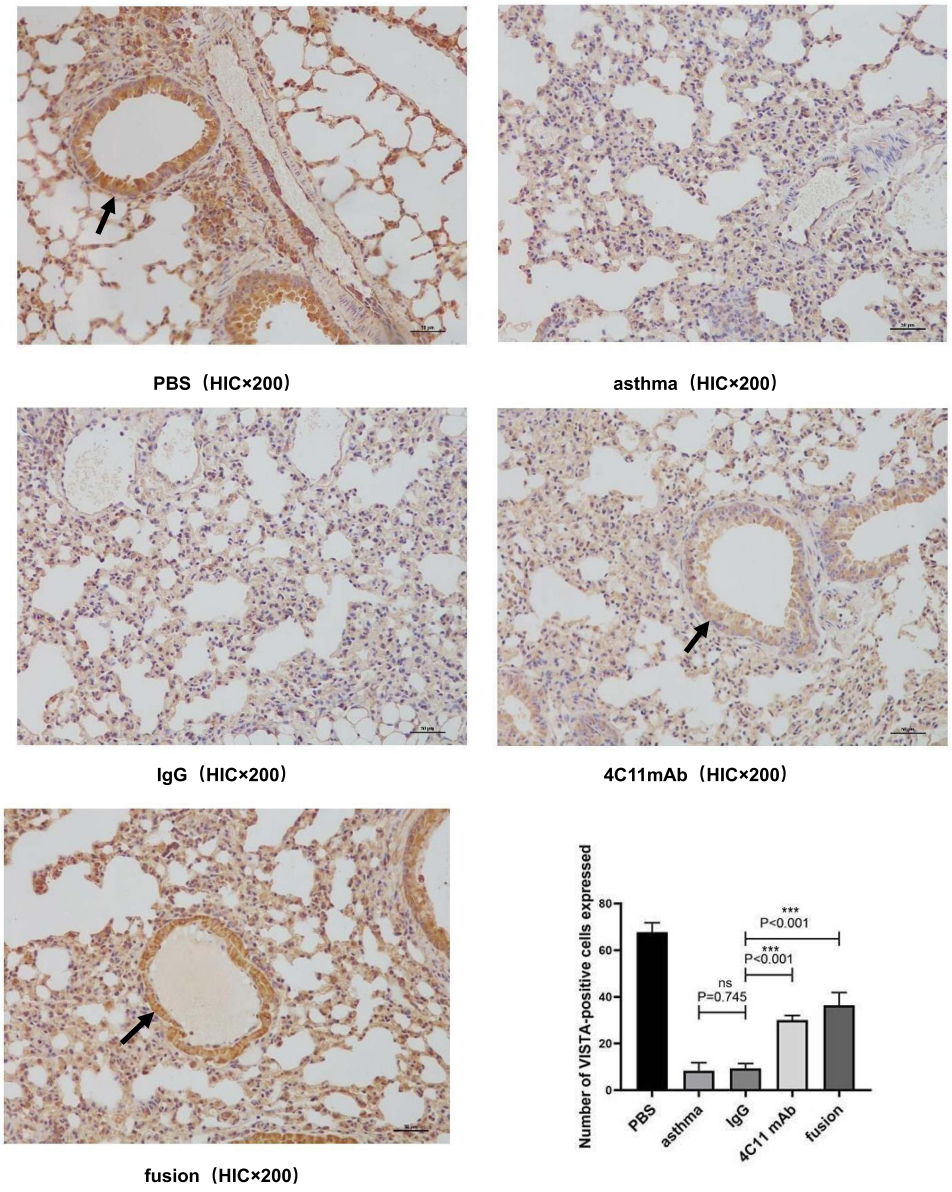

Figure 9 Immunohistochemical results of lung tissue in each group. The arrow indicated the highest proportion of VISTA-positive cells (brown staining) observed in the PBS control group. A moderate number of VISTA-positive cells(arrow) were observed in the 4C11 mAb and fusion protein groups while only a small proportion of VISTA-positive cells were observed in the asthma and IgG groups. (ns=no significance, ***P<0.001).

severe persistent nonatopic asthma where patients treated with humanized anti-IL-6 mAb (Tozumab) demonstrated a significant decrease in the number of hospital admissions and length of stay and a significant improvement in lung function. In a mouse asthma model induced by Aspergillus fumigatus, IL-6 deficiency caused an increase in TGF-β, exacerbated the aggregation of eosinophils and other inflammatory cells in the lungs, increased airway collagen deposition, and induced airway remodeling.[29] CD25, also known as IL-2R, is primarily synthesized by Treg cells and is a good marker of Treg cells, playing an important role in maintaining self-tolerance and homeostasis of the immune system.[30] The genetic defect of CD25 can cause systemic autoimmune disease (AID) in mice.[31] Huss et al[6] reported that the use of anti-CD25 mAb (PC61) in animal experiments blocks the binding of IL-2 to CD25 and inhibits Treg cell activation, while the consumption of CD25+Treg cells causes abnormal T cell activation and disrupts immune tolerance. PD-L2, also known as B7-DC, is highly expressed in dendritic cells(DCs) and macrophages. On interacting with receptor

PD-1, it can inhibit T cell activity and regulate immune response.[32] Lewkowich et al[33] reported that blocking PD-L2 significantly inhibited airway mucus secretion and enhanced AHR in a mouse asthma model.

CD40L is a member of the TNF family, and it is mainly expressed on the activated $CD4^+$T cell surface. By binding to CD40 in vivo and in vitro, CD40L inhibits the induced differentiation of Treg cells and promotes the secretion of cytokines such as IL-8, IL-10, IL-12, TGF- β, TNF-α, and IgE. It induces B cell proliferation and activation and regulates the process of asthma.[7] Another study[34] reported an abnormal proliferation of airway eosinophils and excessive differentiation of Th2 cells in CD40L-KO mice, increasing AHR and more severe asthma progression. Huang Hua Rong et al[35] reported a significant decrease in peripheral blood CD40L levels in children with asthma compared to the normal control group, consistent with the findings of our study. These conflicting studies may be related to the different functions of CD40–CD40L in different cell types or immune response stages. Understanding the detailed mechanism and biological significance of the controversial role of CD40–CD40L interaction in asthma requires further research. Simultaneously, no reports are available on the correlation between CD40L and VISTA, and further research is needed. In the current study, a significant increase in IL-4 and IL-6 levels was detected in the plasma of children with asthma, and the expression of VISTA, CD25, CD40L, and PD-L2 was inhibited, indicating that not only Th2 cell dysfunction but also Treg cell and B cell function inhibition occurred during asthma.

VISTA is a newly discovered synergistic inhibitory molecule that can promote Treg cell differentiation and inhibit T cell activity, thus playing an important role in the progression of diseases such as tumors and AIDS.[36] However, only a few research reports are currently available on the mechanism of the functioning of VISTA in asthma. Inhibition of TNF-α, IL-6, and IFN-γ expression in peripheral blood was detected in mice treated with an anti-VISTA monoclonal antibody (mAb).[37] Liu H et al[38] found that a deficiency of VISTA can increase airway eosinophil infiltration and the levels of IL-5, IL-13, IL-6, and TNF-α in BALF. It further suggested that VISTA mainly exerted immune regulatory effects by inhibiting T-cell activation.

The findings of the current study suggest that the plasma levels of VISTA in the asthma group were significantly reduced compared to those in the control group, while the levels of IL-4 were significantly increased, indicating that VISTA can play a role in the progression of asthma by inhibiting Th2 cell proliferation and differentiation. The plasma VISTA levels in children with asthma are positively correlated with CD40L, which is mainly related to the proliferation and activation of B cells and differentiation of Treg cells. This suggests that VISTA plays a role in regulating the proliferation and activation of B cells and Treg cells through certain pathways in the progression of asthma. VISTA, IL-4, IL-6, CD25, CD40L, and PD-L2 can all participate in the whole course of asthma by regulating Treg differentiation.

Throughout the entire course of asthma, immunological factors play an extremely crucial role. The imbalance of Th1/Th2 and Th17 cell differentiation and proliferation are the main factors.[21] Costimulatory molecules participate in the activation or inhibition of T cell proliferation, thereby affecting the process of immune response.[39,40] As a newly discovered synergistic inhibitory molecule, most research on VISTA is focused on tumors and AIDS.[36,41] Research on elucidating the mechanism of VISTA in asthma is seriously lacking. Herein, we constructed a mouse asthma model sensitized by OVA to intervene with mouse IgG, 4C11 mAb, and VISTA fusion protein during the induction period. Eosinophils and inflammatory cell aggregation, mucus secretion, and VISTA cell distribution in pathological sections of mouse lung tissue were observed. Our experiment detected the Th1, Th2, and Th17 cell levels in plasma and analyzed the secretion levels of Th1, Th2, and Th17 marker cytokines IFN-γ, IL-4, IL-5, IL-13, and IL-17 in BALF and plasma. By measuring the expression levels of the pathway proteins MAPK, NF-κB, and TRAF6, the mechanism of action of VISTA was investigated in a mouse asthma model, and the application prospects of 4C11 mAb and VISTA fusion protein in asthma were explored.

In the mouse asthma model established in the current study, we compared and analyzed the behavioral characteristics of mice in each group. The asthma group mice showed more prominent sensitization reactions and AHR manifestations compared to the PBS control group, with the primary symptoms being restlessness, shortness of breath, cyanosis, frequent scratching of the face and neck skin, poor breathing, and nodding breathing. Significant infiltration of eosinophils and inflammatory cells in the airways, as well as a large amount of airway mucus secretion, were observed in the pathological sections of the lung tissue in the asthma group mice, indicating the success of our experimental modeling and providing reliable support for subsequent experimental research. Based on this, we suggest that the use of

4C11 mAb and VISTA fusion protein intervention can reduce airway inflammatory infiltration and mucus secretion and regulate Th1/Th2/Th17 cell differentiation and related cytokine secretion, partially controlling the occurrence and development of asthma. Furthermore, the measurement of pathway proteins revealed that VISTA inhibits the synthesis of TRAF6 protein, thereby blocking the signal transmission of MAPKs and NF- κB, regulating the release of pro-inflammatory factors, and controlling asthma progression.

VISTA is a newly discovered member of the B7 family that plays a synergistic inhibitory role in the immune response by inhibiting T cell proliferation and activation.[42] Herein, compared with the PBS control group, the asthma group mice showed significant inhibition of Th1 cell differentiation and IFN-γ secretion in BALF and plasma, and a significant increase in the number of Th2 and Th17 cells, as well as levels of IL-4, IL-5, IL-13, and IL-17. This indicates that the asthma group has Th1/Th2/Th17 cell imbalance, reduced Th1 cell differentiation, and significantly increased Th2 and Th17 differentiation. IFN- γ is a symbolic cytokine of Th1 cells, mainly secreted by CD4+ and CD8+T cells, NK cells, B cells, macrophages, monocytes, and DCs. It can form a positive feedback pathway with IL-12, inhibit the differentiation of Th2 cells, reduce the secretion of IL-4 and IgE, control local inflammatory response, and alleviate asthma symptoms.[43] Reports reveal that IFN-γ mainly leads to AHR in the occurrence and development of severe asthma, while IL-17 mainly promotes the progression of airway neutrophil inflammation.[44] IL-4 can promote Th1/Th2 imbalance and induce Th17 cell differentiation, stimulate inflammatory cell production, and increase IgE secretion, thereby promoting the occurrence of asthma.[22] IL-5 is secreted by Th2 cells, NK cells, eosinophils, and basophils, thereby simultaneously promoting eosinophil proliferation and reducing its apoptosis level.[45] Currently, three mAbs targeting IL-5 have been developed, including Mepolizumab, Ruilizumab, and Benazizumab. These have been applied and promoted in the clinical treatment of severe asthma, and have demonstrated good therapeutic effects.[46] IL-13 is mainly secreted by Th2 cells and shares the receptor IL-4R with IL-4 α. It can promote the secretion of IgE, induce the proliferation of airway goblet cells, and increase mucus secretion.[47] Dual vaccination against IL-4 and IL-13 can significantly reduce AHR, airway mucus secretion, and eosinophil aggregation in mice, thereby preventing the occurrence of asthma.[23] IL-17 is the symbolic cytokine of Th17 cells, which can increase the secretion of neutrophil chemokine and produce a local inflammatory reaction, promoting the proliferation of airway goblet cells and mucus secretion. IL-17 can be detected in sputum, nasal cavity, and bronchial biopsy and blood of patients with severe asthma.[48]

In the current study, compared with the IgG intervention, the use of 4C11 mAb and VISTA fusion protein interventions during the induction phase effectively inhibits airway eosinophils aggregation, and mucus secretion, and alleviates asthma symptoms accordingly. Simultaneously, it can induce an increase in Th1 cell differentiation and an increase of IFN- γ in BALF and plasma. Conversely, Th2 and Th17 cell differentiation is inhibited and IL-4, IL-5, IL-13, and IL-17 secretions are reduced. But compared to those in the PBS control group, the levels of IFN- γ in BALF and plasma still decreased, while those of IL-4, IL-5, IL-13, and IL-17 increased, indicating that the 4C11 mAb and VISTA fusion protein partially inhibit asthma progression. Simultaneously, it was revealed that the PBS control group had the highest level of Th1 cells, while the levels of Th2 and Th17 were the lowest. The levels of Th1 cells were the lowest in the asthma and IgG groups, while those of Th2 and Th17 cells were the highest. The levels of Th1, Th2, and Th17 in the 4C11 mAb and fusion protein groups showed values in the middle, with statistical significance compared to the asthma and IgG groups ($p < 0.05$). The findings were consistent with the detection of related cytokines in BALF and plasma. Recently, researchers have found two types of anti-VISTA mAb. One is an inhibitory antibody, including 13F3 and MIH63, which mainly neutralizes the T cell inhibitory effect of VISTA and enhances the body's immunity. The second type is provocative antibodies, mainly, including 4C11 and MH5A, which enhance the T cell inhibitory effect of VISTA and weaken the body's self-immunity.[42] Liu et al[38] established a VISTA-KO mouse asthma model and discovered that the proportion of eosinophils and inflammatory cells in the BALF of mice was significantly increased, and the levels of IL-5, IL-13, and IL-6 were also significantly increased compared to those in the control group. No significant changes were observed in IL-4 and IFN- γ levels, while the percentage of Treg cells was significantly reduced. The changes of cytokine levels (such as IL-4, IL-5 and IFN-γ) indeed differed from our study, but it may only indicate that the regulatory effect of VISTA was not significantly correlated with these cytokines. However, it could not be denied that VISTA had a negative regulatory effect in asthma. During the induction period, the use of 4C11 mAb intervention revealed a significant decrease in the proportion of eosinophils and inflammatory cells in BALF, as well as a decrease in airway

mucus secretion. Therefore, 4C11 is beneficial in blocking OVA-induced Th2-type immune response, thereby suppressing airway inflammatory response and alleviating asthma symptoms, which is consistent with the results of our study. Ohno et al[49] intervened with inhibitory anti-VISTA mAb (MIH63) during the induction period in an OVA-induced mouse asthma model, resulting in a decrease in IFN- γ levels in BALF, increase in IL-13, and no significant changes in eosinophils, which did not exacerbate the asthma response. After intervention with MIH63 during the effective period, eosinophils significantly increased in BALS, while IL-5 and IL-13 secretions increased, AHR increased, and asthma response intensified. The focus of this study was whether VISTA might play a role in different stages of asthma. This suggests that different types of anti-VISTA mAb interventions at different stages of asthma exert different impacts on the occurrence and development of asthma, providing new ideas for subsequent asthma treatment. Han et al[50] reported that lupus-like autoimmune changes can occur in VISTA KO mice. Treatment with MH5A can reduce infiltration of neutrophils and monocytes, reduce the secretion of pro-inflammatory factors such as IL-1 and IL-2, and inhibit lupus progression. LeMercier et al[51] built a mouse model of melanoma and treated it with 13F3, which resulted in the increase of tumor-infiltrating $CD4^+$ and $CD8^+$T cells, and IFN- γ, IL-12, and TNF- α secretions were increased. Wang et al[12] reported that the VISTA fusion protein regulates the secretion of IL-2 and IFN-γ. In the presence of TGF-β in vitro, the VISTA fusion protein promotes the induced differentiation of iTreg cells, which is consistent with the results of the current study. Liu et al[38] injected VISTA fusion protein particles in the tail vein of asthmatic mice during the induction period, and detected high levels of VISTA fusion protein expression in the mouse serum. Consequently, compared with the control group, eosinophils, IL-5, and IL-13 in BALF were significantly increased, which was contrary to our experimental results. The conflicting results regarding VISTA fusion proteins originated from the different preparation and intervention methods of VISTA fusion proteins. Consequently, the VISTA fusion proteins regulated the body's immunity through different pathways. Further research is essential to clarify the mechanism of VISTA fusion proteins in asthma. Anti-VISTA mAb and fusion proteins have been widely used in the diagnosis and treatment of tumors and AIDS, and have demonstrated good therapeutic effects. However, research on the application of Anti-VISTA mAb and fusion proteins in asthma is still in its infancy, and further research should be conducted to open up a new path for the biological treatment of asthma.

In this experiment, the expression levels of MAPK, NF-κB, and TRAF6 pathway proteins were also measured among different groups. The findings reveal that the expression levels of the three proteins were the lowest in the PBS control group, the highest in the asthma and IgG groups, and showed a middle value in the 4C11 mAb and fusion protein groups. The results showed statistical differences ($p < 0.05$). The expression levels were opposite to those of the VISTA protein measured using immunohistochemistry. This suggests that VISTA inhibits the activation of MAPKs and NF-κB signaling pathways by promoting the degradation of TRAF6 protein in vivo, thereby effectively controlling the release of pro-inflammatory factors. Under the mediation of toll-like receptors(TLRs), myeloid differentiation factor 88 (MyD88) combines with IRAK kinase to produce Myddosome, and activates IRAK1 to associate with TRAF6. TRAF6, in turn, cooperates with the ubiquitin-binding enzymes to promote the multi-ubiquitination process of the TAK1 complex. TAK1 can further activate the MAPK/AP-1 and IKK/NF-κB signaling pathways, inducing the secretion of pro-inflammatory factors such as IL-6, IL-12, and TNF-α.[52,53] Xu et al[15] isolated peritoneal macrophages from VISTA-KO mice and stimulated them in vitro with TLR agonists to increase TRAF6 expression and polyubiquitination linked to K63, forming numerous MyD88/IRAK/TRAF6 complexes that mediate MAPK/AP-1 and IKK/NF-κB signaling pathway activation. This significantly increases the expression of pro-inflammatory factors such as IL-6, IL-12, and IFN-γ, consistent with the results of our study. VISTA can regulate the release of pro-inflammatory factors by inhibiting the transmission of MAPKs and NF-κB signaling pathways, thereby playing a role in the occurrence and development of asthma.

Although the current study provided some valuable insights, it also has several shortcomings. First, no blood samples were collected for comparison in the recovery period of the children with asthma. In addition, there was a lack of investigations on changes in the levels of related cytokines, particularly VISTA, in the same child after treatment. Second, the sample size of this experiment was small, which inevitably led to errors. Subsequent large-scale, multicenter studies are needed to reveal the further relationship between VISTA and related cytokines. Several shortcomings were present in our animal experiment. First, sufficient amounts of mouse BLAF and lung homogenate were not collected, and total cell count and classification count analysis were not conducted. Second, no in vitro experiments were conducted to

further analyze the signaling pathway transmission mechanism. A lack of synergy occurred between animal and clinical experiments in detecting cytokines, and no good consistency was present between clinical and animal experiments. Further improvements should be made in the future.

In summary, this study compared and analyzed the differential expression of VISTA, IL-4, IL-6, CD25, CD40L, and PD-L2 in the plasma of children with asthma and the control group. A hyperdifferentiation of Th2 cells was revealed in the asthma group, while Treg cell differentiation and B cell functions were inhibited. The decreased expression of VISTA in children with asthma is positively correlated with the CD40L level in plasma. We also constructed an OVA-induced mouse asthma model and intervened with 4C11 mAb and VISTA fusion protein during the induction period. This can alleviate the pathological changes of asthma in the mouse lungs, increase Th1 cell differentiation and IFN-γ secretion, inhibit Th2 and Th17 cell differentiation and IL-4, IL-5, IL-13, and IL-17 secretion, and partially arrest the progression of asthma. Simultaneously, through the determination of pathway proteins, it was confirmed that VISTA can inhibit the transmission of MAPKs and NF-κB signaling pathways by reducing TRAF6 protein expression, regulating the release of pro-inflammatory factors, and thus affecting the pathological changes of asthma. However, we also found that the intervention of 4C11 mAb and fusion protein groups on asthma mice showed similar levels of control over the progression of asthma, with no significant statistical difference. Further research needs to be conducted to investigate the differences and connections between then and determine whether the simultaneous intervention of the two proteins would exert a better therapeutic effect on the progression of asthma than that obtained by the intervention of a single protein. VISTA is a newly discovered synergistic inhibitory molecule that plays an important role in the progression of asthma. It can serve as a target for the biological therapy of asthma, providing support for asthma prevention and treatment and also providing references for related research.

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Disclosure

Jianqun Yin and Jiawei Chen are co-first authors for this study. The authors report no conflicts of interest in this work.

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